Scientia Horticulturae 197 (2015) 705–711

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Application of potassium polyacrylate increases soil water status and improves growth of bermudagrass (*Cynodon dactylon*) under drought stress condition

Xun Liu [a,b], Zhulong Chan [a,*]

[a] *Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China*

[b] *University of Chinese Academy of Sciences, Beijing 100039, China*

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 21 October 2015
Accepted 23 October 2015
Available online 4 November 2015

Keywords:
Bermudagrass
Drought
Potassium polyacrylate
Super-absorbent polymers
Reactive oxygen species

ABSTRACT

Water availability is the key factor limiting plant growth and development especially in dry and semiarid ecosystems. Super-absorbent polymers help soil keep water after raining or irrigation, and release water gradually during plant growth. This study examined the effect of potassium polyacrylate (K-PAM) on soil water content and physiological changes of bermudagrass in the greenhouse. The results showed that incorporation of K-PAM increased soil water content and survival rate of bermudagrass. Physiological parameter analysis showed that K-PAM treatment resulted in decreased cell membrane damage through modulation of EL and MDA contents in bermudagrass. Plants grown with K-PAM accumulated higher amount of proteins and proline under stress condition, and decreased ROS production. Expression level of several ROS related genes and stress inducible genes were largely up-regulated in plants by drought stress. However, plants grown in K-PAM mixed soil exhibited lower gene expression levels when compared to plants without K-PAM. The results indicated that K-PAM application effectively increased soil water content and improved bermudagrass growth under drought stress condition.

1. Introduction

Of the Earth's ice-free land surface, only 3% have a high level of productivity, while about 30% are too dry (deserts and dryland soils) for agricultural crop production (Osman, 2013). Drought is the most severe abiotic stress which significantly limits plant growth and development. To cope with the harsh environmental conditions including drought, plants develop complex mechanisms to adapt to stress conditions and reduce damages caused by water stress, including modulation of stress responsive genes which resulted in accumulation of osmolytes, detoxification of oxidative stress, modification of cell wall and reduced water loss through regulation of stomatal closure.

Although limiting water loss is important for plant survival under drought stress condition, water availability is the key factor for plant growth especially in dry and semiarid ecosystems (Chirino et al., 2011). Under natural environment condition, drought stress varies in severity, timing and duration because of changes in rainfall patterns (Chenu et al., 2013; Kirkegaard et al., 2007). Recently, increased water deficits associated with over-use of surface water and groundwater are largely threatening the sustainability of agricultural production globally. Therefore, use of super-absorbent polymers (SAPs) is an effective approach to increase soil water availability (Azzam, 1983; Blodgett et al., 1993; Taylor and Halfacre, 1986; Islam et al., 2011a,b,c,d).

SAPs help plants soil keep water after raining or irrigation and release water gradually in soil during plant growth. Application of SAPs enhances seed germination and emergence, crop growth and yield, and reduces the irrigation requirement of plants (Azzam, 1983; Blodgett et al., 1993; Gehring and Lewis, 1980; Yazdani et al., 2007). Because of technical development, costs for SAPs production decreased and the prices became comparatively cheaper (about 5 USD kg^{-1}) (Islam et al., 2011b). Additionally, polymers are safe and non-toxic and will finally decompose to carbon dioxide, water, ammonia, and potassium ion, without any residue (Mikkelsen, 1994). There are no environmental problems for SAPs application in the field.

Bermudagrass (*Cynodon dactylon*) is a widely used warm-season turfgrass on home lawns, golf courses, sport fields and ecosystem

Abbreviations: EL, electrolyte leakage; GR, glutathione reductase; GSH, glutathione; K-PAM, potassium polyacrylate; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; SAPs, super-absorbent polymers; SOD, superoxide dismutase.

* Corresponding author. Fax: +86 27 87510251.
E-mail address: zhulongch@wbgcas.cn (Z. Chan).

http://dx.doi.org/10.1016/j.scienta.2015.10.041
0304-4238/

restoration. As a C_4 grass, bermudagrass is adapted to cultivation in a wide range of hot, dry climatic regions and generally considered to have superior drought resistance to other warm-season turfgrass species (Carrow, 1995, 1996; Qian and Fry, 1997; Shi et al., 2013a,b). However, water consumption of bermudagrass is considerably high, especially for lawn maintaining in arid and semiarid regions.

In this study, potassium polyacrylate (K-PAM) was applied as a SAP during cultivation of bermudagrass in a greenhouse. The soil water content was monitored and plant physiological changes were assayed. Expression level changes of several stress inducible genes were also determined after application of K-PAM.

2. Materials and methods

2.1. Plant materials and growth conditions

The seeds of bermudagrass (provided by American Seed Research of Oregon Company, USA) were first vernalized at 4 °C for 7 days in darkness. Then, the seeds were sterilized with 70% alcohol for 5 min and sown in the nutrient soil (Beilei Fertilizer Company, Zhengjiang, China). The seeds germinated and grew in the growth room which was controlled at an irradiance of about 150 μm quantam^{-2} s^{-1}, 16 h light and 8 h dark cycles, 28 ±2 °C, and about 65–75% relative air humidity.

2.2. Plant treatment

At the 11th day after sowing, the seedlings were transferred to new pots filled with the same nutrient soil with or without 1% K-PAM based on preliminary experiment.

The 21-day-old plants with the same size were subjected to drought treatment by withholding water in the soil for 21 days. For the control treatment, plants were watered every other day. Each treatment contained five pots and each pot had at least 90 seedlings. All the pots were conducted in a completely random design and rotated every day to minimize the environmental deviation. The soil water content was measured at 7, 14 and 21 days after withholding water, using the soil moisture and temperature recorder (L99-TWS-1, Shanghai, China). At 7, 14 and 21 day, the leaf samples were collected for physiological parameter measurement and gene expression analyses. At 2 day after re-watering, the survival rate of each treatment was measured.

2.3. Assay of electrolyte leakage (EL)

For EL assay, 0.15 g plant leaf samples were shaken in Mili-Q water for 6 h at the room temperature to take initial conductivity (C_i). Leaf samples were then boiled for 15 min and cooled to room temperature to take the fully divided conductivity (C_{max}). The electrolyte leakage was calculated as the ratio of C_i and C_{max}.

2.4. Determination of soluble protein and antioxidant enzyme activities

For protein extraction, 1.5 g samples were triturated with liquid nitrogen and homogenized in phosphate buffer. The samples were then centrifuged at 12,000 × *g* for 15 min. The supernatant was used for the measurement of antioxidant enzyme activities, glutathione (GSH) content and H_2O_2 content.

According to the manufacturer's instructions, the peroxidase (POD) and glutathione reductase (GR) activities were assayed using Plant POD Assay Kit (Nanjing Jiancheng, China) and GR Assay Kit (Beyotime, China), respectively. The superoxide dismutase (SOD) activity was measured by the NBT-illumination method as described by Shi et al. (2012).

Table 1
List of primer sequences used for gene expression analysis.

Gene	Primer sequence
SOD	Forward: CTGACTGGGCCTCATTCTATAC Reverse: CCTGCGTTTCCTGTTGATTTAC
POD	Forward: CAGTGCTGGACAACAACTACTA Reverse: TCCTTCCACAGCGTCTCGTT
OSMOTIN	Forward: GTGCCATTGTCCTTCTCTTG Reverse: CGCAGGGATGGTTCTTAGAG
SULFUR E2	Forward: ACGTCATGAAGCTGCAGATC Reverse: CCATTTGTTTTCCCTTCACCC

2.5. Measurement of proline and GSH contents

The proline was extracted using sulfosalicylic acid, and the samples were boiled with acidic-ninhydrin for 40 min. The proline content was measured at 520 nm absorbance after the samples cooling to room temperature (Shi et al., 2012).

GSH content was assayed using the GSH Assay Kit (Nanjing Jiancheng, China) according to the manufacturer's instructions.

2.6. Quantification of malondialdehyde (MDA) and H_2O_2

The MDA of bermudagrass was extracted using thiobarbituric acid (TBA) reagent and MDA content was determined at 450, 532 and 600 nm of absorbance with a spectrometer as described by Yang et al. (2015). The content of H_2O_2 was measured using the titanium sulfate regent as described by Shi et al. (2012).

2.7. Quantitative realtime PCR

Total RNA was isolated from fresh leaves of bermudagrass using TRIzol reagent (Invitrogen, USA). First Strand cDNA was synthesized using the RevertAid^{TM} First Strand cDNA Synthesis Kit (TOYOBO, Japan), according to the manufacturer's instructions. The qPCR was performed by the SYBR-green fluorescence using a CFX96 Real Time PCR Detection System (BIO-RAD, USA). The relative fold change in gene expression was calculated following the $^{\Delta\Delta}$CT method (Livak and Schmittgen, 2001). Three independent biological replicates and three technical replicates for each sample were maintained. The primers for selected genes were designed based on previous RNA sequencing data (Shi et al., 2015) and listed as Table 1.

2.8. Statistical analysis

In this study, SPSS 20.0 software was used for statistical analyses. The treatment differences were performed by ANOVA, and variables contented at least three replicates. Mean separations were performed using Duncan's multiple range test with $P \leq 0.05$.

3. Results

3.1. K-PAM improved drought tolerance of bermudagrass

After 21 days of withholding water, plants in pots without K-PAM were wilted and plant growth was severely retarded, whereas most plants in pots with K-PAM were green and healthy (Fig. 1A). At 2 day after rewatering, most plants in pots with K-PAM survived, but plants in pots without K-PAM were completely dead (Fig. 1A). The soil water content was measured at 7, 14 and 21 day after withholding water. There were significant differences in soil water content between pots with K-PAM and without K-PAM. At 14