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Salicylic acid-induced photosynthetic adaptability of *Zea mays* L. to polyethylene glycol-simulated water deficit is associated with nitric oxide signaling

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Abstract

Salicylic acid (SA) and nitric oxide (NO) form a new group of plant growth substances that cooperatively interact to promote plant growth and productivity. Water deficit (WD) stress is a major limiting factor for photosynthesis, which in turn limits crop yield. However, the mechanism of SA and NO in stimulating photosynthesis has not yet been elucidated. Therefore, in this study, we investigated the SA- and NO-mediated photosynthetic adaptability of maize seedlings to WD in terms of photosynthetic parameters, activities and mRNA levels of CO_2 assimilation enzymes. Our results showed that SA alleviated the WD-induced reduction of photosynthetic performance. The activities of Rubisco and Rubisco activase enzymes increased significantly due to SA pretreatment. Moreover, higher transcription rates of *Rbc* L, *ZmRCAα* and *ZmRCAβ* mRNA further confirmed the effects of SA on CO_2 assimilation. WD or SA-induced decreases or increases of CO_2 assimilation ability were further decreased after c-PTIO addition.

Additional key words: chlorophyll fluorescence transients; gene expression; nitric oxide scavenger; photosynthetic characteristics.

Introduction

Maize (*Zea mays* L.) is one of the most important food crops in China and ranks third worldwide. Maize suffers from biotic and abiotic stress at different growth stages. Water deficit (WD) is one of limiting factors that adversely affect plant growth, development, survival, and crop productivity (Serraj *et al.* 2011). Maize is also considered one of the most WD-sensitive crops. An estimated 15–20% of the grain yield of maize is lost each year due to WD, and such losses may increase further as WD become more frequent and severe with global climate change (FAOSTAT 2010). WD elicits a series of physiological responses including early reduced stomatal conductance (g_s) and photochemical inhibition, and a reduction in photosynthesis (Sikder *et al.* 2015).

Nitric oxide (NO), a gaseous free radical, is a known mediator of many physiological functions in animals. The identification of biological targets and functions of NO in mammals led to the investigation of its role in the physiological processes of plants (Hancock 2012). This led to the finding that plants can produce NO. NO is considered an essential signaling molecule in a wide range of physiological processes, such as seed germination, root organogenesis, stomatal movement, chlorophyll (Chl) biosynthesis, flowering, and senescence (Corpas *et al.* 2011, Liao *et al.* 2012, Tossi *et al.* 2012, Procházková *et al.* 2013). However, some studies also implicate NO as a stress-inducing agent in plants (Bajguz 2014).

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Abbreviations: cGMP – cyclic guanosine monophosphate; Chl – chlorophyll; c-PTIO – 2-4-carboxyphenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide; C_i – intercellular CO_2 concentration; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; LA – leaf area; MAPK – mitogen-activated kinases; NO – nitric oxide; PEG – polyethylene glycol; PI_{ABS} – photosynthesis performance in PSII electron transport; P_N – photosynthetic assimilation rate; RCA – Rubisco activase; RCs – per active reaction centers; RWC – relative water content; SA – salicylic acid; WD – water deficit; WUE_i – intrinsic water-use efficiency.
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Under WD conditions, chloroplasts become one of the endogenous NO cellular sources; it has been assumed that there are binding sites for NO within PSII between the primary and secondary quinone acceptors (Jasid *et al.* 2006). NO protects the critical functional proteins in the PSII complex during drought stress indicating its regulatory role in photosynthesis. For example, in *Triticum aestivum* L, NO restricted the WD-induced reduction in transcription of *Psb* A gene that encodes the D1 protein of the PSII complex (Wang *et al.* 2011, Procházková *et al.* 2013). Additionally, exogenous NO ameliorates WD stress-induced inhibitory growth by enhancing the net photosynthesis, which in turn alleviates the destructive effects on chloroplasts (Kovacs and Lindermayr 2013). The stimulating effect of NO on PSII photochemistry has been correlated with an increase in the proportion of the open PSII reaction centers (Shao *et al.* 2013) and promoting energy balance in the chloroplast (Vanlerberghe *et al.* 2016). Interestingly, the NO scavenger eliminated the effects of endogenous or exogenous NO on the photosynthetic performance to some extent (Chen *et al.* 2014a).

Many studies show that NO acts as a regulator by interacting with secondary messengers, such as Ca^{2+}, cyclic guanosine monophosphate (cGMP), hydrogen peroxide (H_2O_2), phytohormones such as abscisic acid (ABA), protein kinases, such as serine/threonine protein kinase (OST1), and mitogen-activated kinases (MAPK), which in turn can modulate expression of target genes that are involved in stress recovery phytohormones (Cui *et al.* 2011, Baudouin and Hancock 2013). Salicylic acid (SA) is a well-known signaling molecule that plays multifaceted roles in the stress tolerance of plants. Many recent studies have reported the effects of exogenous NO and SA on alleviating oxidative damage induced by environment stresses, such as nickel stress on wheat (Siddiqui et al. 2013), cadmium stress on perennial ryegrass (Wang *et al.* 2013) and peanut (Xu *et al.* 2015), salt stress on cotton seedlings (Liu *et al.* 2014) and soybean (Simaei *et al.* 2011) and rice (Mostofa *et al.* 2015). Most interestingly, NO might be involved in H_2O_2- and SA-induced reduction of oxidative damage through the upregulation of antioxidant defense to confer stress tolerance (Mostofa *et al.* 2015).

It is reported that to adapt to WD stress, plants have evolved several photosynthetic mechanisms, such as, down-regulating the activity of enzymes related to the Calvin cycle, particularly Rubisco and Rubisco activase (RCA) involved in photochemistry (Dias *et al.* 2010, Xu *et al.* 2013). Thus, it is well known that the increase in photosynthesis can be achieved by enhancing the Rubisco and RCA activities (Carmo-Silva *et al.* 2013, Chen *et al*. 2014b). However, the role of SA in inducing photosynthetic ability in response to WD through activation of Rubisco and RCA is not clear. Moreover, the role of NO in the regulation of photosynthetic enzymes is unknown. Therefore, we used exogenous SA and NO scavenger under WD conditions, and then analyze the photosynthetic characteristics, the activities of RCA and Rubisco enzymes, and the expression of genes encoding these enzymes.

Materials and methods

Plant materials and treatments: Maize (*Zea mays* cv. ZhuYu 309) seeds were obtained from Zhumadian Research Institute for Agricultural Sciences. The seeds were soaked in water containing 0.2% (w/v) tiuram and benomyl for 24 h to prevent fungal infection, then placed on moist gauze spread in discs, and finally germinated in the dark at 28°C for 4 d. Uniformly geminated seeds were selected and transplanted to light proof plastic containers (20 × 15 × 14 cm; length × width × height) of aerated half-strength Hoagland nutrient solution with 12 seedlings per container. Seedlings were mounted on floating polyfoam board with sponges; nutrient solutions were renewed every two days and aerated once each day. All plants were grown in a light-and-temperature controlled green house with a 28 ± 2°C/20 ± 2°C day–night regime, a light intensity of 400 μmol(photon) m^{-2} s^{-1}, and a relative humidity of 80 ± 5%.

The 20-d-old plants were transferred in half-strength Hoagland nutrient solution supplemented with or without 5 mM SA for 3 d, and then exposed to 20% polyethylene glycol (PEG)-6000 solution (–0.8 MPa) or 0.4 mM 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) for 3 d. The maize seedlings were treated hydroponically (Gondor *et al*. 2016) as follows:

Treatment	Composition
CK	distilled water as a control
WD	PEG solution for 3 d
SA + WD	SA pretreatment plus PEG
WD + P	PEG plus c-PTIO
SA + WD + P	SA pretreatment plus PEG and c-PTIO

Then, at the third day after treatment, the second uppermost fully expanded leaves of uniform seedlings were sampled, immediately frozen in liquid N_2, and stored at −80°C until (for) analysis. Four biological replicates were performed for each treatment.

Growth parameters and relative water content: Plant growth was characterized by the measurement of plant height and fresh mass in the leaves of 10 plants. The samples were placed in an oven at 105°C for 15 min and dried to a constant mass at 75°C. The length (from ligule to leaf tip) and width (the widest portion of the leaf blade) of each green leaf were measured *in situ*. Individual green leaf area (LA) was estimated as the product of leaf length and maximum breadth, adjusted by a constant coefficient as follows (Elings 2000): LA = length × maximum width × 0.75.

The relative water content (RWC) in leaves was determined by the method described by Wu and Xia (2006). The RWC percentage was calculated using the following formula: RWC [%] = 100% × [(FM − DM)/(SM − DM)]; where FM stands for fresh mass, DM for dry mass, and SM for saturated mass. SM was determined after letting the leaflet float on distilled water for 24 h at room temperature.

Photosynthetic rate, chlorophyll (Chl) *a* fluorescence transients: The leaf gas-exchange parameters, such as net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were determined using *LCA-3* portable photosynthesis system (*ADC,* Hoddesdon, UK) between 11:00 and 13:00 h, intrinsic water-use efficiency ($WUE_i = P_N/g_s$) of each seedling was calculated. The polyphasic rise in the transient fluorescence was measured on the second fully expanded leaves from the top after 30 min of dark adaptation using a plant efficiency analyzer (*PEA*; *Hansatech Instruments*, UK), following the procedures of Strasser *et al.* (2000). The Chl *a* fluorescence transients were obtained by 2-s saturating red light and analyzed with the JIP-test (Strasser *et al.* 2000): the fluorescence intensity at 50 μs, considered F_o, when all PSII RCs are open; the maximal fluorescence intensity, F_m, assuming that the excitation intensity is high enough to close all of the RCs of PSII. $F_v/F_m = (F_m - F_o)/F_m$, represents the maximum photochemical efficiency of PSII of chloroplasts. $PI_{ABS} = RC/ABS\ [\varphi P_o/(1 - \varphi P_o)][\psi_O/(1 - \psi_O)]$, represents the photosynthesis performance in PSII electron transport.

RCA and Rubisco activity, NO concentration: Rubisco activity was measured as described by Jiang *et al.* (2012). RCA activity was determined using a *Rubisco Activase Assay Kit* (*Genmed Scientifics Inc.,* Wilmington, DE, USA). NO content was measured using revolutionary NO measurement system (Cheng *et al.* 2015) that uses micro-ion electrodes to independently measure nitrite (NO_2^-) and nitrate (NO_3^-) ions. The sum of these two ions gives the original amount of NO present in leaves.

Total RNA extraction and gene expression analysis: Total RNA was isolated from wheat leaves with the *Spin Column Plant Total RNA Purification Kit* (*Sangon Biotech,* Shanghai, China) according to the manufacturer's instructions. The cDNA template for real-time RT-PCR was synthesized using the *AMV First Strand cDNA Synthesis Kit* (*Sangon Biotechnology Co.,* Shanghai, China). Real-time RT-PCR was performed with the *ABI StepOne PlusTM Real-Time PCR Detection System* (*ABI, Waltham,* MA, US). Each reaction (20 μL) consisted of 1 μL of diluted cDNA and 10 μL of *SybrGreen qPCR Master Mix* (*ABI*). Actin was used as an internal reference gene to calculate relative transcript levels. Relative gene expression was calculated as described by Livak and Schmittgen (2001).

Statistical analysis: Data was expressed as means ± standard deviation (SD) of independent replicates. Based on sequences in the *GenBank* database, gene-specific primers (Table 1) were designed with *Primer Premier 5.0* and used for amplification. Data was analyzed using one-way analysis of variance (*ANOVA*) by *SPSS* (*version 19.0*) and *Duncan*'s multiple range test to determine significant ($p<0.05$) differences between treatments.

Table 1. Primers sequences used for real-time fluorescence quantitative PCR (qRT-PCR) for detecting gene expression in the leaves of maize plants. T_m – melting temperature.

Gene	Gene description	Sequence (5'-3')	T_m [°C]
actin		F: CTGAACCCCAAGGCAAACA	59.0
		R: ACTGGCGTACAGGGAAAGAA	57.3
Rbc L	Rubisco large subunit	F: CCGTTTCGTCTTTTGTGCC	58.9
		R: TGCGGTGAATCCTCCTGTT	58.3
Rbc S	Ribulose bisphosphate carboxylase small chain	F: CGCTACTGGACCATGTGGAA	59.0
		R: ACTGCGTCTGCTTGATGTTGT	58.1
ZmRCAα	*α*-form Ribulose-1,5-bisphosphate carboxylase/oxygenase	F: TGCAGCTTTCTCCTCCACTTC	60.1
		R: GCCATGGCTCTGACTCTGATG	60.3
ZmRCAβ	*β*-form Ribulose-1,5-bisphosphate carboxylase/oxygenase	F: CCGAACTAAAAAGCACAAGAAATG	60.5
		R: CAGCCATCGCCTTGAACCT	60.3

Results

Growth and RWC: Plant growth can reflect the effects of stress; thus, FW, DW, RWC, and plant height of maize were determined (Table 2). Our results showed that drought inhibited maize growth, showing 51.8, 23.2, 17.7, 4.3, and 11.9% decrease in FM, DM, RWC, plant height, and LA, respectively. However, we found that 0.5 mM SA could relieve the inhibition, and FM, DM, RWC, plant height, and LA of maize seedlings pretreated with 0.5 mM SA followed by exposure to PEG solution were significantly higher (14.6, 2.3, 8.1, 17.8, and 11.4%,

respectively) than those with WD treatment alone. The addition of c-PTIO to WD or WD+SA solution further reduced these indexes as compared with WD and WD + SA treatments, respectively.

Photosynthetic characteristics: WD strongly suppressed photosynthetic capacity. P_N, g_s, PI, and WUE_i of leaves significantly decreased by 53.2, 39.6, 28.8, 25.0%, respectively, as compared with CK treatment (Table 3), but C_i increased by 41.4%. However, WD did not have any effect on F_v/F_m as compared to control. SA pretreatment under WD enhanced the g_s, P_N, F_v/F_m, PI, and WUE_i by 33.6, 48.0, 6.8, 18.1, and 18.0%, respectively, as compared with WD treatment. As an NO scavenger, the addition of c-PTIO to WD or SA solution could decrease these parameters as compared with WD or WD + SA treatments, respectively.

Table 2. Effect of salicylic acid (SA) and 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) on fresh mass (FM), dry mass (DM), relative water content (RWC), plant height, and leaf area of maize seedlings exposed to 20% PEG-stimulated water deficit. Data are the means ± SD of four replicates. *Different letters* in the same columns showed significant differences at $p<0.05$. The 20-d-old plants were pretreated with 5 mM SA for 3 d, then exposed to PEG solution or c-PTIO for 3 d. The maize seedlings were treated as follows: CK – distilled water as a control; WD – 20% PEG-6000 solution for 3 d; SA + WD – 5 mM SA pretreatment plus 20% PEG-6000; WD + P – 20% PEG-6000 + 0.4 mM c-PTIO; SA + WD + P – 5 mM SA pretreatment + 20% PEG-6000 and 0.4 mM carboxy-PTIO.

Treatment	FM [g]	DM [g]	RWC [%]	Plant height [cm]	Leaf area [cm^2]
CK	3.40 ± 0.27^{a}	0.56 ± 0.02^{a}	90.03 ± 5.03^{a}	25.24 ± 1.31^{a}	140.29 ± 3.10^{a}
WD	1.64 ± 0.05^{c}	0.43 ± 0.01^{bc}	74.05 ± 4.10^{c}	18.97 ± 0.73^{c}	123.56 ± 5.01^{b}
SA + WD	1.88 ± 0.10^{b}	0.44 ± 0.00^{b}	80.05 ± 2.35^{b}	22.34 ± 1.02^{b}	137.67 ± 10.34^{ab}
WD + P	1.27 ± 0.12^{d}	0.40 ± 0.01^{c}	68.04 ± 2.09^{d}	17.92 ± 0.62^{c}	110.74 ± 7.81^{c}
SA + WD + P	1.43 ± 0.12^{d}	0.42 ± 0.01^{bc}	76.04 ± 3.03^{c}	20.38 ± 1.02^{bc}	125.65 ± 10.67^{b}

Table 3. Effects of salicylic acid (SA) and 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) on photosynthetic characteristics of maize seedlings exposed to 20% PEG-stimulated water deficit. Data are the means ± SD of four replicates. *Different letters* in the same columns showed significant differences at $p<0.05$. The 20-d-old plants were pretreated with 5 mM SA for 3 d, and then exposed to PEG solution or c-PTIO for 3 d. The maize seedlings were treated as follows: CK – distilled water as a control; WD – 20% PEG-6000 solution for 3 d; SA + WD – 5 mM SA pretreatment plus 20% PEG-6000; WD + P – 20% PEG-6000 + 0.4 mM c-PTIO; SA + WD + P – 5 mM SA pretreatment + 20% PEG-6000 and 0.4 mM carboxy-PTIO.

Treatment	g_s [mmol(H_2O) m^{-2} s^{-1}]	C_i [µmol(CO_2) mol^{-1}]	P_N [µmol(CO_2) m^{-2} s^{-1}]	F_v/F_m	PI	WUE_i [µmol(CO_2) mmol(H_2O)$^{-1}$]
CK	350.31 ± 5.00^{a}	105.71 ± 10.23^{c}	9.58 ± 0.53^{a}	0.79 ± 0.00^{a}	1.63 ± 0.12^{a}	2.52 ± 0.31^{a}
WD	211.56 ± 24.13^{c}	149.52 ± 4.72^{a}	4.48 ± 0.41^{c}	0.73 ± 0.06^{ab}	1.16 ± 0.36^{b}	1.89 ± 0.13^{b}
SA + WD	282.55 ± 13.56^{b}	126.78 ± 5.36^{b}	6.63 ± 0.40^{b}	0.78 ± 0.02^{a}	1.37 ± 0.42^{a}	2.23 ± 0.12^{a}
WD + P	180.01 ± 2.89^{d}	150.01 ± 9.54^{a}	4.08 ± 0.20^{d}	0.67 ± 0.04^{b}	0.77 ± 0.29^{c}	1.69 ± 0.02^{c}
SA + WD + P	188.94 ± 1.77^{cd}	148.33 ± 8.54^{a}	5.02 ± 0.56^{c}	0.75 ± 0.04^{a}	1.66 ± 0.28^{a}	2.04 ± 0.02^{b}

Activities of Rubisco and RCA enzymes, and NO content: We determined Rubisco and RCA activities to study further the mechanism by which SA regulates CO_2 fixation. Rubisco and RCA activity were respectively decreased by 45.3 and 39.9% in response to WD (Fig. 1). However, SA-pretreated maize plants had higher Rubisco and RCA activities than plants without SA treatment under WD. We found that as compared with WD and WD + SA treatments, the Rubisco activity was decreased by 15.5 and 17.9% with WD + c-PTIO and WD + SA + c-PTIO treatments, respectively. Also, RCA activity was reduced by 40.0 and 36.6% in WD + SA + c-PTIO treatments, respectively. In addition, determination of NO content showed that WD increased NO production, especially SA + WD treatment increased NO con tent by 4.1 fold and 1.2 fold compared with CK and WD.

Expression of *Rbc* and *RCA* genes: To evaluate further the molecular response of maize photosynthesis to SA and c-PTIO under WD, we analyzed the genes encoding the *RCA* and *Rbc* subunits at the transcriptional level using qRT-PCR (Fig. 2). Although under WD the *ZmRCAα*, *ZmRCAβ*, *Rbc* L and *Rbc* S levels were decreased by 57.4, 87.4, 37.9, and 59.3%, respectively, they were significantly down-regulated except *Rbc* S when maize plants were treated with c-PTIO. However, transcript levels of *ZmRCAα*, *ZmRCAβ* and *Rbc* L in SA + WD treatment were higher than those in WD treatment but decreased due to the addition of c-PTIO.

Discussion

WD is one of most damaging of environmental stresses for plant growth, development, and yield formation. Plant responses to WD stress are complicated and involve processes regulated by signaling molecules such as NO and SA (Iqbal *et al.* 2014, Qiao *et al.* 2014). Our results showed that WD resulted in the loss of leaf water content and inhibition of plant growth; however, SA pretreatment significantly relieved WD-induced inhibition of growth and resulted in increased FW, DW, LA, and plant height. Interestingly, these stimulating effects were suppressed by the NO scavenger c-PTIO. This indicated that NO signal might be involved in the adaptation of SA-regulated maize seedling growth to WD.

Photosynthesis, the main force driving crop production, is one of the key processes to be affected by WD (Ashraf *et al.* 2013). Photosynthetic CO_2 assimilation per unit area and time, *i.e.*, P_N is inhibited by rapidly developing WD in physiological studies, so it is implicated in a decrease in dry matter production (Tatar *et al.* 2016). In this study, substantial decrease of photosynthesis under WD condition was due to nonstomatal factors, the photosynthetic capacity was impaired significantly, because the chloroplast envelope was possibly affected by low RWC (< 80%). However, SA pretreatment could reverse such changes. Moreover, SA enhanced P_N by maintaining hydraulic factors (RWC), which serves to regulate water loss in relation to water uptake. Therefore, RWC and WUE_i in WD + SA pretreatment decreased very little compared with control. NO signal is produced in the chloroplast, and is thought to regulate photosynthesis relying on nonstomatal factors under stresses (Kausar and Shahbaz 2013, Procházková *et al.* 2013). NO was also involved in CTK regulation of photosynthetic adaptability to WD in our previous experiment (Shao *et al.* 2010). Our study suggests that SA-induced improvements in CO_2 assimilation may also be related to NO signals. Therefore, NO scavenger may manifest changes in CO_2 fixation rate and electron transport ability.

The activation of Rubisco by RCA serves an important regulatory function in linking the rate of CO_2 fixation to the rate of electron transport activity *via* the production of ATP (Dias *et al.* 2010, Parry *et al.* 2013). Rubisco catalyzes the first step in the net photosynthetic CO_2 assimilation (Chen *et al.* 2014b), and its activation state is regulated by RCA (Boex-Fontvieille *et al.* 2014). In this study, water loss caused de-activation of Rubisco and RCA, which in turn caused a metabolic limitation to photosynthesis, resulting in a drop of net formation of carbohydrates, such as dry mass. However, there was no significant difference in Rubisco and RCA activity between SA + WD and WD treatments regarding an increase in CO_2 assimilation rate. These results suggest that SA improves the efficiency of photosynthetic carbon fixation by activating the photosynthetic enzymes, which restore the supply of CO_2 to Rubisco enzyme and help in overcoming stomatal limitations.

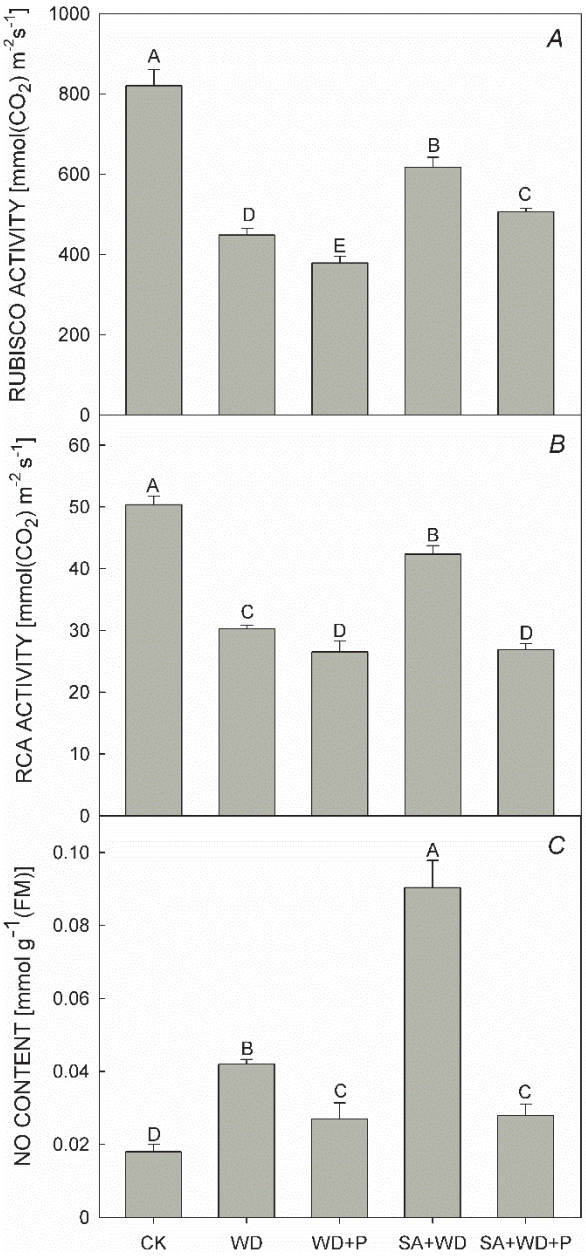

Fig. 1. Effects of salicylic acid (SA) and 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) on Rubisco activity (*A*), Rubisco activase activity (*B*), and nitric oxid (NO) content (*C*) of maize seedlings under 20% polyethylene glycol (PEG)-6000 induced water-deficit stress. Data are the means ± SD of three replicates. *Different letters* in the same columns showed significant differences at $p<0.05$. The 20-d-old plants were pretreated hydroponically with 5 mM SA solution for 3 d, and then exposed to 20% PEG solution or 0.4 mM c-PTIO for 3 d. The maize seedlings were treated as follows: CK – distilled water as a control; WD – 20% PEG-6000 solution for 3 d; SA + WD – 5 mM SA pretreatment plus 20% PEG-6000; WD + P – 20% PEG-6000 + 0.4 mM c-PTIO; SA + WD + P – 5 mM SA pretreatment + 20% PEG-6000 and 0.4 mM c-PTIO.

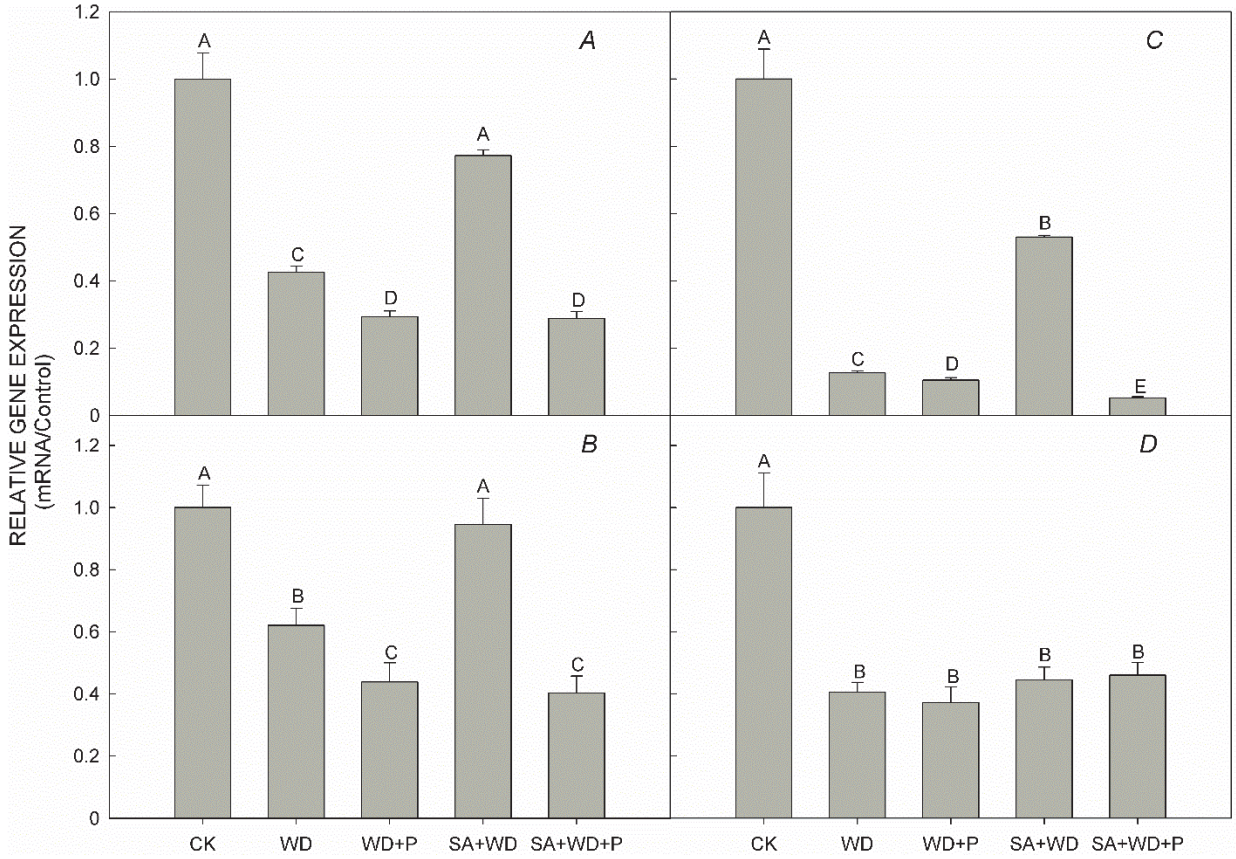

Fig. 2. Effects of salicylic acid (SA) and 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) on the expression of *ZmRCAα* (*A*), *Rbc* L (*B*), *ZmRCAβ* (*C*), and *Rbc* S (*D*) of maize seedlings under 20% polyethylene glycol (PEG)-6000 induced water-deficit stress. Data are the means ± SD of three replicates. *Different letters* in the same columns showed significant differences at $p<0.05$. The 20-d-old plants were pretreated hydroponically with 5 mM SA solution for 3 d, then exposed to 20% PEG solution or 0.4 mM c-PTIO for 3 d. The maize seedlings were treated as follows: CK – distilled water as a control; WD – 20% PEG-6000 solution for 3 d; SA + WD – 5 mM SA pretreatment plus 20% PEG-6000; WD + P – 20% PEG-6000 + 0.4 mM c-PTIO; SA + WD + P – 5 mM SA pretreatment + 20% PEG-6000 and 0.4 mM c-PTIO.

The amount of Rubisco synthesized is primarily determined by the levels of Rubisco subunits (*Rbc* L, *Rbc* S) mRNA (Suzuki and Makino 2013). In the present study, *Rbc* S showed some upregulation, but *Rbc* L was significantly up-regulated by SA, suggesting that SA could enhance Rubisco biosynthesis under WD. Two RCA genes, *ZmRCAα* and *ZmRCAβ*, encoding larger and smaller polypeptides of approximately 46 and 43 kD were found in maize (Yin *et al.* 2014), which ensure a stable RCA structure and maintain initial Rubisco activity under stress conditions (Wang *et al.* 2010, Chen *et al.* 2014b). Different RCA subunits may play different roles in photosynthetic acclimation. Under drought, RCAβ subunit was reported to decrease significantly in wheat (Zhao *et al.* 2016). In the present study, although *ZmRCAα* and *ZmRCAβ* genes expression were down-regulated under WD, a significant increase in these genes (consistent with an increase in Rubisco activity) was observed after SA pretreatment. Therefore, we speculated that enhanced transcription of *ZmRCAα* and *ZmRCAβ* mRNA under WD caused an increase in the initial Rubisco activity after SA treatment. However, c-PTIO lowered the transcription of *ZmRCAα* and *ZmRCAβ* mRNA and was accompanied by the down-regulation of P_N and photosynthetic performance implying that SA improving photosynthetic adaptability might be related to the NO signal. Moreover, our results show that NO content under WD conditions were significantly different from those in SA or c-PTIO treatments, which further implicated NO in SA-induced photosynthetic adaptability.

In conclusion, WD significantly decreased photosynthesis characteristics due to nonstomatal limitations, such as leaf CO_2 assimilation rate, PSII activity, the amount, and activity of Rubisco and RCA enzymes. However, SA exerts a positive effect on photosynthesis under WD condition, which occurs mainly through stimulating the transcription of *Rbc* L, *ZmRCAα*, and *ZmRCAβ* mRNA and enhancing the activity of Rubisco and RCA. Moreover, the NO scavenger highlights an important role for NO in SA-regulated leaf photosynthesis adaptability to WD stress.

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