Plant Physiology and Biochemistry 111 (2017) 129–143

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Molecular and physiological responses of Iranian Perennial ryegrass as affected by Trinexapac ethyl, Paclobutrazol and Abscisic acid under drought stress

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ARTICLE INFO

Article history:
Received 3 September 2016
Received in revised form
29 October 2016
Accepted 22 November 2016
Available online 27 November 2016

Keywords:
Abscisic acid
Drought stress
Perennial ryegrass
Paclobutrazol
Trinexapac-ethyl

ABSTRACT

Drought stress is the major limiting factor which affects turfgrass management in area with restricted rainfall or irrigation water supply. Trinexapac ethyl (TE), Paclobutrazol (PAC) and Abscisic acid (ABA) are three plant growth regulators (PGRs) that are commonly used on turf species for increasing their tolerance to different environmental stresses such as drought. However, little is known about the impact of PGRs on stress tolerance of Iranian Perennial ryegrass (*Lolium perenne*). The present study was conducted to examine the visual and physiological changes of Iranian Perennial ryegrass in response to foliar application of TE, PAC, and ABA under drought stress conditions. According to the obtained results, application of all three PGRs considerably restored visual quality of drought exposed plants. TE treatment increased chlorophyll content, proline content and resulted in less malondialdehyde (MDA) in drought stressed Perennial ryegrass. Application of all PGRs enhanced the relative water content (RWC) and decreased the electrolyte leakage (EL) and Hydrogen peroxide contents (H_2O_2 content) of plants under drought stress, though the impact of TE was more pronounced. Throughout the experiment, TE- and ABA-treated plant showed greater soluble sugar (SSC) content as compared to the control. Antioxidant enzymes activities of drought exposed plants were considerably increased by PGRs application. Catalase (CAT) and Superoxide dismutase (SOD) activities were greater in TE-treated grasses followed by PAC-treated plants. Ascorbate peroxidase (APX) and peroxidase (POD) activities were significantly enhanced by TE and ABA application. The results of the present investigation suggest that application of TE, ABA and PAC enhances drought tolerance in Perennial ryegrass. TE, PAC and ABA were all effective in mitigating physiological damages resulting from drought stress, however the beneficial effects of TE were more pronounced. The result obtained of real time-PCR suggested that regulation of *CAT*, *APX*, *POD* and *SOD* genes expression at translational levels highly depended on the application of TE, PAC and ABA. Also, the results showed that deletion mutation in SOD and POD genes were not leading to enzyme inactivation.

Chemical names used

Trinexapac-ethyl (TE) [4-(cyclopropyl-α-hydroxy-nietliylene)-3,5-dioxocyclohexanecarboxylic acid ethyl ester]; Paclobutrazol (PAC) [(2RS,3RS)-l-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-tri-azol-l-yl)pentan-3-oI]; Abscisic acid (ABA) [ACC – 1-aminocyclopropane-1-carboxylic acid]; DAB – 3,3'dia-minobenzidine; H_2O_2 – hydrogen peroxide

Abbreviations: (TE), Trinexapac ethyl; (PAC), Paclobutrazol; (ABA), Abscisic acid; (PGRs), plant growth regulators; (RWC), relative water content; (EL), electrolyte leakage; (H_2O_2 content), Hydrogen peroxide contents; (MDA), malondialdehyde; (SSC), soluble sugar content; (SOD), superoxide dismutase; (APX), ascorbate peroxidase; (POD), peroxidase; (CAT), catalase.

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http://dx.doi.org/10.1016/j.plaphy.2016.11.014
0981-9428/

1. Introduction

Perennial ryegrass (*Lolium perenne* L.) is a cool-season turfgrass which is widely used for golf courses, parks and athletic fields in cool and warm climatic regions (Turgeon, 2005). Reduction of turf quality, thinned turf canopy, leaf senescence and leaf wilting under drought stress are important concerns with Perennial ryegrass management in a wide range of geographic locations (Krishnan et al., 2013). Therefore, increasing drought tolerance of Perennial ryegrass is of significant importance for both water conservation and maintaining plant growth in water-limiting environments.

Molecular and Physiological factors also involve in drought stress injury and should thus be considered for describing effect of plant growth regulator on drought resistance (Etemadi et al., 2015). Stressful conditions causes damage to plant cells through excess accumulation of reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), which can cause oxidative damage to cellular constituents for example, ROS can affect the antioxidative enzyme activities leading to lipid peroxidation and malondialdehyde (MDA) formation (Gao et al., 2008). MDA, a main byproduct of peroxidation of membrane lipids, is generally utilized as a biomarker of cell membrane damage in plants (Filek et al., 2012). Plants have the ability to sense ROS and re-programme their gene expression in response to changing conditions in their environment, accordingly evolve an efficient antioxidative defense system, consists of several antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) and non-antioxidant enzymes like glutathione to scavenge and decomposition these ROS (Ben Amora et al., 2005; Farooq et al., 2009). Increased accumulation of osmolytes in response to drought stress, such as proline and soluble sugar (SSC), helps to osmotic adjustment, detoxifies ROS, and protects membrane integrity in many plants (Pessarakli, 2008; Pireivatloum et al., 2010). Various methods have been applied for increasing drought tolerance of turfgrasses. Among them, application of plant growth regulators (PGRs) have been reported to be a promising way of reducing drought stress impacts (Lu et al., 2009; Samaranayake et al., 2008).

Trinexapac-ethyl (TE) is well-known as an anti-gibberellin plant growth regulator. TE is foliarly absorbed and acts by inhibiting the 3β-hydoxylase enzyme reaction that converts GA_{20} to GA_1 late in the gibberellins biosynthesis pathway (Ervin and Koski, 2001; McCarty et al., 2004). TE increases the turf density and quality, accelerates root growth, shortened internodes, enhances turfgrass tolerance to stress and reduces shoot extension rate (Baldwin et al., 2006; Ervin et al., 2004; Fagerness et al., 2002; McCann and Huang, 2007; Williams et al., 2010). Paclobutrazol (PAC) is well-known as an anti-gibberellin plant growth retardant. PAC disrupts GA biosynthesis by blocking the ent-kaurene oxidase that converts ent-kaurene to ent-kaurenoic acid in the gibberellins biosynthesis pathway (Buchanan et al., 2000; Li et al., 2009; Mahoney et al., 1998). PAC is a widely used growth retardant in some systems of turfgrass management for clipping reduction, seed head suppression, improving turf performance under stress and reducing the number of required mowing (Blank et al., 2009; Liya et al., 2010; McCullough et al., 2005). Abscisic acid (ABA) is a plant hormone and growth regulator which known to regulate plant adaptive responses to various environmental stresses and diverse physiological and developmental processes. ABA induces stomatal closure and reduces water loss via transpiration, which consequently increases plant tolerance to drought stress (Bright et al., 2006; DaCosta and Huang, 2007; Wilkinson and Davies, 2002).

One of the most important responses to drought stress is altered mRNA steady-state levels. Modern methods such as real-time RT-PCR provide exact measurements of mRNA levels of genes when expression levels are compared under different conditions or treatments.

Data describing the effects of PGRs application on **Iranian** Perennial ryegrass (*Lolium perenne* L.) drought tolerance are limited. Therefore, the present study was aimed at investigating the effect of exogenous applications of TE, PAC and ABA on turf visual quality, different physiological responses and expression levels of genes related to drought tolerance in Perennial ryegrass, and to determine the best PGR for increased drought tolerance of this turfgrass.

2. Materials and methods

2.1. Plant materials and growth conditions

This research was performed during 2013 and 2014 in Department of Horticulture at Isfahan University of Technology, Isfahan, Iran under greenhouse conditions. Polyvinyl chloride (PVC) pots (40-cm length, 10-cm diameter) filled with sterilized sandy loam soil were seeded using seeds of Iranian Perennial ryegrass (*Lolium perenne* L.) collected from turfgrass research farm in Fereydan, Isfahan. A nylon screen lidded the bottom of each PVC tube to preserve the soil and allow for drainage of water from the tubes. Plants were maintained in a greenhouse under natural light conditions (13-h photoperiod, relative humidity of 50%–60%, photosynthetic photon flux density of 500 μmol m^{-2}. s^{-1} at the canopy level) with temperatures of 23 °C day/15 °C night at Isfahan University of Technology to allow for plant establishment.

Irrigation was applied as needed to prevent any visible drought stress during grass establishment. In general, turfs were watered three times weekly to maintain plants under well-watered conditions and soil moisture at field capacity. Plants were maintained at a cutting height of 4 cm and mowed once a week using a reel-type mower. A 10-5-10 (N-P_2O_5-K_2O) fertilizer was applied at 15 g m^{-2} rate once every 2 weeks to provide nutrients and to facilitate plant establishment before initiation of treatments.

2.2. Treatments and experimental design

An experiment based on randomized complete block design with three replications was used to test the effects of TE, PAC and ABA on drought stress responses of Iranian Perennial ryegrass (*Lolium perenne* L.), and five pots were used for each replications (numbers of plant sampled = 75). The experiment consisted of five treatments: 1) well-watered plants without PGR treatment (control), 2) drought stress without PGR treatment (drought), 3) drought stress with TE application (drought + TE) 4) drought stress with PAC application (drought + PAC), 5) drought stress with ABA application (drought + ABA). The concentrations of applied PGRs were as follow: TE at 0.25 kg ai ha^{-1}; 2) PAC at 0.42 kg ai ha^{-1}; 3) ABA at 0.054 kg ai ha^{-1}. PGRs treatments were applied once 14 days before the beginning and once on the day of drought stress treatment, according to Bian et al. (2009). PGRs were foliage sprayed using a CO2 pressurized backpack sprayer (35 psi) equipped with an 8002VS nozzle (Spraying Systems Co., Wheaton, IL) calibrated to deliver 81 l ha^{-1} of spray volume. This rate of TE, PAC and ABA had been previously found to be effective in promoting stress tolerance in grass (Beasley and Branham, 2007; Bian et al., 2009; McCann and Huang, 2008; Roohollahi et al., 2010). Each time TE, PAC and ABA was applied in treatments number 3, 4 and 5, the same volume of water as PGRs volume was sprayed on grasses in treatments number 1 and 2. In addition, control plants were sprayed with clear water. Following the last application of PGRs, drought stress was imposed by withholding irrigation for 45 days while the control plants (treatment 1) received irrigation three times a week