



Abscisic acid and brassinolide combined application synergistically enhances drought tolerance and photosynthesis of tall fescue under water stress



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ABSTRACT

Tall fescue (*Festuca arundinacea* Schreb.) is a widely used cool-season turfgrass, and its growth is mainly limited by water deficit. Abscisic acid (ABA) and brassinolide (BR) are two important stress hormones regulating plant physiological processes and growth under water deficit. To investigate effects of exogenous ABA and BR on physiological and photosynthetic performance of tall fescue under water stress conditions, ABA (10 and 20 mg L⁻¹) and BR (0.4 and 0.8 mg L⁻¹) were applied individually and in combination (0.4 mg L⁻¹ BR and 10 mg L⁻¹ ABA) under three soil water regimes [75 ± 5% FC (HW), 50 ± 5% FC (MW) and 25 ± 5% FC (LW)] in the greenhouse. Results revealed that ABA and BR application markedly decreased the relative conductivity and malondialdehyde, notably increased leaf relative water content, antioxidant enzyme activity and proline content under water stress. ABA plus BR application was equally effective in improving the activities of antioxidant enzyme as ABA at 20 mg L⁻¹. ABA application reduced stomatal conductance and decreased both transpiration and net photosynthetic rate (P_n), while BR application significantly increased P_n and water use efficiency (WUE) by enhancing chlorophyll content. The ABA and BR combination application showed higher P_n and WUE as well as BR single application. It indicated that ABA and BR combination application increased photosynthetic capacity, and reduced the effect on photosynthetic loss caused by ABA under water stress. All these confirmed that ABA plus BR application exhibited a synergistic interaction on enhancing drought tolerance and photosynthesis of tall fescue under water stress.

1. Introduction

Water is the primary factor limiting plant growth and production in arid and semiarid regions. Plants have evolved different adaptabilities including morphological, physiological, biochemical and molecular mechanisms in response to water stress (Fariduddin et al., 2009; Haisel et al., 2006). As important chemicals involved in many plant developmental processes, hormones play vital roles in regulating plant growth and physiological process under stress environment (Anuradha and Rao, 2003; Achuo et al., 2006; Houimli et al., 2010; Pattanagul, 2011). In which, abscisic acid (ABA) and brassinolide (BR) are two important

hormones and take part in many plant physiological processes such as osmotic adjustment, antioxidant protection, stomatal regulation and photosynthesis (Jiang and Zhang, 2002; Shakirova et al., 2016; Wani et al., 2017).

Endogenous ABA level would increase when plants are subjected to drought stress (Jiang and Zhang, 2002). Drought-induced ABA can trigger the generation of H₂O₂ and NO to activate antioxidant enzymes gene expression and improve antioxidant capacity, and can promote the biosynthesis of dehydrin proteins to reduce osmotic pressure (Han and Kermodé, 1996; Zhang et al., 2007; Hu et al., 2013). It has been reported that ABA application to *Cotinus coggygria* seedlings can improve

Abbreviations: ABA, abscisic acid; BR, brassinolide; Chl, chlorophyll; CAT, catalase; DW, dry weight; FC, field capacity; FW, fresh weight; G_s , stomatal conductance; MDA, malondialdehyde; RWC, relative water content; P_n , net photosynthetic rate; POD, peroxidase; RC, relative conductivity; ROS, reactive oxygen species; SOD, superoxide dismutase; T_r , transpiration rate; TW, turgor weight; WUE, instantaneous water use efficiency

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antioxidant enzyme activities and reduced glutathione contents under drought-shade combined stress (Li et al., 2011). Furthermore, as a signal transduction, ABA can induce the Ca^{2+} and K^+ outflow in guard cell membrane and regulate stomatal closure to reduce plant transpiration (Pei et al., 2000; Romano et al., 2000; Schroeder et al., 2001), thereby causing photosynthesis inhibition and decline under water stress (Popova et al., 2006; Zhou et al., 2006). Brassinolide (BR) is closely associated with cell enlargement and division, and can regulate antioxidant mechanism and photosynthetic capacity to improve plant stress-resistance (Creelman and Mullet, 1997). Behnamnia (2015) reported that BR could protect photosynthetic apparatus from oxidative toxicity and increase chlorophyll stability under drought stress by enhancing the activities or expression of protective enzyme in tomato leaves. In addition, BR also can increase leaf area, rubisco and nitrate reductase activities to promote photosynthesis (Anuradha and Rao, 2003). Fariduddin et al. (2009) found that BR notably increased the net photosynthetic rate and water use efficiency by elevating the activity of nitrate reductase and carbonic anhydrase in *Brassica juncea* seedlings under drought stress.

Different hormones have diverse functions on plant growth and physiological regulation, and their combined application may cause synergistic effect or counter effect (Peleg and Blumwald, 2011). For example, gibberellin counteracted ABA-induced increase in intracellular malate for controlling extracellular pH in barley aleurone (Heimovaara-Dijkstra et al., 1994). Sadak et al. (2013) found that indole acetic acid and kinetin synergistically improved photosynthetic pigments, free amino acid, proline and phenolic contents in faba bean under salt stress, while the synergistic effect under salt stress was significantly decreased when the kinetin application level up to 100 mg L^{-1} . In addition, Rajagopal and Andersen (2010) found that superimposed effect of ABA and ethylene on root formation largely depended on the degree of water stress, and the promotion of ABA and ethylene on root formation decreased significantly in severely-stressed pea than those under moderate or mild stress. The inconsistent interaction of hormones in response to stress may be caused by differences in molecular structure, environmental stress types, stress degree, hormone dosage and treatment time (Hu et al., 2013; Sadak et al., 2013).

Turf has been widely used in urban greening, soil erosion control, dust stabilization, noise abatement and recreation area (Beard and Green, 1994). Sufficient water supply is fundamental for turf establishment and prolonging their green period (Pan, 2011). Daily water consumption of turf could reach about 3.0–8.0 mm, and some even up to 10 mm in dry season, and which consumes a huge amount of water and increases the investment (Jiang, 1998). Tall fescue (*Festuca arundinacea* Schreb.) is the most commonly used cool-season turfgrass in dry areas due to their strong tramp resistance, anti-adversity and strong adaptability (Thompson et al., 2001). Limitations in water availability for irrigation due to drought and water-use regulations necessitate rational approaches to reduce water use and increase drought tolerance for their growth and health (Sermons et al., 2017).

Hormone application has been tested as an easy and economical way to increase drought tolerance and reduce irrigation frequency and water requirement in turf management (Lee, 2011). Little information is available about the effect of ABA and BR combination application on improving drought resistance of turfgrass. In this study, the physiological response and photosynthetic capacity of tall fescue treated by various concentrations of ABA and BR individual or mixed were investigated under different water supply regimes. Based on the physiological function of ABA and BR, we hypothesized that ABA and BR mixed application would exhibit additive improvement effect on anti-oxidation, and the BR could compensate photosynthetic loss caused by ABA under water stress, while their effects depend on water availability and their concentration. Our objectives were: 1) to evaluate differences in physiological and photosynthetic response to BR and ABA single application under different soil water regimes; 2) to clarify the additive effect of combining BR and ABA on drought tolerance and

photosynthesis of tall fescue.

2. Materials and methods

2.1. Plant materials and growth conditions

Tall fescue, Houndog 5, was obtained from DLF-Trifolium Seed Company (Beijing, China). The seed purity and germination rate were 93.2% and 86.5%, respectively. Seeds were sterilized in 10% H_2O_2 for 10 min, and then rinsed several times using distilled water. The sterilized seeds were sown at a density of 28 g m^{-2} in $18 \text{ cm} \times 20 \text{ cm}$ (inner diameter \times height) pots containing soil: sand (3:1, v/v) on March 29, 2015. The soil organic matter content was 18.36 g kg^{-1} , total N, P and K contents were 0.97, 0.43 and 11.54 g kg^{-1} respectively, and available N, P, and K contents were 34.26, 36.74, and 98.91 mg kg^{-1} respectively. Its field capacity (FC) is 22.74%. The plants were grown in the research greenhouse in Northwest A&F University at a day/night temperature of 25/20 °C, with 70% relative humidity and 14-h photoperiod at PPFD of $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

2.2. Water and hormone treatments

Soil water content was maintained at $75 \pm 5\%$ FC level by watering every day during March 29 to May 31. Watering was taken on by sprinklers and water was slowly and evenly irrigated from community ground to avoid runoff and plant interception loss at 18:00 on each day. On May 12, 45 days after sowing, plants were mown to 5 cm to ensure a uniform growth for the experiment. Since 1 June, three water regimes were conducted, and which were: sufficient water supply (HW, $75 \pm 5\%$ FC), moderate water stress (MW, $50 \pm 5\%$ FC), and severe water stress (LW, $25 \pm 5\%$ FC). The three soil water content (SWC) regimes were induced by withholding watering and weighing the pots daily, and the water treatments lasted for 15 d. ABA and BR were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (China). The stock solutions of ABA and BR were prepared by dissolving 0.04 g BR and 1.0 g ABA in 10 mL of ethanol. Final volume was made by using distilled water in 100 mL volumetric flask. The ABA and BR desired concentrations was prepared by the dilution of stock solution with distilled water. Five hormone treatments were applied, and which were BR_{0.4} (0.4 mg L^{-1} BR), BR_{0.8} (0.8 mg L^{-1} BR), ABA₁₀ (10 mg L^{-1} ABA), ABA₂₀ (20 mg L^{-1} ABA), BR_{0.4} \times ABA₁₀ (mixture of 0.4 mg L^{-1} BR and 10 mg L^{-1} ABA). Hormone applications were conducted on the 5th (June 4), 10th (June 9) and 15th (June 14), respectively, since the last sufficient irrigation day (May 31). Each pot was uniformly foliar sprayed with 30 mL of respective solution. The CK treatment was sprayed with 30 mL distilled water added with equal quantity of ethanol. The experiment was a completely random design. There were 18 treatments (3 water regimes \times 6 hormone application treatments) with three replications, and totally there were 54 pots.

The first and second fully expanded leaves were sampled in each pot on June 15, and some were used directly for relative water contents (RWC) and relative conductivity (RC) measurements, and the left were frozen in liquid nitrogen immediately, then stored at $-80 \text{ }^\circ\text{C}$ for malondialdehyde (MDA), proline, chlorophyll and antioxidant enzyme activity tests.

2.3. Relative water content (RWC)

Leaf samples (about 0.1 g fresh weight: FW) were rinsed 5 times and then soaked in deionized water for 24 h at room temperature (about 20 °C), the turgor weight (TW) was determined. Then the dry weight (DW) was recorded after oven drying at 80 °C for 24 h (Pattanagul, 2011). RWC was calculated using the following formula: $\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100\%$.

2.4. Relative conductivity (RC)

The relative conductivity (RC) was determined according to the method described by Chu et al. (2016). Approximately 0.1 g of fresh leaves was sampled and cut into 20 mm pieces. They were rinsed several times and then incubated in 10 mL deionized water for 3 h at 20 °C. After incubation, electrical conductivity was determined (I_1) using DDS-307 conductivity meter (INESA, Shanghai, China). Subsequently, the soak solution was placed in a boiling water bath for 20 min, and the electrical conductivity after boiling (I_2) was obtained after cooling. RC was calculated from the equation: $RC = I_1/I_2 \times 100\%$.

2.5. Malondialdehyde (MDA) content

The level of membrane lipids peroxidation was estimated by the MDA concentration using a slightly modified thiobarbituric acid (TBA) method following Talaat et al. (2015). Fresh leaves (0.5 g) were homogenized in 5 mL 5% trichloroacetic acid (TCA), and the homogenate was centrifuged at 3000 r min⁻¹ for 10 min. After centrifugation, the reaction mixture (containing 2 mL of 0.67% TBA and 2 mL of supernatant) was placed in boiling water bath for 30 min. The mixture was centrifuged at 3000 r min⁻¹ for 10 min again after cooling. The absorbance of supernatant was recorded at 450 nm, 532 nm and 600 nm, respectively, and the MDA concentration of supernatant was calculated by: $C(\text{MDA})/\mu\text{mol L}^{-1} = 6.45(A_{532} - A_{600}) - 0.56A_{450}$. The MDA content of unit fresh weight was calculated by: Leaf MDA content ($\mu\text{mol g}^{-1}$) = $C(\text{MDA}) \times V(\text{supernatant})/\text{sample weight}$.

2.6. Proline content

The proline content was measured adopting the acidic-ninhydrin staining method (Liu et al., 2016). Leaf samples (0.5 g) were homogenized with 5 mL sulfosalicylic acid (3%), after that the homogenate was centrifuged at 3000 r min⁻¹ for 5 min. 2 mL supernatant was added to 2 mL of glacial acetic acid containing 2 mL of acidic-ninhydrin. The mixture was put at 100 °C water for 30 min, then 4 mL of toluene was added after cooling, and the supernatant was carefully transferred to the 10 mL centrifuge tube and centrifuged at 3000 r min⁻¹ for 5 min. The absorbance of supernatant was recorded at 520 nm, and the toluene was used as control. The proline in supernatant (μg) was determined from a standard curve plotted by using L-proline, and proline content of unit fresh weight was calculated by: leaf proline content ($\mu\text{g g}^{-1}$) = proline of supernatant (μg)/sample weight (g) (Woodward and Bennett, 2005).

2.7. Antioxidant enzyme activity

Fresh leaves (0.2 g) were homogenized at 4 °C for 10 min in 1.6 mL 50 mmol L⁻¹ potassium phosphate buffer (pH 7.8) containing 0.2 mmol L⁻¹ EDTA and 1% (m/v) polyvinylpyrrolidone. The homogenate was centrifuged at 5000 r min⁻¹ for 20 min at 4 °C, and the supernatant was used for the superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activity measurements.

SOD activity was assayed using the nitroblue tetrazolium (NBT) method (Chu et al., 2016). Enzyme reaction mixture contained 50 mmol L⁻¹ phosphate buffer (pH 7.8), 14.5 mmol L⁻¹ methionine, 75 $\mu\text{mol L}^{-1}$ NBT, 0.1 $\mu\text{mol L}^{-1}$ EDTA, 4 $\mu\text{mol L}^{-1}$ riboflavin and enzyme extract. The reaction mixture was placed under the 4000 lux fluorescent lamp for 20 min. One unit of SOD was defined as the amount of enzyme required to inhibit 50% the photochemical reduction of NBT at 560 nm, the SOD activity was expressed as activity unit (U g⁻¹). CAT activity was estimated according to the consumption of H₂O₂ at 240 nm for 4 min, the reaction mixture was consisted of 50 mmol L⁻¹ phosphate buffer (pH 7.0), and 12.5 mmol L⁻¹ H₂O₂, the unit CAT activity was calculated as U g⁻¹ min⁻¹ (Chu et al., 2016). POD activity was measured by the oxidation of guaiacol in the presence

of H₂O₂. The reaction mixture was consisted of 28 mmol L⁻¹ guaiacol, 5 mmol L⁻¹ H₂O₂ and 50 mmol L⁻¹ phosphate buffer (pH 7.0), the increase in absorbance was recorded at 470 nm, the POD activity was expressed as activity unit (U g⁻¹ min⁻¹).

2.8. Chlorophyll a and b content

Leaf samples (0.2 g) were homogenized with 80% acetone and thereafter the homogenate was centrifuged at 3000 r min⁻¹ for 5 min. The absorbance of was read at 663.2 nm and 646.8 nm, and the chlorophyll a and b concentration of extract were calculated according to: $C(\text{Chl } a)/\text{mg L}^{-1} = (12.25A_{663.2} - 2.79A_{646.8})$, $C(\text{Chl } b)/\text{mg L}^{-1} = (21.21A_{646.8} - 5.1A_{663.2})$. The chlorophyll a and b content of unit fresh weight was calculated by: Chl a content (mg g^{-1}) = $C(\text{Chl } a) \times V(\text{supernatant})/\text{sample weight}$; Chl b content (mg g^{-1}) = $C(\text{Chl } b) \times V(\text{supernatant})/\text{sample weight}$ (Behnamnia, 2015).

2.9. Photosynthetic parameters

The photosynthetic parameters were obtained using LI-6400XT photosynthesis system (LI-COR, Lincoln, Nebraska, USA). The measurements were taken between 9:00 and 11:00 am on three consecutive sunny days (14–16 June). Three newly-fully expanded leaves in each plots were randomly chosen, and totally 9 replications were conducted. Net photosynthetic rate (P_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate (T_r , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and stomatal conductance (G_s , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured at a PPFD source of 800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Instantaneous water use efficiency (WUE, $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) was calculated by $WUE = P_n/T_r$. The CO₂ concentration of reference chamber was $450 \pm 10 \mu\text{mol mol}^{-1}$ and relative humidity was 60%–70%.

2.10. Statistical analysis

Data were processed using Microsoft Office Excel 2010. Treatments means were compared using one-way analysis of variance (ANOVA, Duncan's multiple range tests) at $p = 0.05$ level. Two-way ANOVA followed by least significant different (LSD) test was used to partition the main effects of water regime, plant hormone treatment and their interactive effect, and the statistical significance was set at $p < 0.05$ or $p < 0.01$. SPSS 17.0 was used for all statistical analysis and SigmaPlot 12.5 was used for drawing.

3. Results

3.1. Relative water content (RWC)

The RWC values decreased significantly ($p < 0.05$) for all hormone treatments as soil water regime declined (Fig. 1). Under HW regime, there were no significant differences in RWC values between hormone treatments. Under MW regime, ABA treated plants (ABA₁₀, ABA₂₀ and BR_{0.4} × ABA_{0.8}) had significant higher ($p < 0.05$) RWC than without any hormone application. Under LW regime, plants without any hormone application had significant lower ($p < 0.05$) RWC than that of each hormone treatment, while there was no significant differences among all five hormone treatments (Fig. 1). Water regime, hormone treatment and their interaction significantly affected leaf RWC values of tall fescue (Table 1).

3.2. Malondialdehyde (MDA) content and relative conductivity (RC)

Malondialdehyde (MDA) content increased significantly as soil water regime declined (Fig. 2A). Hormone application significantly ($p < 0.05$) decreased MDA content under each water regime. Under MW and LW regimes, The combination application of BR and ABA (BR_{0.4} × ABA₁₀) and ABA₂₀ treatment resulted in significant lower

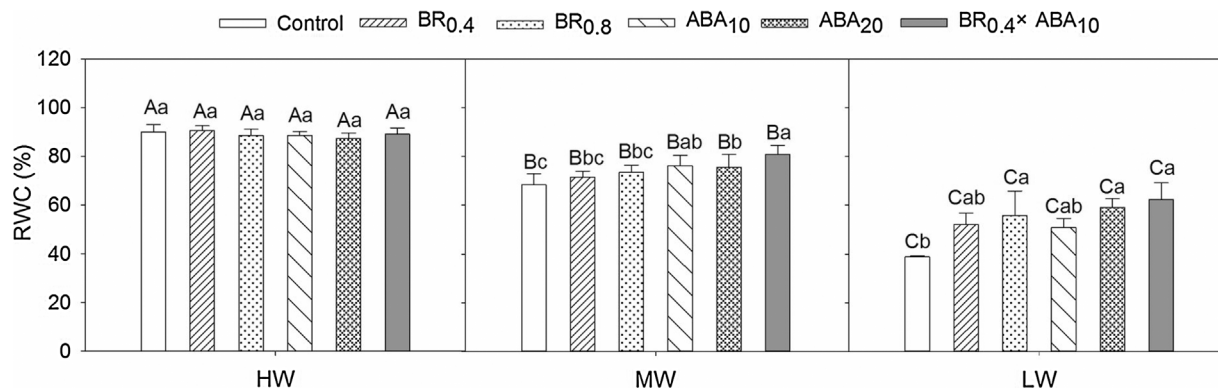


Fig. 1. Effects of ABA and BR application on leaf relative water content (RWC) of tall fescue under each water regime (HW: sufficient water supply, $75 \pm 5\%$ FC; MW: moderate water stress, $50 \pm 5\%$ FC; LW: severe water stress, $25 \pm 5\%$ FC. BR_{0.4}: 0.4 mg L^{-1} BR; BR_{0.8}: 0.8 mg L^{-1} BR; ABA₁₀: 10 mg L^{-1} ABA; ABA₂₀: 20 mg L^{-1} ABA; BR_{0.4} × ABA₁₀: 0.4 mg L^{-1} BR and 10 mg L^{-1} ABA). Vertical bars represent the means \pm SD ($n = 4-6$). Capital letters above the bars indicate significant difference among water regimes under same hormone treatment, while small letters indicate significant difference among hormone treatments under same water regime ($p < 0.05$).

MDA content, comparing with other hormone treatments (Fig. 2A). Relative conductivity (RC) had similar trend with MDA under each water regime and hormone treatment (Fig. 2B). Water regime, hormone treatment and their interaction had significant effects on leaf MDA and RC (Table 1).

3.3. Antioxidant enzymes activity

Compared with HW regime, the antioxidant enzyme activities increased significantly under MW and LW regimes. Under HW regime, there were no significant differences among hormone application treatments in antioxidant enzymes activity (Fig. 3). Individual application of ABA resulted in significant higher SOD activity than individual application of BR, and which were 349.94 and 251.84 U g^{-1} for ABA₁₀, 355.91 and 284.46 U g^{-1} for ABA₂₀, 247.44 and 223.81 U g^{-1} for BR_{0.4}, and 287.25 and 219.37 U g^{-1} for BR_{0.8} under MW and LW regimes, respectively. Under MW and LW regimes, ABA₂₀ and BR_{0.4} × ABA₁₀ showed similar effects in improving antioxidant enzyme activities (Fig. 3). Water regime, hormone treatment and their interactive effects on SOD, CAT, and POD activity were notable (Table 1).

3.4. Proline content

Proline accumulated significantly ($p < 0.05$) as soil water regime decreased for each hormone treatment (Fig. 4). The maximum proline were obtained at BR_{0.4} × ABA₁₀ application (58.74 , 124.98 , $182.59 \mu\text{g g}^{-1}$ for the HW, MW and LW regimes, respectively) ($p < 0.05$). BR applied individually (87.99 , $129.05 \mu\text{g g}^{-1}$ for BR_{0.4}; 103.55 , $138.63 \mu\text{g g}^{-1}$ for BR_{0.8}) had more positive effect on proline accumulation compared with ABA single application (82.17 , $107.63 \mu\text{g g}^{-1}$ for ABA₁₀, 87.10 , $106.08 \mu\text{g g}^{-1}$ for ABA₂₀) under MW and LW regimes (Fig. 4). Water regime, hormone treatment or their interaction statistically significantly effected proline content in tall fescue (Table 1).

Table 1

Analysis of variance results (F values) for the effects of water regime and hormone treatments on relative water content (RWC), malondialdehyde (MDA) content, relative conductivity (RC), proline, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities of tall fescue.

Factor	DF	RWC (%)	MDA ($\mu\text{mol g}^{-1}$)	RC (%)	Proline ($\mu\text{g g}^{-1}$)	SOD (U g^{-1})	CAT ($\text{U g}^{-1} \text{ min}^{-1}$)	POD ($\text{U g}^{-1} \text{ min}^{-1}$)
WR	2	309.50**	375.50**	110.41**	418.02**	89.17**	141.35**	53.34**
HT	5	8.45**	28.46**	16.21**	67.88**	30.84**	40.19**	27.96**
WR × HT	10	3.57**	8.20**	4.52**	11.24**	7.04**	8.57**	5.93**

ns, * and ** indicated non-significant, significant at $p < 0.05$ and 0.01 , respectively.

WR: water regime; HT: hormone treatment; WR × HT: water regime × hormone treatment.

3.5. Chlorophyll content

The chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) contents decreased significantly as soil water content decreased under each hormone treatment ($p < 0.05$) (Fig. 5A, B). There were no significant differences in Chl *a*, Chl *b* and their ratio among hormone treatments under HW regime. Compared with the control treatment, Chl *b* significantly increased 36.81 , 41.61 , 22.97 , 22.49 , 41.66% after BR_{0.4}, BR_{0.8}, ABA₁₀, ABA₂₀ and BR_{0.4} × ABA₁₀ treatments under MW regime, concomitant with declines in Chl *a*/Chl *b* by 22.20 , 19.85 , 27.84 , 25.92 , 37.10% , while the Chl *a* contents only increased notably by 11.82 , 19.01% at BR individual treatment ($p < 0.05$). Under LW regime, Chl *a* (0.66 , 0.64 and 0.68 mg g^{-1}) and Chl *b* contents (0.39 , 0.39 , 0.41 mg g^{-1}) were significant higher at BR_{0.4}, BR_{0.8} and BR_{0.4} × ABA₁₀ treatments than the others (Chl *a*: 0.46 , 0.49 , 0.53 mg g^{-1} and Chl *b*: 0.24 , 0.31 , 0.35 mg g^{-1} for control, ABA₁₀ and ABA₂₀, respectively) ($p < 0.05$). No significant differences were detected in Chl *a*/Chl *b* among hormone treatments under each water regime (Fig. 5C). Water regime and hormone treatment significantly affected chlorophyll *a* and *b* content and their ratio, while their interactive effect was only significant on Chl *a* content (Table 2).

3.6. Photosynthetic parameters

The net photosynthesis rate (P_n) and transpiration rate (T_r) decreased significantly under each hormone treatment as soil water regimes declined ($p < 0.05$). For each water regime, BR_{0.8} (9.93 , 7.76 , $4.53 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the HW, MW and LW regimes, respectively) and BR_{0.4} × ABA₁₀ (10.25 , 7.86 , $4.61 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were more effective treatments in improving the P_n as compared with the other hormone treatments ($p < 0.05$). The P_n of ABA₁₀ (7.12 , 4.64 , $2.68 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and ABA₂₀ (6.60 , 4.01 , $2.85 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) treated plants showed a decline compared to the control (7.59 , 6.21 , $3.13 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Fig. 6A). The WUE of BR_{0.8} and BR_{0.4} × ABA₁₀ treated plants showed a consistent trend with P_n , whereas there were no differences between ABA application and

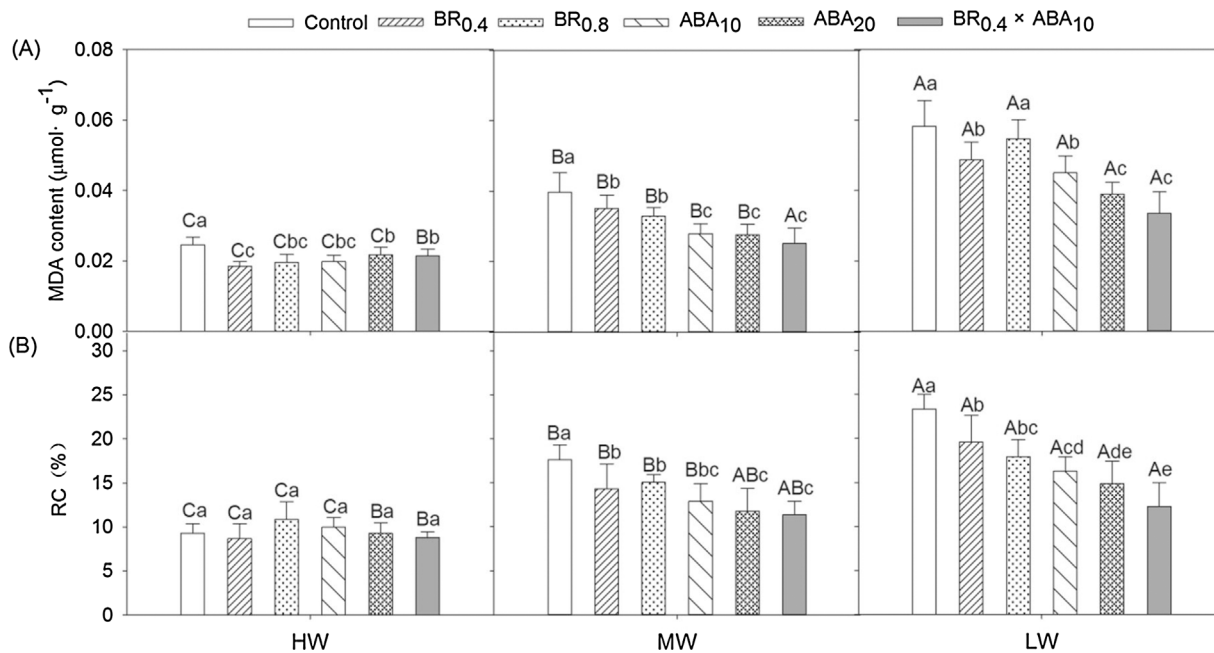


Fig. 2. Effects of ABA and BR application on malondialdehyde (MDA) and relative conductivity (RC) content of tall fescue under each water regime (HW: sufficient water supply, $75 \pm 5\%$ FC; MW: moderate water stress, $50 \pm 5\%$ FC; LW: severe water stress, $25 \pm 5\%$ FC. BR_{0.4}: 0.4 mg L^{-1} BR; BR_{0.8}: 0.8 mg L^{-1} BR; ABA₁₀: 10 mg L^{-1} ABA; ABA₂₀: 20 mg L^{-1} ABA; BR_{0.4} × ABA₁₀: 0.4 mg L^{-1} BR and 10 mg L^{-1} ABA). Vertical bars represent the means ± SD (n = 4-6). Capital letters above the bars indicate significant difference among water regimes under same hormone treatment, while small letters indicate significant difference among hormone treatments under same water regime (p < 0.05).

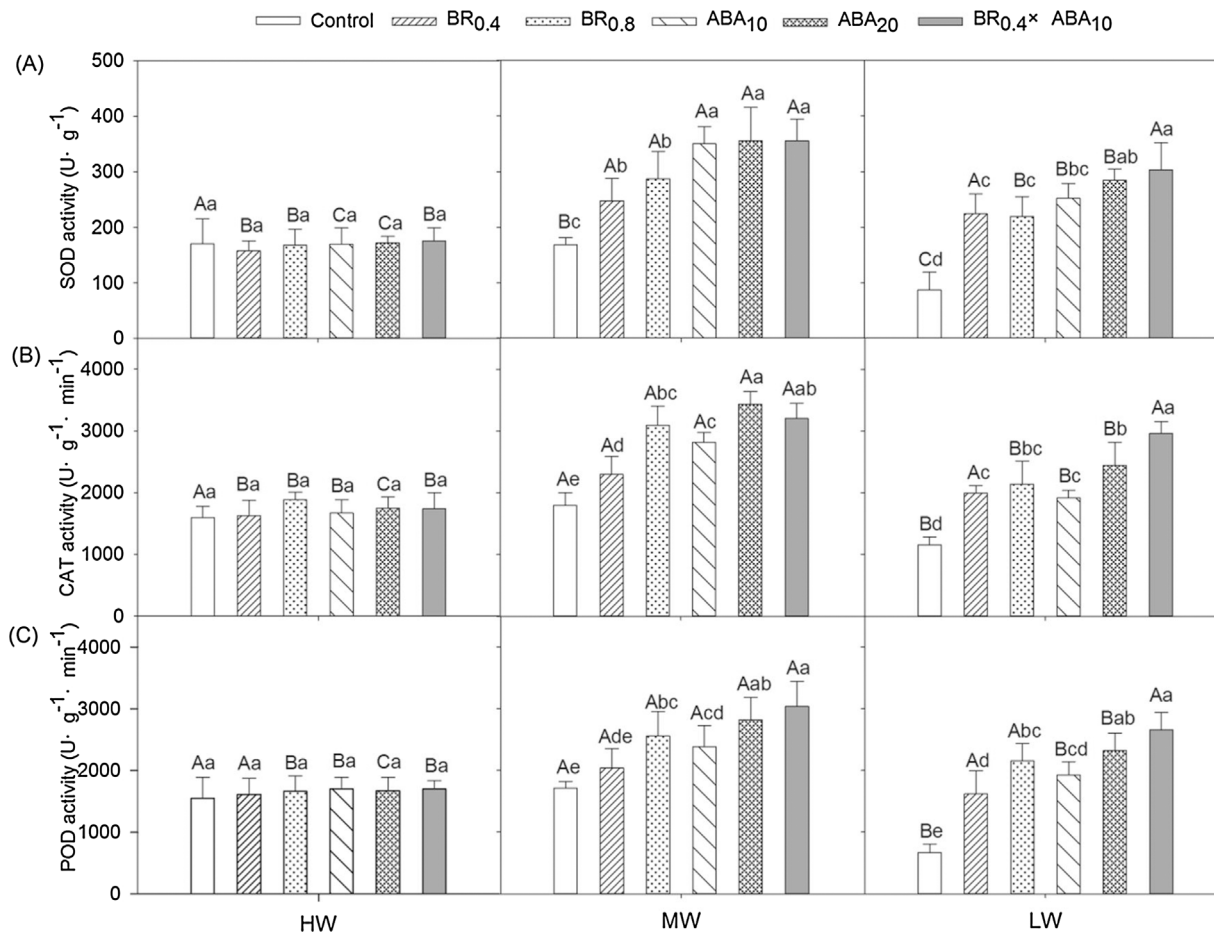


Fig. 3. Effects of ABA and BR application on superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities of tall fescue under each water regime (HW: sufficient water supply, $75 \pm 5\%$ FC; MW: moderate water stress, $50 \pm 5\%$ FC; LW: severe water stress, $25 \pm 5\%$ FC. BR_{0.4}: 0.4 mg L^{-1} BR; BR_{0.8}: 0.8 mg L^{-1} BR; ABA₁₀: 10 mg L^{-1} ABA; ABA₂₀: 20 mg L^{-1} ABA; BR_{0.4} × ABA₁₀: 0.4 mg L^{-1} BR and 10 mg L^{-1} ABA). Vertical bars represent the means ± SD (n = 4-6). Capital letters above the bars indicate significant difference among water regimes under same hormone treatment, while small letters indicate significant difference among hormone treatments under same water regime (p < 0.05).

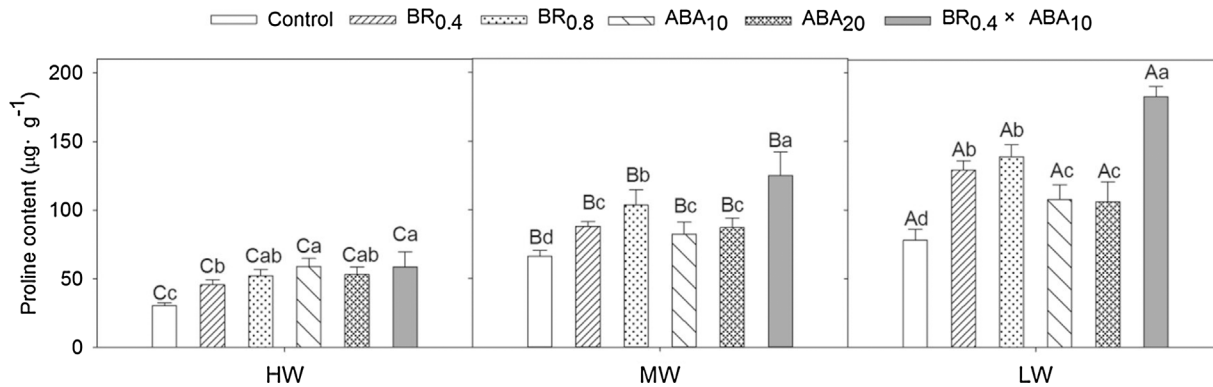


Fig. 4. Effects of ABA and BR application on proline content of tall fescue under each water regime (HW: sufficient water supply, $75 \pm 5\%$ FC; MW: moderate water stress, $50 \pm 5\%$ FC; LW: severe water stress, $25 \pm 5\%$ FC. BR_{0.4}: 0.4 mg L^{-1} BR; BR_{0.8}: 0.8 mg L^{-1} BR; ABA₁₀: 10 mg L^{-1} ABA; ABA₂₀: 20 mg L^{-1} ABA; BR_{0.4} × ABA₁₀: 0.4 mg L^{-1} BR and 10 mg L^{-1} ABA). Vertical bars represent the means \pm SD (n = 4-6). Capital letters above the bars indicate significant difference among water regimes under same hormone treatment, while small letters indicate significant difference among hormone treatments under same water regime ($p < 0.05$).

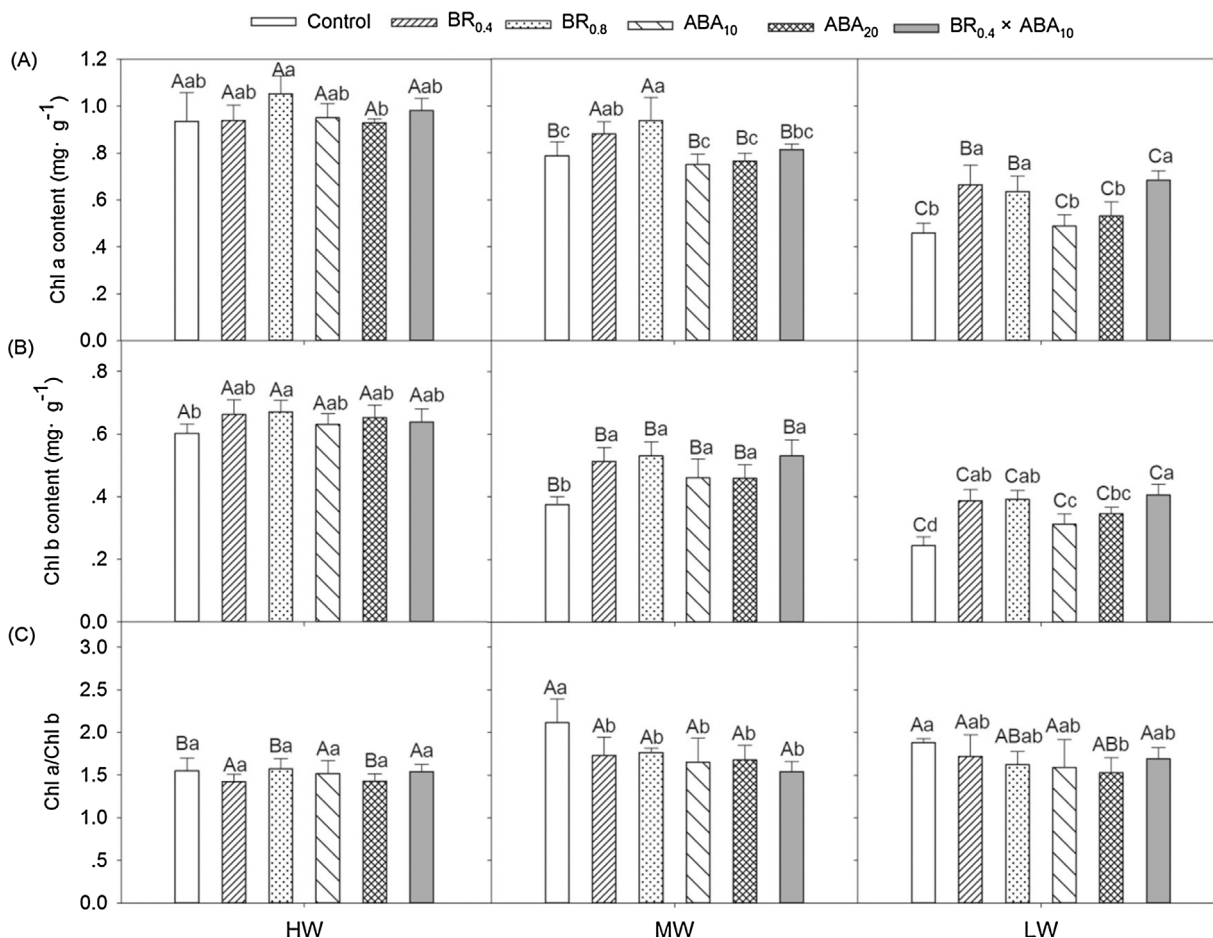


Fig. 5. Effects of ABA and BR application on chlorophyll a (Chl a) and chlorophyll b (Chl b) content, and Chl a/Chl b ratio of tall fescue under each water regime (HW: sufficient water supply, $75 \pm 5\%$ FC; MW: moderate water stress, $50 \pm 5\%$ FC; LW: severe water stress, $25 \pm 5\%$ FC. BR_{0.4}: 0.4 mg L^{-1} BR; BR_{0.8}: 0.8 mg L^{-1} BR; ABA₁₀: 10 mg L^{-1} ABA; ABA₂₀: 20 mg L^{-1} ABA; BR_{0.4} × ABA₁₀: 0.4 mg L^{-1} BR and 10 mg L^{-1} ABA). Vertical bars represent the means \pm SD (n = 4-6). Capital letters above the bars indicate significant difference among water regimes under same hormone treatment, while small letters indicate significant difference among hormone treatments under same water regime ($p < 0.05$).

control treatments (Fig. 6C).

All the hormone treatments significantly decreased T_r as compared to the control across three water regimes ($p < 0.05$). Under HW and MW regimes, ABA single application (ABA₁₀: $2.98, 2.11 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, ABA₂₀: $3.21, 2.01 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) decreased significantly more ($p < 0.05$) T_r relative to BR single application (BR_{0.4}: $4.03, 2.57 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, BR_{0.8}: $3.60, 2.53 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). There were no significant differences in T_r among hormone treatments

under LW regime (Fig. 6B). Similar trends were found for G_s as with T_r under each treatment. Water regime, hormone treatment and their interaction significantly affected P_n and T_r values. Water regime and hormone treatment exhibited significant effects on G_s values, and only hormone showed significant effects on WUE of tall fescue (Table 2).

Table 2

Analysis of variance results (*F* values) for the effects of water regime and hormone treatments on chlorophyll *a* (Chl *a*) content, chlorophyll *b* (Chl *b*) content, Chl *a*/Chl *b*, net photosynthetic rate (*P_n*), transpiration rate (*T_r*), instantaneous water use efficiency (WUE) and stomatal conductance (*G_s*) of tall fescue.

Factor	DF	Chl <i>a</i> (mg g ⁻¹)	Chl <i>b</i> (mg g ⁻¹)	Chl <i>a</i> /Chl <i>b</i>	<i>P_n</i> (μmol CO ₂ m ⁻² s ⁻¹)	<i>T_r</i> (mmol H ₂ O m ⁻² s ⁻¹)	WUE (μmol CO ₂ mmol ⁻¹ H ₂ O)	<i>G_s</i> (mmol H ₂ O m ⁻² s ⁻¹)
WR	2	229.41**	323.65**	10.21**	450.04**	376.88**	0.93 ^{ns}	306.19**
HT	5	11.84**	16.72**	4.04**	70.24**	27.80**	17.17**	29.14**
WR × HT	10	2.37*	1.56 ^{ns}	1.23 ^{ns}	3.42**	5.44**	0.68 ^{ns}	1.32 ^{ns}

ns, * and ** indicated non-significant, significant at *p* < 0.05 and 0.01, respectively.

WR: water regime; HT: hormone treatment; WR × HT: water regime × hormone treatment.

4. Discussion

Different hormones would have different effects on plant response to water deficit, and to use them together may result in synergistic or antagonistic interaction (Hoffmann et al., 2011; Peleg and Blumwald, 2011). In this study, BR and ABA application alone or together

significantly affected the physiological and photosynthetic processes in tall fescue, and the effects were closely related with soil water regimes and their concentrations. The effects were mostly obviously under low soil water supply conditions (*i.e.* MW and LW regimes), suggesting that BR and ABA have positive effects in improving drought tolerance and water use efficiency in tall fescue under water stress.

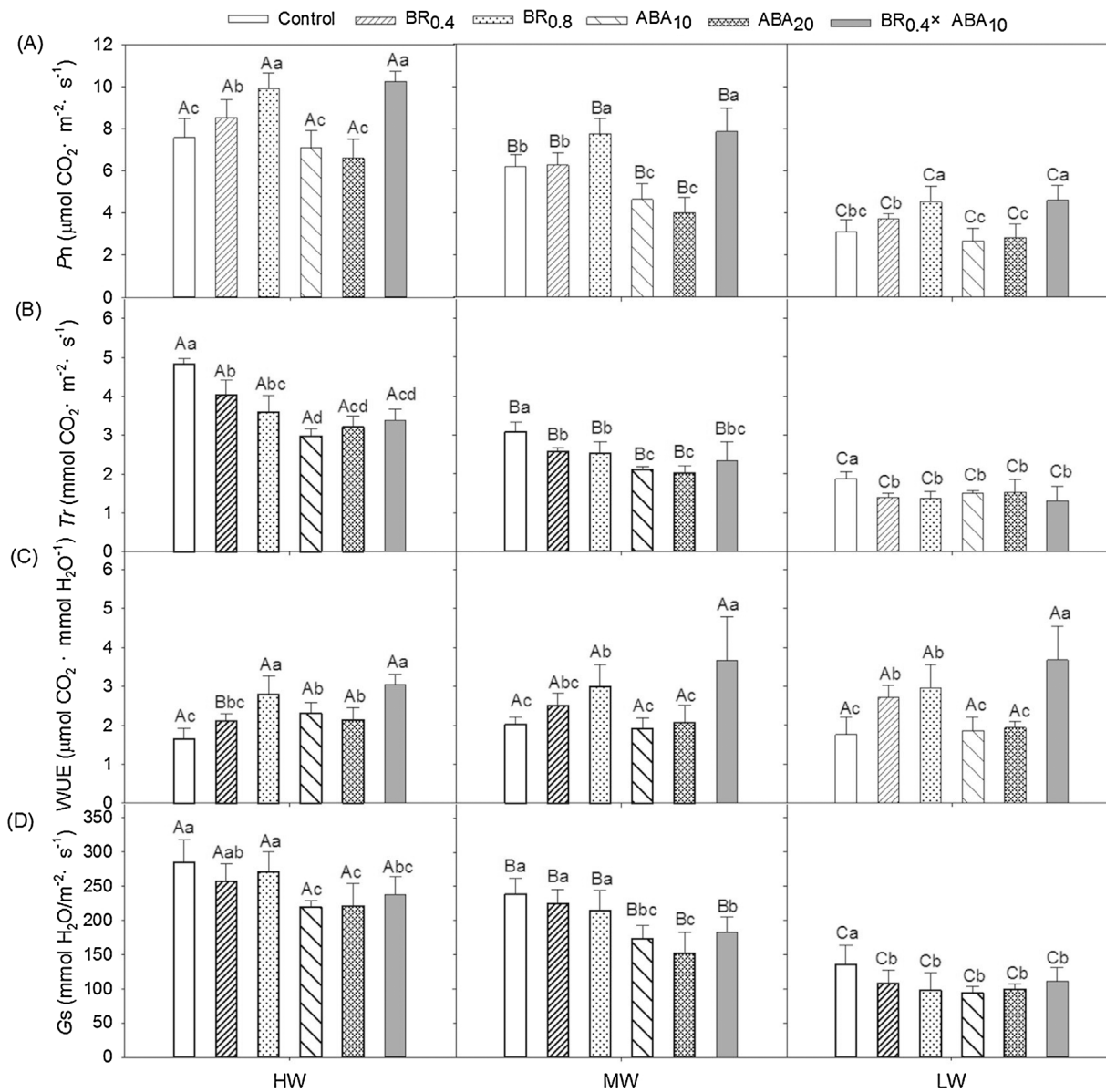


Fig. 6. Effects of ABA and BR application on leaf net photosynthesis rate (*P_n*), transpiration rate (*T_r*), instantaneous water use efficiency (WUE) and stomatal conductance (*G_s*) of tall fescue under each water regime (HW: sufficient water supply, 75 ± 5% FC; MW: moderate water stress, 50 ± 5% FC; LW: severe water stress, 25 ± 5% FC. BR_{0.4}: 0.4 mg L⁻¹ BR; BR_{0.8}: 0.8 mg L⁻¹ BR; ABA₁₀: 10 mg L⁻¹ ABA; ABA₂₀: 20 mg L⁻¹ ABA; BR_{0.4} × ABA₁₀: 0.4 mg L⁻¹ BR and 10 mg L⁻¹ ABA). Vertical bars represent the means ± SD (n = 4-6). Capital letters above the bars indicate significant difference among water regimes under same hormone treatment, while small letters indicate significant difference among hormone treatments under same water regime (*p* < 0.05).

ABA application (alone or with BR) significantly increased leaf RWC under lower soil water supply (i.e. MW and LW regimes) (Fig. 1). The increase in RWC of ABA treated plants was consistent with decline in G_s and T_r , which may be presumably due to that ABA application triggered closing of stomata and reduced transpiration water loss (Fig. 6) (Wang et al., 2003; Aldesuquy, 1999). Researches showed that BR could reduce water loss by promoting stomatal closure in fava and tomato (Haubrick et al., 2006; Yuan et al., 2010). Here, only under LW regime plants treated with BR alone showed higher RWC and significant lower G_s , meaning that the effects of BR may be affected by its concentration or soil water conditions. By using ABA-deficient tomato mutant and its original genotype, Yuan et al. (2010) reported that BR application could reduce water loss by inducing endogenous ABA synthesis under drought stress, implied that BR could enhance drought-protective effect of ABA by stimulating its biosynthesis (Choudhary et al., 2011). These indicated that ABA and BR combined application may exhibit a synergistic effect on water maintaining.

Under stress conditions, over-production of reactive oxygen species (ROS) could cause oxidative system damage and membrane destruction in plants and ROS level is controlled by the antioxidant protective enzymes activities (Fazeli et al., 2007). The superoxide dismutase (SOD) is the first line of defence to catalyze the conversion of O_2^- to H_2O_2 , subsequently the H_2O_2 is converted to H_2O by peroxidase (POD) and catalase (CAT) (Alscher et al., 2002). In our study, the promotion of ABA and BR application on SOD activity was more obvious than those of POD and CAT under MW and LW regime (Fig. 3). The BR and ABA improved the antioxidant enzymes activities under adversity stress largely through modulating the protein conformation, functionality or the expression of enzymes gene (Wang et al., 2003; Choe, 2006; Zhang et al., 2008). Guo et al. (2012) found that exogenous application of ABA enhanced stress tolerance by inducing gene expression, such as that of *DHAR*, *Mn-SOD* and *POD*, involved in oxidative stress defense mechanisms. The elevation in SOD and CAT activity by BRs may be the consequence of enhanced expression of *det2* gene (Cao et al., 2005). Thus, the additive oxidation resistance of the combined application may be attributed to the cooperation of ABA and BR-mediated transcriptional pathway.

Proline has been considered as a stabilizer for membranes, a protective osmolyte and a free radical scavenger (Fariduddin et al., 2013). The proline accumulation is an enzyme-regulation process, and its biosynthesis was correlated with stress degree, such as in this study, proline concentration was notably higher under MW and LW regimes (Fig. 4) (Sumithra and Reddy, 2004). ABA and BR application caused a considerable proline accumulation under each water regime (Fig. 4). Savoure et al. (1997) found that exogenous ABA application could trigger the expression of proline biosynthesis genes (*At-P5S*, *At-P5R*) by using *Arabidopsis thaliana* wild type and its ABA-deficient mutant. Meanwhile, Sharma et al. (2011) reported that BR application increased proline level, and which may be attributed to the stimulation of Δ^1 pyrroline-5-carboxylate synthase responsible for proline synthesis. The combination of BR and ABA resulted in significant higher proline accumulation than their individual application, which showed that BR and ABA could induce different biosynthetic pathway of proline or their combination colligate their respective stimulated effect on proline biosynthesis (Fig. 4) (Savoure et al., 1997; Sharma et al., 2011).

Chlorophylls are the core components of pigment-protein complexes and play a major role in photosynthesis. Chloroplast is a major source of ROS in plant cell during drought stress, and the chlorophyll would degrade seriously as ROS level increased (Iturbe-Ormaetxe et al., 1998). The exogenous BR could activate the activities of chlorophyll biosynthesis enzymes and antioxidant enzymes in chloroplast and thereby protect photosynthetic chloroplast against drought-induced pigment destruction (Haisel et al., 2006). In this study, the application of BR and ABA did not show any effects on Chl *a* and Chl *b* contents under sufficient water supply, while under lower soil water regimes (i.e. MW and LW) the Chl *a* and Chl *b* contents in plants treated with BR increased

significantly (Fig. 5). It was also observed that the affection was stronger on the Chl *b* content than that Chl *a*, and Chl *a*/Chl *b* increased notably, which may be due to the conversion of Chl *b* to Chl *a* under water deficit condition (Houimli et al., 2010).

It has been confirmed that ABA induced by drought could facilitate stomatal closure in guard cells to reduce water loss (Schroeder et al., 2001). The single ABA application noticeable reduced G_s , P_n and T_r values under each water regime (Fig. 6). Similar results were reported in barley plants under drought (Popova et al., 2006). On the contrary, single BR application only significantly reduced G_s values under LW regime, and T_r values under MW and LW regimes. One interesting phenomenon was that under each water regime, although G_s , T_r values decreased, P_n values significantly increased at 0.8 mg L⁻¹ BR treatment (Fig. 6). This may be due to that exogenous BR application could promote the chlorophyll synthesis and improve rubisco and nitrate reductase activities, and thus to improve photosynthetic rate and water use efficiency (Yu et al., 2004).

The results showed that ABA and BR had specific effects on regulating physiological and photosynthetic processes in tall fescue, and the effects depended on water regime level and the hormone concentration. Combined application of ABA and BR exhibited similar antioxidant enzymes activities as with higher concentration ABA, and combined application of ABA and BR showed the highest proline accumulation, implying that BR and ABA had the synergistic effect on enhancing antioxidant capacity when applied together. Meanwhile, higher P_n , similar T_r , and significant bigger WUE values under ABA and BR combined application treatment under LW regime, confirmed that BR application could compensate the photosynthetic losses caused by ABA (Hu et al., 2013). Kurepin et al. (2008) and Choudhary et al. (2011) reported that BR application could enhance the stress tolerance by increasing endogenous ABA biosynthesis under short-term stress. All these suggested that the ABA and BR combination showed synergistic effects in improving the drought resistance and photosynthetic capacity in tall fescue (Xu et al., 1994; Hu et al., 2013).

5. Conclusion

Our results clearly showed that exogenous ABA and BR application notably improved water maintaining capacity and antioxidant system activities of tall fescue under drought stress. The combination of ABA and BR colligated their respective effect on enhancing antioxidant system. Moreover, BR application compensated the photosynthetic losses caused by ABA under drought stress by improving the chlorophyll contents. The results suggested that the combined application of ABA and BR at proper concentration could complementarily and synergistically enhance the antioxidant activity and photosynthesis of turfgrass in response to water deficit, which will be helpful for turfgrass establishment and management under water limited environment.

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