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Paclobutrazol improves leaf carbon-use efficiency by increasing mesophyll conductance rate, while abscisic acid antagonizes this increased rate

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Abstract

Our experiment was conducted to understand the effects of foliar sprays of paclobutrazol (PBZ), its mixture with abscisic acid (ABA), or water (control) on leaf carbon-use efficiency (CUE_l), photosynthesis, and respiration of *Euonymus japonicus* seedlings. Plants were pot-grown in a greenhouse. The study revealed that elevated stomatal conductance, mesophyll conductance, and maximum rate of Rubisco carboxylation (V_{cmax}) in plants treated with PBZ could strongly improve net photosynthetic rate (P_N). ABA could antagonize the increase of mesophyll conductance and stomatal conductance by PBZ, thereby inhibiting the improvement of photosynthesis by PBZ. The combination of PBZ and ABA significantly reduced day respiration (R_d) and dark respiration (R_{dk}), resulting in the increased CUE_l, P_N/R_d, and P_N/R_{dk}. The increased P_N in the PBZ treatment led to slightly increased P_N/R_d and CUE_l. Thus, the CUE_l could be improved by PBZ and its mixtures with ABA by regulating leaf photosynthesis and respiration.

Additional key words: gas exchange; leaf respiration; photosynthetic respiration ratio; plant growth inhibitors.

Introduction

In order to maintain the landscape of some ornamental plants for urban greening, frequent pruning is needed to control the growth of ornamental plants, and as such growth can be regulated by applying chemical inhibitors (Papafotiou and Chronopoulos 2015). Paclobutrazol (PBZ) commonly referred as a plant growth regulator is effective and universal in regulating plant size of urban ornamental plants (Fletcher *et al*. 2000). In addition to being effective in controlling plant growth, PBZ could affect leaf carbon-use efficiency (CUE_l) which depends on the dynamic balance between photosynthesis and respiration. The CUE_l is an important indicator of carbon balance in leaves which could determine the carbon budget at individual and ecosystem level (Gifford 2003). A PBZ-mediated improvement of CUE_l may result from enhanced photosynthesis (Dwivedi *et al*. 2017) and decreased plant respiration (Zeng *et al*. 1992). There have been many studies on the effect of PBZ on photosynthesis. Previous studies found that the enhanced photosynthesis may result from an increase of chlorophyll (Chl) content and synthesis and stomatal opening and density (*see e.g.*, Tari 2003, Jaleel *et al*. 2007). However, the changes in biochemical components [including maximum electron transport rate (J_{max}) and maximum rate of Rubisco carboxylation (V_{cmax})] and mesophyll conductance (g_m), which are important photosynthesis-limiting factors, have been overlooked. Researchers often use V_{cmax} and J_{max} to illustrate the photosynthetic capacity (Sago 1994). Mesophyll conductance is considered as another limiting factor of photosynthetic efficiency which could be equally important as stomatal conductance (g_s) (Flexas *et al*. 2012). In addition, photorespiration (R_p) is also considered to be closely related to photosynthesis, and the reduction of photorespiration is conducive to improving photosynthetic efficiency (Zelitch 1989). However, the effects of PBZ on R_p have not been studied. The effect of PBZ on photosynthesis has been investigated extensively, whereas the research of respiratory responses in leaves is relatively scarce. Respiration which mainly includes day respiration (R_d) and dark respiration (R_{dk}) is a way of releasing carbon (Gonzàlez-Meler *et al*. 2009). There are only a few studies on the effects of PBZ on R_{dk} (Zeng *et al*. 1992), and the studies on R_d are missing. R_d is directly related to CUE_l.

Abscisic acid (ABA), an antagonist of auxin synthesis,

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Abbreviations: ABA – abscisic acid; C_c – CO_2 concentration in the chloroplasts; C_i – intercellular CO_2 concentration; CUE_l – leaf carbon-use efficiency; F_v/F_m – maximal quantum yield of PSII photochemistry; g_m – mesophyll conductance; g_s – stomatal conductance; J_{max} – maximum electron transport rate; PBZ – paclobutrazol; PGI – plant growth inhibitor; P_N – net photosynthetic rate; R_d – day respiration; R_{dk} – dark respiration; R_p – photorespiration rate; V_{cmax} – maximum rate of Rubisco carboxylation.

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commonly referred as a plant growth inhibitor (PGI), is also often used to control plant growth, and according to Horrell *et al*. (1990), the mixtures of PBZ and ABA might better control plant growth. However, when mixed application of PBZ and ABA is used, the change of $\mathrm{CUE_l}$ is not clear.

To our knowledge, ABA could significantly influence photosynthesis through affecting stomatal conductance, photosynthetic electron transport chain and/or activities of photosynthetic enzymes (Sharma *et al*. 2011, Hu *et al*. 2013). Some studies have confirmed that ABA could decrease the photosynthetic rate and dark respiration rate in leaves (Pandey and Srivastava 1997, Zhou *et al*. 2006, Šafránková *et al*. 2007). However, it is unclear how ABA affects $\mathrm{CUE_l}$, and what effect have the mixtures of PBZ and ABA on photosynthesis, respiration, and $\mathrm{CUE_l}$. Hence, this study focused on the following issues: What are the effects of g_m, V_cmax, J_max, and R_p on net photosynthetic rate (P_N) after the application of PBZ? Would the addition of ABA promote the improvement of $\mathrm{CUE_l}$ and photosynthesis by PBZ when PBZ and ABA are mixed and what are the impact mechanisms?

Euonymus japonicus Thunb. is widely used in urban landscapes in China as a shrub or small tree, mainly because of its evergreen leaves, rapid growth, plasticity, and tolerance to urban landscapes (Zhong *et al*. 2008, Choi and Park 2016). Height growth of this species is often regulated by foliar application of plant growth regulators (Zhong *et al*. 2008, Papafotiou and Chronopoulos 2015). To the best of our knowledge, the effect of PBZ and the combination of PBZ and ABA on $\mathrm{CUE_l}$ of *Euonymus japonicus* has been seldom investigated. Therefore, the aim of this study was to improve our understanding of the mechanisms of photosynthesis under PBZ treatment and the influence of mixtures of PBZ and ABA on $\mathrm{CUE_l}$, photosynthesis, and respiration of *Euonymus japonicus*.

Materials and methods

Plant material and experimental treatments: The experiment was carried out in the greenhouse of the Miaofeng Mountain, located in the western region of Beijing, China (39°54'N, 116°28'E). Three-year-old *Euonymus japonicus* seedlings from Dadongliu nursery in Beijing were selected for their dimensional uniformity [height = 88.9 ± 1.26 cm, $n = 48$] and individually transplanted into 14-L pots; then they were placed in the greenhouse. Greenhouse conditions were regulated; day/night temperature and relative humidity were set at 25–28°C/15–18°C and 70–80%, respectively, during the experimental period. Each pot contained 70% field soil (mainly eluvial cinnamon soil) and 30% sand by volume. The seedlings were grown under natural light and were kept well-watered over the duration of the experiment to avoid moisture stress. Twelve replicates were maintained for each treatment including control plants. The treatment was given when seedlings grew for 45 d after transplanting.

Treatment combinations were varying concentrations of paclobutrazol (PBZ, purity > 98%) and abscisic acid (ABA, purity > 98%, *Sigma-Aldrich*). Treatments were PBZ alone (PBZ) or combined with ABA at different concentrations (PA1 and PA2, respectively).

Treatment	PGIs used	Concentration [mg $\mathrm{L^{-1}}$]
Control	-	-
PBZ	PBZ	800
PA1	PBZ + ABA	800 + 10
PA2	PBZ + ABA	800 + 100

The treatments were applied as foliar sprays until the leaf was wet to the point of runoff. Control plants received clean water foliar sprays, the clean water was from garden hose. The concentrations of PBZ and ABA applied in this study referred to the study of Huang *et al*. (2011) and Huang *et al*. (2009), respectively. The spraying was done once every seven days, a total of three times. An isolation barrier was set up between seedlings to avoid contamination of adjoining plants during spraying. In this study, PBZ is regarded as a PGI, however, it is commonly also referred as a plant growth regulator.

Gas exchange, fluorescence, and respiration: Thirty days after the last spraying, photosynthesis and fluorescence were measured using an infrared gas analyzer (*GFS-3000/FL* gas exchange system, *Heinz Walz GMBH*, Effeltrich, Germany). Gas-exchange parameters were recorded in parallel with chlorophyll (Chl) fluorescence measurements on full-grown leaves, and the leaf position in different treatments was the same. All the data were collected using a 3-$\mathrm{cm^2}$ chamber. Four replicates of each treatment were collected for analysis. Prior to measuring, the temperature of the clamped leaves (T_leaf) was adjusted to 25°C, and the concentration of $\mathrm{CO_2}$ in the chamber (C_a) was set to 400 ± 5 μmol($\mathrm{CO_2}$) $\mathrm{mol^{-1}}$. First, the values of R_dk and minimal fluorescence yield of the dark-adapted state ($\mathrm{F_0}$) were measured more than 30 min after leaves had been placed in darkness, then maximal fluorescence yield of the dark-adapted state ($\mathrm{F_m}$) was measured after a saturating light pulse. The maximum quantum yield of PSII ($\mathrm{F_v/F_m}$) was calculated as $(\mathrm{F_m - F_0})/\mathrm{F_m}$, which describes the potential maximum photosynthetic capacity of plants (Buschmann 2007). Then net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular $\mathrm{CO_2}$ concentration (C_i), maximal fluorescence yield of the light-adapted state ($\mathrm{F_m}$'), and the steady-state fluorescence yields in light (F) were recorded simultaneously in the light-saturated state of photosynthesis [$\mathrm{PAR_{top}}$ of 1,500 μmol(photon) $\mathrm{m^{-2}\ s^{-1}}$]. The photon yield of PSII photochemistry (Φ_PSII) was determined as $1 - \mathrm{F/F_m'}$. The rate of electron transport passing PSII [J_f, μmol($\mathrm{e^-}$) $\mathrm{m^{-2}\ s^{-1}}$] was calculated as follows: $J_\mathrm{f} = \varepsilon \times \Phi_\mathrm{PSII} \times \alpha_\mathrm{l} \times \mathrm{PAR_{top}}$, where α_l (usually 0.85) is the absorbance by leaf photosynthetic pigments, ε is the fraction of absorbed irradiance partitioned to PSII, ε is generally 0.5 when assuming that the light is distributed equally to PSI and PSII (Niinemets *et al*. 2005).

Light-response curves were determined by varying photosynthetically active radiation ($\mathrm{PAR_{top}}$). The irradiance was set at 2,000; 1,800; 1,500; 1,200; 1,000; 800, 600, 400, 300, 200, 100, 50, and 0 μmol(photon) m^{-2} s^{-1}, while keeping C_a and T_{leaf} as above. Data were recorded when steady-state conditions had been attained for at least 2 min at each light level. Day respiration (R_d) was estimated from the light-response curves using the Yin *et al.* (2009) method. Dark respiration (R_{dk}) was measured from dark adaptation (> 30 min). Photorespiration (R_p) was calculated using the method of Valentini *et al.* (1995). With R_d, the CUE_l, which could simply indicate the state of balance between photosynthesis and respiration (Gifford 2003, Galmés *et al.* 2007), was calculated by using following formula: $CUE_l = 1 - R_d/P_N$. In addition, the P_N/R_{dk} ratio and P_N/R_d ratio, which could indicate the leaf carbon-use efficiency, were also calculated.

CO_2-response curves were measured by varying C_a between 50 and 2,000 μmol(CO_2) mol^{-1} at a T_{leaf} of 25°C and a saturating PAR_{top} of 1,500 μmol(photon) m^{-2} s^{-1}. The values of C_a were 400, 300, 200, 150, 100, 50, 400, 400, 600, 800; 1,200; 2,000 μmol(CO_2) mol^{-1}. Data were recorded after steady-state conditions had been attained for at least 4 min at each CO_2 concentration.

Photosynthesis model calculation: The Farquhar-von Caemmerer-Berry (FvCB) photosynthetic biochemical model is widely used to describe leaf photosynthesis in plants. The C_3 photosynthesis model assumes that photosynthesis was limited by the amount and activity of Rubisco (A_c) and ribulose-1,5-bisphosphate (RuBP) regeneration (A_j) (Farquhar *et al.* 1980, Sharkey 1985). We replaced the C_i in the original model with C_c using the method proposed by Flexas *et al.* (2008). The foliar net photosynthetic rate (P_N) was determined by the above limiting rates from a P_N/C_c curve:

$$P_N = \min\{A_c, A_j\} - R_d \tag{1}$$

The net photosynthetic rate was mainly limited by the number and activity of Rubisco at low CO_2 concentration. According to FvCB model, A_c was calculated as:

$$A_c = \frac{(C_c - \Gamma^*)V_{cmax}}{C_c + K_c\left[1 + \frac{O}{K_o}\right]} - R_d \tag{2}$$

where V_{cmax} [μmol(CO_2) m^{-2} s^{-1}] is the maximum rate of Rubisco carboxylation, K_c and K_o are Michaelis-Menten constants of carboxylation and oxygenation, respectively. O (usually 210 mmol mol^{-1}) is the partial pressure of O_2 at Rubisco. C_c [μmol(CO_2) mol^{-1}] is CO_2 concentration at the carboxylation sites of Rubisco (the calculation method of C_c is listed in the Eq. 4). Γ^* (37.43 μmol mol^{-1} at 25°C) is the C_c based on CO_2 compensation point in the absence of R_d (Bernacchi *et al.* 2002). The photosynthesis is mainly limited by RuBP regeneration capacity at higher CO_2 concentration, the A_j model was an extension of the generalized stoichiometry (Yin *et al.* 2006):

$$A_j = \frac{J_{max}(C_c - \Gamma^*)}{4(C_c + 2\Gamma^*)} - R_d \tag{3}$$

where J_{max} [μmol(e^-) m^{-2} s^{-1}] is the maximum electron transport rate.

The CO_2 concentration of Rubisco carboxylation site (C_c) was determined as:

$$C_c = C_i - \frac{P_N}{g_m} \tag{4}$$

where g_m [mol m^{-2} s^{-1}] is mesophyll conductance that was estimated with the method of Harley *et al.* (1992) as:

$$g_m = \frac{P_N}{C_i - \frac{\Gamma^*[J_f + 8(P_N + R_d)]}{J_f - 4(P_N + R_d)}} \tag{5}$$

Statistical analysis: Normality test of data and variance homogeneity were examined by *Kolmogorov-Smirnov* and *Levene*'s methods. One-factorial analysis of variance (*ANOVA*) was performed to test for significant photosynthesis and respiration parameters (*e.g.*, P_N, g_s, g_m, C_i, C_c, J_{max}, V_{cmax}, R_{dk}, R_d, R_p) and leaf carbon-use efficiency indicators (*e.g.*, P_N/R_d, P_N/R_{dk}, and CUE_l) among different treatments. Significant differences were determined at $P<0.05$ and mean separation used *Fisher*'s protected LSD method. Correlation analyses were conducted to study the correlation between P_N, CUE_l, and indicators related to photosynthesis and respiration. Statistical analyses were performed using *SPSS version 18.0* (*SPSS*, *IL*, Chicago, USA).

Results

Net photosynthetic rate, respiration rate, and CUE_l: We found the highest mean value of P_N in PBZ-treated plants, which was significantly different from those in control and PA2-treated plants (Fig. 1*A*). R_d and R_{dk} were significantly lower after treatment with inhibitor combinations of PBZ and ABA than that in either PBZ treatment or control; the differences of R_d and R_{dk} between PBZ treatment and control were not significant (Fig. 1*B*). P_N/R_{dk} and P_N/R_d were both higher in treated plants when compared to control samples, and there were significant differences in P_N/R_{dk} and P_N/R_d between combination treatments and control (Fig. 1*C*). The variation trend of CUE_l was basically the same as that of P_N/R_d and P_N/R_{dk}, CUE_l was significantly higher in treatment of combinations of PBZ and ABA than that in control (Fig. 1*D*).

Photosynthetic parameters: The g_s was significantly higher in PBZ-treated plants than that in control samples and PA2-treated plants, and was significantly lower in PA2-treated plants than in PBZ and PA1-treated plants, but similar to the control (Fig. 2*A*). We found the highest mean values of g_m, C_i, and C_c in PBZ-treated plants, which were significantly different from those in control and other treated plants (Fig. 2).

The V_{cmax} was significantly higher in the treated plants than that in control samples (Fig. 3*A*). J_{max} in both treatments with inhibitor combinations of PBZ and ABA were significantly higher than that in either PBZ treatment or

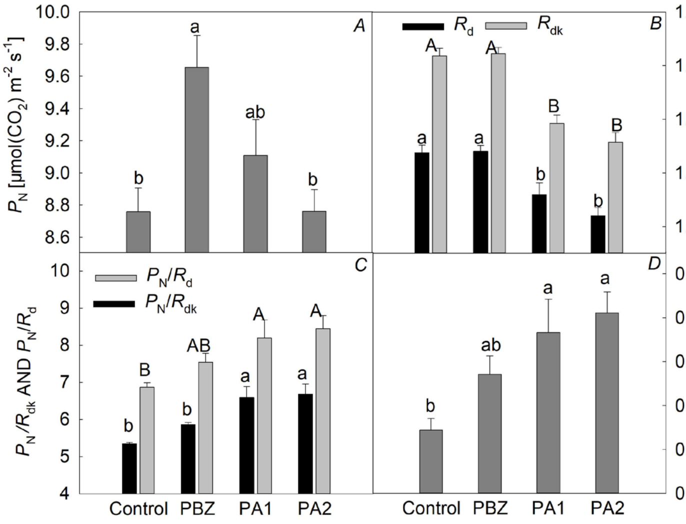

Fig. 1. Changes of net photosynthetic rate (P_N) (*A*), day respiration (R_d) and dark respiration (R_{dk}) (*B*), P_N/R_{dk} and P_N/R_d (*C*), and leaf carbon-use efficiency (CUE$_l$) (*D*) under treatments with PBZ, PA1, and PA2. *Different lowercase letters* on P_N (*A*), R_d (*B*), P_N/R_{dk} (*C*), and CUE$_l$ (*D*) indicate significant difference at $P<0.05$ (*ANOVA*) between different treatments; *different capital letters* on R_{dk} (*B*) and P_N/R_d (*C*) indicate significant difference at $P<0.05$ (*ANOVA*) between different treatments using a *Fisher*'s protected LSD test. Vertical bars indicate SEs of the means. PBZ – 800 mg L^{-1} paclobutrazol; PA1 – 800 mg L^{-1} paclobutrazol + 10 mg L^{-1} ABA; PA2 – 800 mg L^{-1} paclobutrazol + 100 mg L^{-1} ABA.

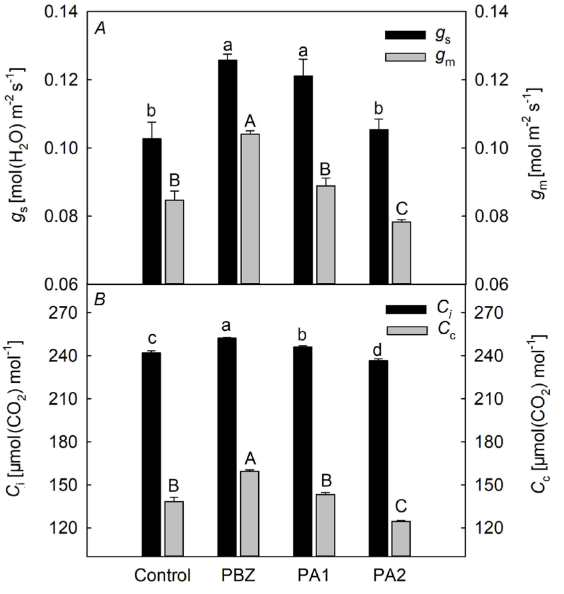

Fig. 2. Changes of stomatal conductance (g_s) and mesophyll conductance (g_m) (*A*), intercellular CO$_2$ concentration (C_i), and CO$_2$ concentration in the chloroplasts (C_c) (*B*) under treatments with PBZ, PA1, and PA2. *Different capital letters* on g_m (*A*) and C_c (*B*) indicate significant difference at $P<0.05$ (*ANOVA*) between different treatments, *different lowercase letters* on g_s (*A*) and C_i (*B*) indicate significant difference at $P<0.05$ (*ANOVA*) between different treatments using a *Fisher*'s protected LSD test. Vertical bars indicate SEs of the means. PBZ – 800 mg L^{-1} paclobutrazol; PA1 – 800 mg L^{-1} paclobutrazol + 10 mg L^{-1} ABA; PA2 – 800 mg L^{-1} paclobutrazol + 100 mg L^{-1} ABA.

control (Fig. 3*B*). We found the lowest mean value of R_p in PBZ-treated plants, which were significantly different from those in control and PA2-treated plants, but similar to the PA1-treated plants (Fig. 3*C*).

Correlation analysis: P_N was significantly positively correlated with g_s, g_m, C_i, C_c, and V_{cmax} under PBZ treatment; CUE$_l$ was significantly positively correlated with P_N, g_s, g_m, C_i, and V_{cmax} under PBZ treatment. P_N was significantly positively correlated with g_s, g_m, C_i, and J_{max}, and significantly negatively correlated with R_p under the combination of PBZ and ABA, while CUE$_l$ was significantly negatively correlated with R_{dk} and R_d under the combination of PBZ and ABA (Table 1).

Discussion

In our study, PBZ could significantly improve the P_N of *Euonymus japonicus* seedlings. This was consistent with Jaleel *et al*. (2007). The increase of P_N was significantly correlated with the increase of g_m, g_s, and V_{cmax} under PBZ treatment. The elevated g_m and g_s enhanced the CO$_2$ concentration in the chloroplasts in the PBZ treatment compared with the control and other treatment groups. The increase in g_s in plants treated with PBZ agrees with the finding of Xia *et al*. (2018). The larger stomatal opening and increased stomatal density under PBZ treatment may be the reason for the increase of stomatal conductance (Fernández *et al*. 2006, Waqas *et al*. 2017, Xia *et al*. 2018). The increase in g_m in plants treated with PBZ might be related to the increase of leaf thickness caused by PBZ. The g_m is mainly affected by biofilm permeability (Boron *et al*. 2011), carbonic anhydrase (Tholen and Zhu 2011), aquaporin (Terashima and Ono 2002), and leaf structure which mainly involves the thickness of cell wall and the surface area of chloroplast facing the intercellular space (Terashima *et al*. 2011, Flexas *et al*. 2012, Ort *et al*. 2015). PBZ could increase the leaf thickness (Jaleel *et al*. 2007), and the increase of leaf thickness can increase the surface area of chloroplast facing the intercellular space, thereby increasing the g_m (Flexas *et al*. 2013). In addition, the increased photosynthetic activity in the PBZ treatment occurred also due to the increased V_{cmax}. PBZ could increase Rubisco activity (Van den Boogaard 1994), and higher Rubisco activity may be the main reason for the increase of V_{cmax}. We found PBZ had no significant effect on J_{max}. According to Epron *et al*. (1995), the electron transport is used for photosynthesis and photorespiration. And our results showed that PBZ significantly increased

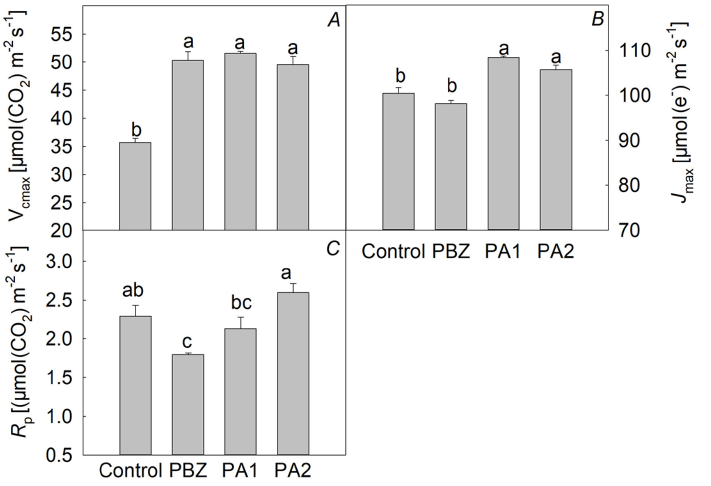

Fig. 3. Changes of maximum rate of Rubisco carboxylation (V_{cmax}) (*A*), maximum electron transport rate (J_{max}) (*B*), and photorespiration (R_p) (*C*) under treatments with PBZ, PA1, and PA2. *Different lowercase letters* indicate significant difference at $P<0.05$ (*ANOVA*) between different treatments using a *Fisher*'s protected LSD test. Vertical bars indicate SEs of the means. PBZ – 800 mg L^{-1} paclobutrazol; PA1 – 800 mg L^{-1} paclobutrazol + 10 mg L^{-1} ABA; PA2 – 800 mg L^{-1} paclobutrazol + 100 mg L^{-1} ABA.

Table 1. Correlational relationship between net photosynthetic rate (P_N) and leaf carbon-use efficiency (CUE$_l$) to P_N, stomatal conductance (g_s), mesophyll conductance (g_m), intercellular CO$_2$ concentration (C_i), CO$_2$ concentration in the chloroplasts (C_c), maximum rate of Rubisco carboxylation (V_{cmax}), maximum electron transport rate (J_{max}), and day respiration (R_d), dark respiration (R_{dk}), and photorespiration (R_p). ** represents significant correlation at 0.01 level. * represents significant correlation at 0.05 level.

Treatment	Index	P_N	g_s	g_m	C_i	C_c	V_{cmax}	J_{max}	R_{dk}	R_d	R_p
PBZ	P_N	1	0.948**	0.926**	0.980**	0.950**	0.928**	–0.077	0.569	0.076	–0.543
	CUE$_l$	0.855**	0.743*	0.715*	0.741*	0.614	0.843**	–0.178	0.379	–0.450	–0.488
PBZ + ABA	P_N	1	0.945**	0.820*	0.764*	0.606	0.472	0.740*	–0.383	–0.321	–0.925**
	CUE$_l$	0.671	0.457	0.180	0.119	–0.106	0.307	0.180	–0.903**	–0.917**	–0.335

P_N. Therefore, we concluded that PBZ can inhibit the R_p, and this is confirmed by our results.

The relatively low g_s and g_m in the combination of PBZ and ABA treatments resulted in a significantly lower C_c than that in the PBZ treatment, which might partly explain why the P_N under combination of PBZ and ABA treatments were lower than that of PBZ treatment in our experiment. The lower g_s and g_m might occur due to the reduction of g_s and g_m by ABA. ABA could reduce g_s by affecting stomatal guard cells (Islam *et al*. 2010). Flexas *et al*. (2006) also showed that ABA application to soybean and tobacco decreased g_m. Therefore, we concluded that ABA could antagonize the increase of g_m and g_s by PBZ, thereby inhibiting the improvement of photosynthesis by PBZ. Compared to PBZ treatment, with the increase of the concentration of ABA added in combination treatment, V_{cmax} seemed to decrease, but it was not obvious. Compared to PBZ treatment, the increased J_{max} in combination of PBZ and ABA treatments might be due to the significant promotion of photorespiration by ABA. As mentioned above, the electron transport is used for photosynthesis and photorespiration (Epron *et al*. 1995). According to our results, compared with PBZ, the P_N under combination of PBZ and ABA decreased, indicating that electron transport flow was more used for photorespiration. We also found that the values of R_p in the combination of PBZ and ABA were significantly higher than that of PBZ in our experiment, which might be due to the increase of R_p by ABA. Popova *et al*. (1987) pointed out that the rate of photorespiration increased by ABA treatment in barley leaves.

We found that the effect of PBZ on R_d and R_{dk} was not significant. The combination of PBZ and ABA could significantly reduce R_d and R_{dk}. The decreased R_d and R_{dk} might be mainly due to the inhibitory effect of ABA on leaf respiration (Pandey and Srivastava 1997). The increase of photosynthesis/respiration ratio indicates the increase of leaf carbon-use efficiency (Poorter and Bongers 2006). In our study, the elevated P_N/R_{dk} ratio and P_N/R_d ratio in the combination of PBZ and ABA underline the fact that both PBZ and its mixture with ABA could improve CUE$_l$. However, we noticed that the R_d calculation by this common method of Laisk (1977) is not very reliable (Gong *et al*. 2018). So, further studies on accurate measurement of R_d were carried out (Gong *et al*. 2018).

Correlation analysis showed that the combination of PBZ and ABA could elevate CUE$_l$ mainly by significantly reducing R_{dk} and R_d, respectively. The plants under the PBZ treatment showed a mild promotion effect on CUE$_l$ which was the result of the high photosynthetic rate, high g_m, high g_s, and high V_{cmax}. It can be seen that the PBZ and the combination of PBZ and ABA could improve CUE$_l$ by regulating photosynthesis and respiration.

In addition, it should be noted that the values of F_v/F_m in this study were all higher than 0.75, which indicated that plants grew normally without stress. The typical value range for F_v/F_m is between 0.75 and 0.85 (Sperlich *et al*. 2014).

In our study, PBZ could affect CUE$_l$ by increasing g_s,

g_m, and V_{cmax}. Mixing PBZ with ABA could further improve CUE_l by significantly reducing respiration rates. Compared to PBZ, mixing PBZ with ABA could decrease g_s and g_m rates, and increase photorespiration rate. Hence, while the application of PBZ led to reduced photorespiration rate and increased g_s and g_m, the addition of ABA may mediate these reduced and increased rates, respectively. Therefore, ABA could antagonize the improvement of photosynthesis by PBZ. Findings from this study improved our understanding of the impact mechanism of PBZ, ABA, and their mixtures on leaf CUE_l.

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