South African Journal of Botany 92 (2014) 83–88

Contents lists available at ScienceDirect

South African Journal of Botany

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

Physiological and biochemical changes of common bermudagrass (*Cynodon dactylon* [L.] Pers.) under combined salinity and deficit irrigation stresses

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ARTICLE INFO

Article history:
Received 16 July 2013
Received in revised form 27 January 2014
Accepted 3 February 2014
Available online 13 March 2014

Edited by Z-H Chen

Keywords:
Antioxidant
Common bermudagrass
Deficit irrigation
Turfgrass
Water salinity

ABSTRACT

Experiments were conducted to evaluate the effects of combined water salinity and deficit irrigation on common bermudagrass in order to address the shortage of water resources and increasing water and soil salinity in arid and semi-arid zones. This study was carried out under greenhouse conditions in a completely randomized design with factorial arrangements. Treatments included 4 water salinity levels (0.5, 3, 6 and 9 dS m^{-1}) and 4 deficit irrigation regimes (2, 4, 6 and 8 day intervals). Results indicated a rise in ion leakage, soluble sugars and proline concentrations; and a drop in leaf relative water content (RWC), leaf chlorophyll content and photosynthetic rate with increasing levels of both stresses. Antioxidant enzymes, superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7) and ascorbate peroxidase (APX, EC 1.11.1.11) showed higher activity under moderate drought or salinity conditions. However, these enzyme levels dropped at higher levels of these two stresses. Based on the results of the present study, common bermudagrass could be grown under moderate combined water and salinity stresses without considerable damage to plants at the physiological and/or biochemical level. This is the first report of applying combined water and salinity stresses to an important turfgrass species. Further studies are needed to evaluate the effects of these stresses on characteristics of bermudagrass at the molecular and ultrastructural levels.

1. Introduction

Lack of water resources and high water salinity levels are among the most important growth-restricting factors for plants species in some arid and semi-arid regions of the world (Levitt, 1980). Knowledge of relative salinity and drought tolerance among turfgrass species/cultivars is important for selecting turfgrasses that persist during drought and salinity stress. Turfgrasses are the most important groundcover-like plants in the world. Common bermudagrass (*Cynodon dactylon* [L.] Pers.) is widely distributed throughout the world between 45°N and 45°S (Harlan and de Wet, 1969). *Cynodon* spp. is frequently used in the transition zone where it can provide an excellent surface for golf course fairways and athletic fields (Munshaw et al., 2006). Salinity tolerance in different bermudagrass species ranges from 6 to 10 dS m^{-1}; and are classified as semi-tolerant to tolerant species to drought stress (Kamal Uddin et al., 2012). Drought or salinity tolerance, especially in grasses, depends on plant morpho-physiological features (Bahrani et al., 2010). Although the general effects of drought and salinity stresses on plant growth and development have been studied, their influence at the physiological and biochemical levels is not well understood (Jaleel et al., 2008). However, Du et al. (2012) studied metabolic responses of hybrid bermudagrass to short-term and long-term drought stresses. Salinity stress may result in the destruction of osmotic balance and ion toxicity (Turkan and Demiral, 2009). Studies have shown that the ability of plants to tolerate saline conditions, may alleviate the adverse effects of stresses such as salinity and drought (Kamal Uddin et al., 2012). The plants are able to ameliorate the stresses by synthesizing metabolites such as protein to prevent enzyme degradation through reduction of turgor changes within the cells (Kamal Uddin et al., 2012; Harivandi, 1988). Therefore, limited changes occur in growth rate compared to sensitive plants (Harivandi, 1988). Salinity and drought stresses were reported to act via overproduction of reactive oxygen species (ROS) (Gomez et al., 2004) which leads to oxidative stress (Mane et al., 2011). Accumulation of ROS may cause destruction of cell structures and molecules such as proteins and nucleic acids (Mittler, 2006). Hu et al. (2012) determined that ROSs are harmful to membranes, thus to scavenge them, plants have a well-developed complex antioxidant

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; EL, electrolyte leakage; POD, peroxidase; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase.

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http://dx.doi.org/10.1016/j.sajb.2014.02.006
0254-6299/

defense system including enzymatic and non-enzymatic antioxidant processes. The antioxidant enzymes that are found in plants include SOD, CAT, POD and APX (Apel and Hirt, 2004). Hu et al. (2012) further suggested that the activity of these antioxidants increased in some *Lolium perenne* L. cultivars after 4 days of treatment with 250 mM NaCl. DaCosta and Huang (2007) observed the same using drought stress, but stated that severe drought stress resulted in the reduction of antioxidant enzymes in *Agrostis* spp. Recent studies of salinity or drought effects on turfgrasses have focused on growth response mechanisms (Marcum, 1999). This research was designed to investigate the effects of combined drought and water salinity stresses on common bermudagrass and to investigate their biochemical and physiological responses under these conditions.

2. Materials and methods

2.1. Plant materials and experimental conditions

Experiments were conducted in the research greenhouse of the Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran (52°32_E and 29°36_N, 1810 m asl). The loamy soil was collected from the top 20 cm layer of the Department's research field. Some of the physico–chemical properties of this soil are shown in Table 1. The soil was air-dried and crushed to pass through a 10-mm sieve. Eighty plastic pots (with holes in the bottom), 35 cm in height, 25 cm in diameter, and top area of 0.0314 m^2 were filled with 5 kg of air-dried soil with a layer of gravel filter (2–4 mm gravel and 2 cm deep) at the bottom. Seeds of common bermudagrass 'California origin' were weighed and sown at the rate of 0.3 g pot^{-1}. Irrigation was carried out daily before seed germination and after turf establishment using tap water (EC 0.5 dS m^{-1}). During this period, the soil water content was kept at field capacity level (measured FC and PWP were 29% and 19%, respectively) by adding tap water. Deficit irrigation treatments consisted of four irrigation levels W_0, W_1, W_2 and W_3 with 2, 4, 6 and 8 day watering intervals, respectively.

The saline irrigation water treatments were tap water (control) and 0.5, 3, 6 and 9 dS m^{-1} (S_0, S_1, S_2, and S_3) obtained by the addition of equal proportions of NaCl and $CaCl_2$ to the tap water. Both deficit irrigation and salinity stresses were applied at the same time (after turfgrasses established). This study was carried out in a completely randomized design with factorial arrangements. Each treatment consisted of 5 replications. The amount of water for each irrigation treatment was determined by weighing the pots. Thirty percent more water was applied to leach accumulated salt from the pots. The chemical analysis of saline irrigation water is shown in Table 2. The maximum and minimum of day and night temperatures in the greenhouse were 34 ± 3 and 28 ± 3 °C, respectively. Relative humidity was about 75%. Electrical conductivity was determined in the drainage water during the growing season. The experiment was repeated in two consecutive years (2011 and 2012). Treatments lasted about 8 weeks. Data were analyzed using SAS software (ver. 9.1), and means were compared using the least significant difference (LSD) test at $p < 0.05$.

Table 1
Physico-chemical properties of the soil used in the experiment.

Physical property		Chemical property	
Sand (%)	33	Ca (meq l^{-1})	3.60
Silt (%)	47	Cl (meq l^{-1})	1.90
Clay (%)	20	Na (meq l^{-1})	1.40
Field capacity (cm^3 cm^{-3})	0.29	SO_4 (meq l^{-1})	1.90
Permanent wilting point (cm^3 cm^{-3})	0.19	Mg (meq l^{-1})	1.70
		HCO_3 (meq l^{-1})	2.40
		EC (dS m^{-1})	1.16

Table 2
Chemical analysis of the saline irrigation water used in the experiment.

EC (dS m^{-1})	pH	Cl (meq l^{-1})	Na (meq l^{-1})	Ca (meq l^{-1})	HCO_3 (meq l^{-1})
0.5	8.2	7.1	3.5	3.1	2.7
3	8	105	51	40	6.7
6	7.9	191	131	52	5.5
9	7.6	293	205	78	4.1

2.2. Relative water content (RWC) and electrolyte leakage (EL)

Relative water content estimation was performed by incubating 0.2 g of fresh leaf sample in 50 mL of distilled water for 4 h. Then the turgid weights of leaf samples were measured. The leaf samples were oven dried at 60°C for 48h for dry weight calculation at 60 °C for 48 h. RWC was calculated by the following equation:

$$RWC(\%) = (fw-dw)/(tw-dw) \times 100$$

where fw, dw, and tw are fresh, dry and turgid weights, respectively (Sairam et al., 2002). Electrolyte leakage in leaves was measured as described by Saadalla et al. (1990), using an electrical conductivity meter (Metrohm 644, Swiss) and calculated with the formula:

$$\text{Electrolyte leakage} = EC1/EC2 \times 100,$$

where EC1 is conductivity reading at room temperature, and EC2 is conductivity reading at 120 °C.

2.3. Chlorophyll content and photosynthetic rate

Chlorophyll content was measured according to the method of Saini et al. (2001) using the formula:

$$\text{mg Chl/g fw} = ([20.2\,(OD645\ \text{nm}) + (8.02(OD663\ \text{nm})] \times V)/(fw \times 1000),$$

where OD is optical density, V is the final solution volume in mL and fw is tissue fresh weight in mg. Photosynthesis rate was measured with a photosynthesis meter (LCi, England) at 10:30–12:00 AM.

2.4. Proline content and reducing sugars

Proline content was determined according to the method described by Bates et al. (1973) using a spectrophotometer (UV-120-20, Japan) at a 520 nm wavelength and the appropriate proline standards. A phenol–sulfuric acid method was used to determine the reducing sugar content (Dubois et al., 1956). Shoot and root samples were oven dried at 60 °C for 48 h and ground to make a powder using an electric mill. Powdered samples (0.2 g) were centrifuged with 80% ethanol. The final volume was brought to 25 mL using 80% ethanol. Then, 1 mL of extract was poured into test tubes and 1 mL of 5% phenol was added. 5 mL of concentrated sulfuric acid was added to tubes and were immediately stirred. Light absorption at 490 nm was measured by a spectrophotometer. A standard curve was drawn using glucose standards.

2.5. Measurement of antioxidant enzyme activity

2.5.1. Enzyme extraction

To extract antioxidant enzymes, bermudagrass fresh leaf samples (0.5 g) were collected and crushed using liquid nitrogen in a mortar and then homogenized with a cold enzyme extraction buffer [0.5% polyvinylpyrrolidone (PVP), 3 mM EDTA, and 0.1 M potassium phosphate buffer, pH = 7.5]. The extracted samples were centrifuged at 13,500 rpm for 10 min at 2–4 °C and kept on ice. The supernatant was used for enzyme analysis.