Plant Physiology and Biochemistry 95 (2015) 57–64

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Genotypic variation in growth and metabolic responses of perennial ryegrass exposed to short-term waterlogging and submergence stress

Mingxi Liu [a], Yiwei Jiang [b,*]

[a] Department of Grass Science, Hunan Agricultural University, Changsha, 410128, PR China
[b] Department of Agronomy, Purdue University, West Lafayette, IN, 47907, USA

ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form
17 June 2015
Accepted 6 July 2015
Available online 9 July 2015

Keywords:
Antioxidant metabolism
Lolium perenne
Fast and slow growing
Water excess
Water-soluble carbohydrate

ABSTRACT

Physiological mechanisms of waterlogging (WL) and submergence (SM) tolerance are not well understood in perennial grasses used for turf and forage. The objective of this study was to characterize growth, antioxidant activity and lipid peroxidation of perennial ryegrass (*Lolium perenne*) exposed to short-term WL and SM. 'Silver Dollar' (turf-type cultivar), 'PI418714' (wild accession), 'Kangaroo Valley' (forage-type cultivar) and 'PI231569' (unknown status) varying in growth habits and leaf texture were subjected to 7 d of WL and SM in a growth chamber. Plant height was unaffected by WL but was significantly reduced by SM for all grasses except PI418714. The SM treatment caused greater reductions in leaf chlorophyll and total carotenoid concentrations. Substantial declines in water-soluble carbohydrate concentrations were found in the shoots and roots under SM, particularly in Kangaroo Valley and PI231569, two relatively fast-growing genotypes. Significant increases in malondialdehyde concentrations were noted in the shoots and roots of all genotypes exposed to WL and SM, but to a greater extent in Kangaroo Valley and PI231569 under SM. Shoot activities of catalase (CAT) and peroxidase (POD) increased under SM, more pronounced in Silver Dollar and PI418714, two relatively slow-growing genotypes. Waterlogging or SM stresses decreased root activities of superoxide dismutase, CAT, POD and ascorbate peroxidase, especially for Kangaroo Valley and PI231569. The results indicated that maintenance of antioxidant activity and carbohydrate and minimization of lipid peroxidation could contribute to better waterlogging or submergence tolerance of perennial ryegrasses.

1. Introduction

Flooding stress occurs to the plants due to frequent, heavy rainfall or over-irrigation followed by slow drainage. As a result, plants may be exposed to waterlogged or submerged conditions. Waterlogging is defined as the saturation of the soil with water around the roots, while submergence describes the condition in which the whole plant is completely covered by water. Either form of excess water stress can negatively affect plant growth and physiology. The capability to adapt to excess water conditions is crucial for increasing survival of the plants, particularly for those plants growing in coastal and flood-prone plains where frequent flooding stress may occur.

Responses of plant growth and leaf color to flooding stress vary with stress intensity and plant species. Waterlogging reduced shoot and root dry weight in cool-season perennial grass species including creeping bentgrass (*Agrostis stolonifera*) (Huang et al., 1998; Jiang and Wang, 2006) and Kentucky bluegrass (*Poa pratensis*) (Wang and Jiang, 2007). In creeping bentgrass, a decline in turfgrass quality occurred even when the water level was maintained at 15- or 1-cm below the soil surface under cool temperatures (Jiang and Wang, 2006). However, waterlogging stimulated plant growth in the tolerant warm-season perennial grass species such as Knotgrass (*Paspalum paspaloides*) and spiny mudgrass (*Pseudoraphis spinescens*), while reductions in growth were observed in the intolerant seashore paspalum (*Paspalum vaginatum*) and centipedegrass (*Eremochloa ophiuroides*) (Zong et al., 2015). A study by Barney et al. (2009) found that lowland ecotypes of switchgrass (*Panicum virgatum*) had higher tiller production and length, leaf area and biomass than the upland ecotypes under flooded conditions, demonstrating growth variations of

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; Chl, chlorophyll; HT, plant height; MDA, malondialdehyde; POD, peroxidase; SM, submergence; SOD, superoxide dismutase; WL, waterlogging; WSC, water-soluble carbohydrate; Car, total carotenoid.

* Corresponding author.
E-mail address: yjiang@purdue.edu (Y. Jiang).

http://dx.doi.org/10.1016/j.plaphy.2015.07.008
0981-9428/

different types of grasses in response to excess water conditions. Reduced concentrations of leaf chlorophyll and shoot water soluble carbohydrate were also found in creeping bentgrass (Jiang and Wang, 2006) and Kentucky bluegrass (Wang and Jiang, 2007), but to a much lesser extent in the tolerant cultivars.

Submergence stress either inhibits or enhances plant growth, depending on type of species and survival strategy (Bailey-Serres and Voesenek, 2008). Under submersion, some plant species or ecotypes elongated their leaf blades to above the water, while others showed minimum growth possibly for the purpose of conserving energy (Bailey-Serres and Voesenek, 2008; Perata and Voesenek, 2007; Yu et al., 2012). Enhanced shoot elongation, leaf blade and tiller production has been observed in flooding tolerant perennial grass species (Banach et al., 2009;Barney et al., 2009; Mollard et al., 2008). However, in rice (*Oryza sativa*), submergence tolerance was achieved by minimizing shoot elongation and by increasing dry matter weight underwater (Kawano et al., 2009). In addition, some cultivars or ecotypes within a plant species exhibited little or no changes in leaf growth (Yu et al., 2012). Collectively, variable growth responses and changes of chlorophyll and carbohydrate status are essential for flooding tolerance of a particular plant to waterlogged or submergence stress.

Diverse physiological responses to waterlogged or submerged conditions have been found in different plant species (Bailey-Serres and Voesenek, 2008; Wang and Jiang, 2007; Zong et al., 2015). One of the fundamental metabolic changes under excess water stresses is antioxidant metabolism. Waterlogging or submergence stress may increase production of active oxygen species (ROS) such as superoxide (O^{2-}) and hydrogen peroxide (H_2O_2), which can cause lipid peroxidation and oxidative damages to the plant cell (Mittler, 2002). Plants have evolved enzymatic defense systems to protect cells against oxidative injury by removing, decomposing or scavenging ROS. In this system, superoxide dismutase (SOD) plays a central role in catalyzing the dismutation of $O_2^{\cdot -}$ to H_2O_2 and oxygen (Bowler et al., 1992), and then H_2O_2 can be decomposed by several pathways including CAT, POD and ascorbate peroxidase (APX). Qi et al. (2014) reported that ROS detoxification is one of the primary mechanisms for surviving waterlogging for a hybrid of baldcypress (*Taxodium distichum*) and montezuma cypress (*Taxodium mucronatum*). It has been found that root SOD activities increased in both tolerant and intolerant cultivars of creeping bentgrass, but to a greater extent in the tolerant cultivar exposed to waterlogging stress (Wang and Jiang, 2007). Similarly, the increased leaf and root activities of SOD and POD were higher in the waterlogging tolerant knotgrass and spiny mudgrass than that of sensitive seashore paspalum and centipedegrass (Zong et al., 2015). However, the decreased and unchanged activities of SOD, CAT, POD, and APX to waterlogging or submergence have also been found in different plant species (Ahmed et al., 2002; Arbona et al., 2008; Lin et al., 2004; Tan et al., 2010; Wang and Jiang, 2007). The results suggest that responses of antioxidant enzymes to flooding stress are complex, varying with plant species, cultivars and stress intensity. Along with growth and other physiological parameters, differential responses of antioxidant enzymes to waterlogging and submergence stress are not fully understood, especially in perennial grasses.

Perennial ryegrass is a popular and important cool-season turf, forage and pasture grass that is widely used around the world. This species adapts to well-drained soil conditions, but some cultivars and wild accessions differed in submergence tolerance (Yu et al., 2012). Little is known about physiological mechanisms associated with waterlogging or submergence tolerance of perennial ryegrasses varying in growth habits, leaf texture and flooding response. The objective of this study was to characterize growth, antioxidant activity and lipid peroxidation of perennial ryegrasses exposed to short-term waterlogging and submergence stresses. We hypothesized that perennial ryegrass accessions differing in growth habits significantly varied in carbohydrate and antioxidant metabolism to waterlogging and submergence stress. Through investigation of shoot and root metabolism in different genotypes, the study would reveal the mechanisms of grass adaptation to saturated or flooded soils.

2. Materials and methods

2.1. Plant materials and growth conditions

Silver Dollar is a turf-type commercial cultivar developed from the Turf-Seed Company (Gervais, Oregon, USA). Kangaroo Valley is a forage-type variety. Along with accessions PI231569 (uncertain status) and PI418714 (wild), they were obtained from the USDA National Plant Germplasm System at the Western Regional Plant Introduction Station in Pullman (Washington, USA). These four perennial ryegrasses vary in growth habits, leaf texture, color and flooding responses (Yu et al., 2012). The seeds were sown in sand in plastic pots (4-cm diameter, 9-cm deep) in a greenhouse on 20 November 2013 at Purdue University, West Lafayette, IN, USA. On April 7, 2014, each genotype was propagated into the same-size pots through tillers, and each pot contained 8–10 tillers. Tiller plants were watered as needed with 1/2 Hoagland solution and cut twice a week to 7 cm for Kangaroo Valley, PI231569 and PI418714 and to 6 cm for Silver Dollar. The average air temperatures and photosynthetic photon flux density (PPFD) in the greenhouse were 20 °C ± 1.5 °C and 450 μmol m^{-2} s^{-1}, respectively. On 23 May 2014, the grasses were transferred to a growth chamber at 20 °C/15 °C, with a 12 h photoperiod of 500 μmol m^{-2} s^{-1} for 7 d before stress treatments were imposed.

2.2. Waterlogging and submergence treatments

Waterlogging and submergence treatments started on 30 May 2014 and ended on 06 June 2014 when severe damage and loss of leaf color occurred to the submerged grasses (visual observation). Waterlogging treatment was imposed by placing the pots into plastic containers (58-cm length × 35-cm width × 28-cm depth) and tap water (pH = 6.5) was added to the containers until the water level was at the soil surface of each pot. Submergence stress was imposed by submerging the grass pots in same-size containers with tap water. The water level was kept at 8 cm above the grass canopy. No nutrients were supplied to the plants and water was not changed during the period of stress treatments (7 d). Algae were removed if they accumulated. The control pots were well-drained and received normal watering.

2.3. Measurements

Plant height (HT) in each pot was recorded to identify growth during the period of stresses. At the end of 7 d, plants were harvested and roots were washed free of sand. A portion of the shoots and roots was immediately frozen in liquid nitrogen and stored at −80 °C until further use. A portion of the leaves were randomly selected for chlorophyll (Chl) and carotenoid (Car) extraction. Leaf Chl and Car were extracted by soaking approximately 50 mg leaf samples in 15 mL dimethyl sulfoxide (DMSO) in the dark for 48 h. The absorbance was then read at 665 nm, 649 nm and 480 nm and concentrations of Chl and Car were calculated using the method of Wellburn (1994).

Total water-soluble carbohydrate (WSC) concentration was measured using the anthrone method (Koehler, 1952) with some modifications (Yu et al., 2012). Briefly, WSC was extracted from