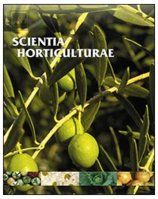
Scientia Horticulturae 228 (2018) 1–9

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



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

Zhifei Chen[a,b], Zhi Wang[b,c], Yungui Yang[a,**], Min Li[b], Bingcheng Xu[b,c,*]

[a] College of Animal Science and Technology, Northwest A & F University, Yangling 712100, Shaanxi, People's Republic of China
[b] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A & F University, Yangling 712100, Shaanxi, People's Republic of China
[c] Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, Shaanxi, People's Republic of China

ARTICLE INFO

Keywords:
Abscisic acid
Brassinolide
Drought stress
Antioxidant enzymes
Photosynthesis
Synergistic interaction

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 H_2O_2 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 Kermode, 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

[**] Corresponding author at: Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, Shaanxi, People's Republic of China.

[*] Corresponding author. State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A & F University, Yangling 712100, Shaanxi, People's Republic of China.

E-mail addresses: yungui999@163.com (Y. Yang), bcxu@ms.iswc.ac.cn (B. Xu).

http://dx.doi.org/10.1016/j.scienta.2017.10.004
Received 24 August 2017; Received in revised form 1 October 2017; Accepted 3 October 2017

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 (Sermonsa 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 antioxidation, 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 cm × 20 cm (inner diameter × 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 μmol m^{-2} s^{-1}.

2.2. Water and hormone treatments

Soil water content was maintained at 75 ± 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 l2, 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 ± 5% FC), moderate water stress (MW, 50 ± 5% FC), and severe water stress (LW, 25 ± 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), ABA_{10} (10 mg L^{-1} ABA), ABA_{20} (20 mg L^{-1} ABA), $BR_{0.4}$ × ABA_{10} (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 × 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 °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: $RWC = (FW - DW)/(TW - DW) \times 100\%$.