

The Enhancement of Drought Stress Tolerance of Kentucky Bluegrass by Prohexadione-Calcium Treatment

Javad Rezapour Fard, Mohsen Kafi* and Roohangiz Naderi

Department of Horticultural Science, Faculty College of Agricultural Science and Natural Resources, University of Tehran, Karaj, Iran

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*Corresponding author's email: mkafi@ut.ac.ir

Abstract

Kentucky bluegrass (*Poa pratensis* L.) is one of the most widely used turfgrasses for home lawns, golf courses, parks, and athletic fields in temperate climates. Oxidative stress caused by drought stress is a major limiting factor for grass cultivation in arid and semi-arid regions. The objective of this study was to investigate whether Prohexadione-Calcium (Pro-Ca) may play a role in promoting drought tolerance in kentucky bluegrass. Pro-Ca was exogenously applied as a foliar spray at the rate of 0, 7.5, 15 or 25 mg a.i./m² to well established kentucky bluegrass under well-watered (100 of field capacity) or drought-stressed (70 and 40% of field capacity) conditions. The effect of Pro-Ca on the growth physiology, drought stress response, antioxidant activity, and lipid peroxidation of kentucky bluegrass exposed to drought stress was measured during 28 days at 7 days interval. Pro-Ca treated kentucky bluegrass exposed to drought stress had higher relative water content (RWC) and turf quality, and lower electrolyte leakage and malondialdehyde (MDA) content as membrane integrity indicators, compared with untreated plants. Pro-Ca application significantly increased the antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) activities under well-watered and drought conditions, but this increase is more evident in drought stressed plants. Pro-Ca application at 15 mg/m² was found to be more effective in alleviating drought stress damage in kentucky bluegrass. The results from this study suggest that Pro-Ca enhanced drought stress tolerance in kentucky bluegrass by maintaining higher RWC and membrane stability.

Keywords: Antioxidant enzymes, Membrane integrity, Oxidative stress, Prohexadione-Calcium, Turfgrass.

INTRODUCTION

In turfgrass management, drought stress is a major limiting factor for grass growth. Water availability for irrigation of turfgrass is becoming increasingly limited, making water conservation a main concern of turfgrass growers and managers across many areas of the world, especially arid and semi-arid regions (Shaoyun *et al.*, 2009). Environmental stresses lead to intensive production of reactive oxygen species (ROS) setting off progressive oxidative damage and finally cell death (Asada, 1999; Reddy *et al.*, 2004; Sharma *et al.*, 2012; Ashraf and Harris, 2013). Drought stress promotes the production of superoxide (O_2^-), singlet oxygen (1O_2), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2), which can be detrimental to proteins, lipids, carbohydrates, and nucleic acids (Smirnoff, 1993). The plant cells are normally protected against this oxidative damage by antioxidant enzymes which detoxify reactive oxygen species (Foyer *et al.*, 1994; Møller *et al.*, 2007; Bian and Jiang, 2009; Ashraf and Harris, 2013). Antioxidant enzymes' activity under drought stress depends on plant species, stress intensity and duration (DaCosta and Huang, 2007), maintaining a high level of antioxidant activity increases the capacity of protection mechanisms for drought tolerance (Sharma and Dubey, 2005; Turkan *et al.*, 2005). Antioxidant enzymes' activity has a beneficial function on plant tolerance in drought stress (Møller *et al.*, 2007; Bian and Jiang, 2009). SOD scavenges O_2^- to H_2O_2 (Bowler *et al.*, 1992), and POD, CAT and APX detoxify H_2O_2 to H_2O at different cellular locations (Mittler, 2002). While genetic engineering may improve drought tolerance, applications of plant growth regulators and antioxidant materials to protect plants from drought damage could be another choice (Jiang and Zhang, 2001).

Prohexadione-calcium (Pro-Ca) mimics the structure of 2-oxoglutaric acid, and inhibits 2-oxoglutarate-dependent dioxygenases activity, which are essential for formation of growth-active gibberellins and flavonoids metabolism (Rademacher, 2000). Pro-Ca as a bioregulator affects plant metabolism such as hormonal balance. Pro-Ca blocks particularly 3β -hydroxylation, thereby inhibiting the formation of highly active GAs from inactive precursors (Rademacher, 2000). Furthermore, Pro-Ca has very favorable toxicological and eco-toxicological features, a low propensity for crop residues and no health risk for user or consumer is indicated (Winkler 1997; Evans *et al.*, 1999).

Therefore, the objectives of this study were to evaluate responses of perennial ryegrass to foliar application of Pro-Ca under drought stress and to examine whether improved stress tolerance was associated with the mitigation of electrolyte leakage and MDA content as membrane integrity indicators and enhancement in the activity of antioxidant enzymes. If it is the case, Pro-Ca could be used to alleviate plant damage caused by ROS in kentucky bluegrass under drought stress.

MATERIALS AND METHODS

Plants and experiment conditions

The seeds of *P. pratensis* L. 'Barimpala' were cultivated in well drained polyvinyl chloride tubes (60 cm in depth and 15 cm in diameter) filled with the sandy-loam soil (63.2% sand, 23.4% silt, 13.4% clay) in April 2013 at a greenhouse conditions. After 2 months growth, shoots were clipped twice at 10 days intervals to a height of 5 cm above the soil surface in order to reach a uniform establishment and get ready for the treatments. The air temperatures were set at 23–28 °C and 20–25 °C during day/night time with a relative humidity of 45%.

Water stress treatments

Water stress was implemented during experiment period from June, 2014, at three levels as well-watered (100% of field capacity), mild (70% of FC), and relatively severe (40% of FC). Soil volumetric water content was determined by weighting the pots during the experimental period for each treatment.

Pro-Ca application

Prior to the drought stress treatments, in each pot 100 ml of freshly prepared Pro-Ca solutions was applied as foliar spray at 7.5, 15 and 25 mg a.i./m² concentrations, after 1 day from the last mowing. Deionized water was used as solvent and control for the treatments.

Turf quality and RWC analysis

The grass quality was visually rated based on NTEP on 1 to 9 scales, where 1 brown leaves, dead grass; 9 turgid green leaves, optimum density and uniformity (Turgeon, 2002). RWC% was calculated according to Barrs and Weatherley (1962). Leaf samples were detached from the plants and immediately weighted to determine fresh weight (FW). Samples were placed into covered petri dishes filled with distilled-deionized water for leaves to reach full turgid. After approximately 24 h at 4°C, leaf samples were blotted dry with paper towels and weighted to determine turgid weight (TW). Leaf tissue was then dried in an oven at 80°C for 48 h to determine dry weight (DW). Leaf RWC was calculated as $(FW-DW)/(TW-DW) \times 100$.

Electrolyte leakage and MDA measurement

For EL analysis, whole fully expanded leaves (0.1 g from each pot) were incubated in 15 ml distilled water on a shaker for 24 h. The conductance of the incubation solution was measured as the initial level of EL (C_i) using a conductance meter. Leaf tissue in the incubation solution was killed in an autoclave at 120 °C for 30 min. The conductance of the incubation solution with killed tissues (C_{max}) was determined following 24 h incubation on a shaker. Relative EL was calculated as $(C_i/C_{max}) \times 100$ (Blum and Ebercon, 1981).

The lipid peroxidation was measured in terms of MDA content (Dhindsa *et al.*, 1981). For measurement of MDA content, 3 ml of 20 % trichloroacetic acid containing 0.5 % thiobarbituric acid was added to a 1 ml aliquot of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. The tube was centrifuged at $10,000 \times g$ for 10 min, and then absorbance of the supernatant was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of MDA was calculated using MDA extinction coefficient of 155 mM cm^{-1} .

Antioxidant enzymes activity

For enzyme extraction, 0.5 g leaf was extracted with 4 mL of extraction buffer (50 mM potassium phosphate, 1 mM EDTA, 1% PVP, 1 mM DTT, and 1mM PMSF, pH 7.8). The extractions were centrifuged at $15,000 \times g$ for 30 min at 4 °C, and supernatant was collected for enzyme assay. The protein content was determined using Bradford's method (1976).

1. Catalase assay

Activity of CAT was measured in a cuvette using the method of Dhindsa *et al.* (1981). The CAT reaction solution (1 mL) of 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2 was mixed rapidly with 50 μL enzyme extract. Reaction was initiated by adding the enzyme extract. Changes in absorption at 240 nm were read for 1 min with a UV-visible recording spectrophotometer (UV-160A, SHIMADZU, Japan). One unit CAT activity was defined as an absorbance decrease of $0.01 \text{ unit min}^{-1}$.

2. Superoxide dismutase assay

The SOD activity was determined by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) (McCord and Fridovich, 1969). Each 1 mL reaction solution contained: 75 μM NBT, 13 mM methionine, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), and 50 μL enzyme extract. Riboflavin (2 μM) was added at the last step to the reaction. Microtubes containing the reaction mixture were irradiated under fluorescent lamps at $78 \mu\text{mol m}^{-2} \text{ s}^{-1}$ for 15 min. The light absorbance at 560 nm was determined with spectrophotometer. One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction.

3. Ascorbate peroxidase assay

The ascorbate peroxidase (APX) activity was determined according to the method of Nakano and Asada (1981). The reaction solution (1 mL) contained 50 mM phosphate buffer (pH

7.0), 0.1 mM EDTA, 0.5 mM sodium ascorbate, 0.1 mM H₂O₂ and 50 µL enzyme extract. The light absorbance of the reaction solution was read for 1 min at 290 nm.

Experimental design and statistical analysis

The experiment was set out as split-plot based on a randomized complete block design (RCBD) with three replications for each treatment. Analysis of variance was carried out on the collected data and the mean value of data was compared at 0.01 and 0.05 levels of probability to detect the difference between treatment means using Fisher's protected least significant difference (LSD).

RESULTS

Drought stress decreased grass quality but grass treated with Pro-Ca exhibited higher quality during drought stress (Fig. 1). The plants sprayed with Pro-Ca maintained significantly higher RWC level under drought-stress conditions, compared to control plants (Fig. 2). The best protection against drought stress was found with 15 mg a.i./m² Pro-Ca. After drought stress treatment, ion leakage increased significantly. The plants sprayed with Pro-Ca significantly have reduced ion leakage, compared to controls (Fig. 3). The same as electrolyte leakage, MDA contents which is membrane lipid peroxidation indicator, increased in response to drought stress and Pro-Ca treatments decreased MDA content during stress (Fig. 4).

The best protection against drought stress was found with 15 mg/m² Pro-Ca. Superoxide

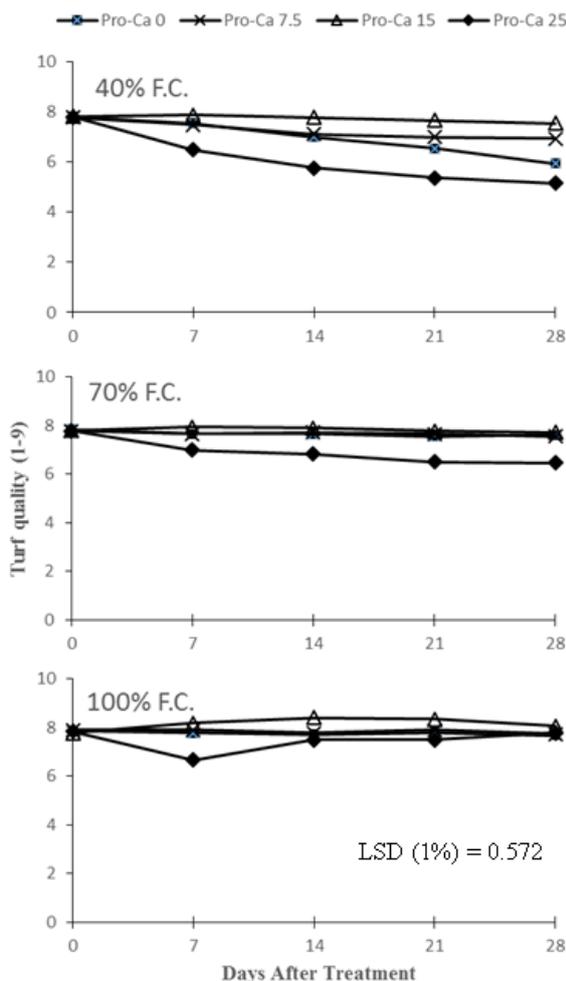


Fig. 1. Turf quality of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg a.i./m² under given drought conditions.

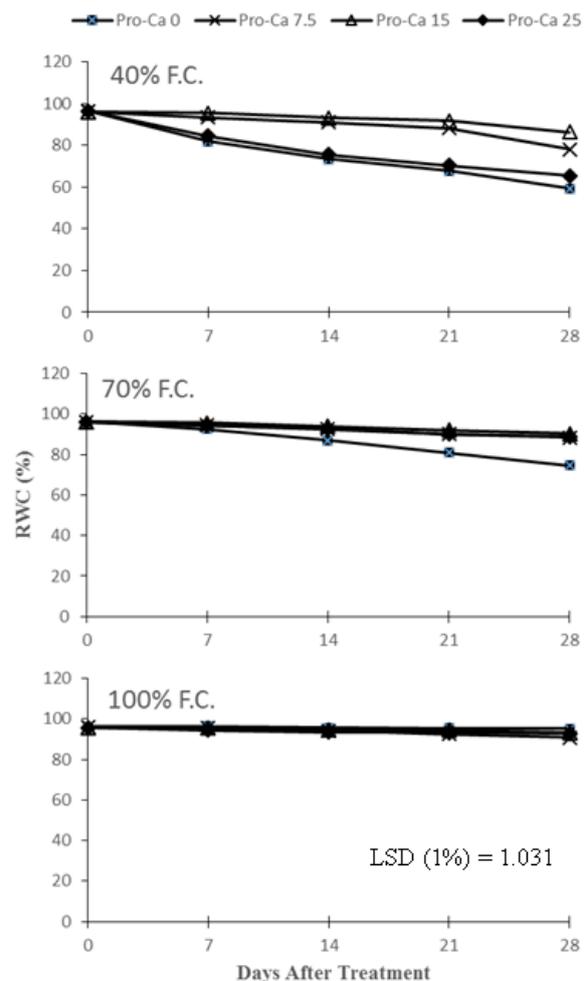


Fig. 2. Relative water content of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg a.i./m² under given drought conditions.

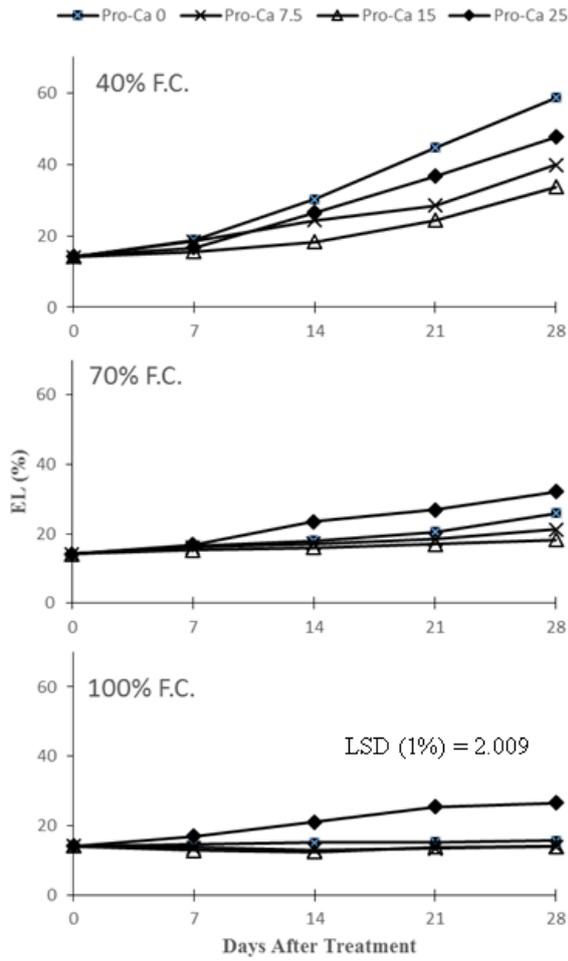


Fig. 3. Electrolyte leakage of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg a.i. m⁻² under given drought conditions.

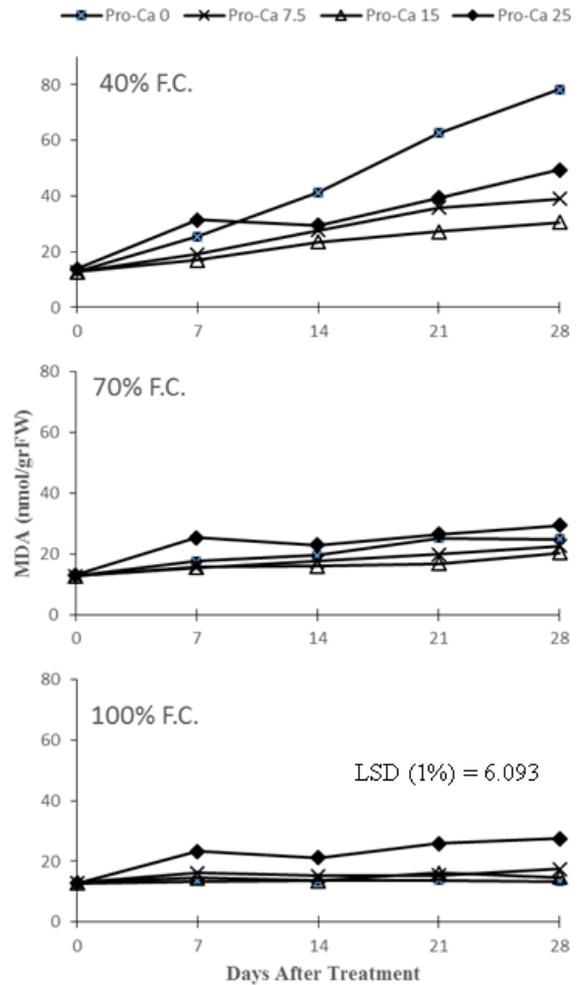


Fig. 4. Malondialdehyde content of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg a.i. m⁻² under given drought conditions.

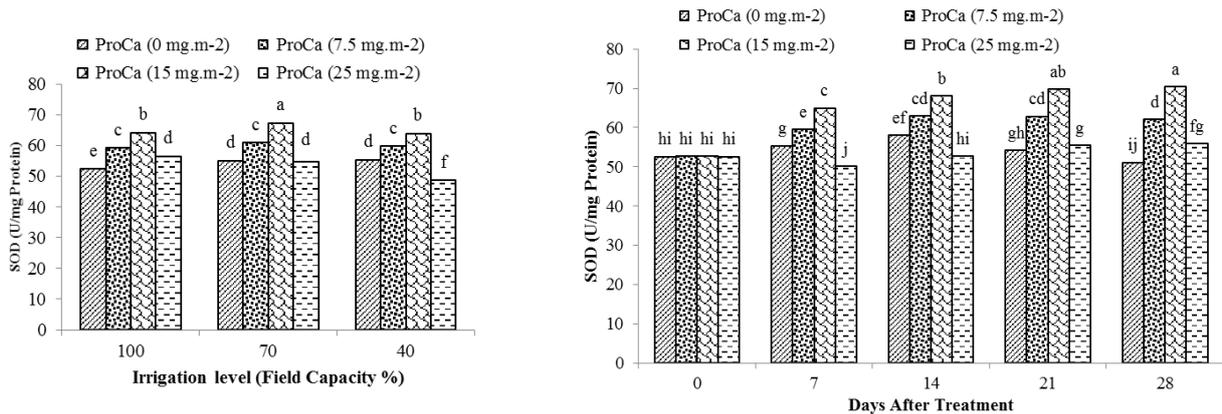


Fig. 5. Superoxide dismutase activity of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg m⁻² a.i. under given drought conditions (left) and during stress time (right).

dismutase (Fig. 5), ascorbate peroxidase (Fig. 6) and catalase activities were significantly increased in Pro-Ca treated grass under drought stress (Fig. 7). The magnitude of this increase in antioxidant enzymes activities was showed in 15 mg/m³ Pro-Ca treatment.

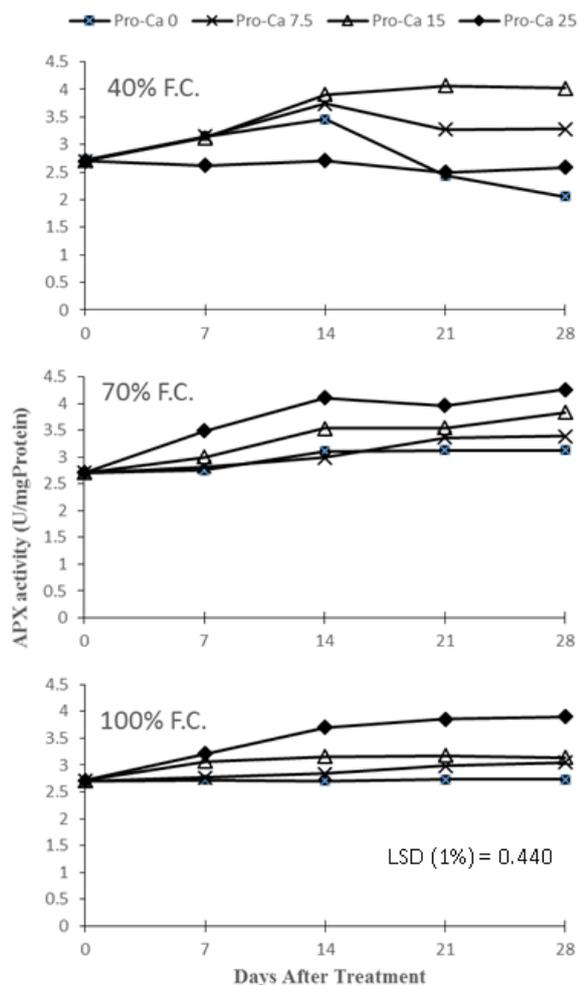


Fig. 6. Ascorbate peroxidase activity of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg m⁻² a.i. under drought conditions.

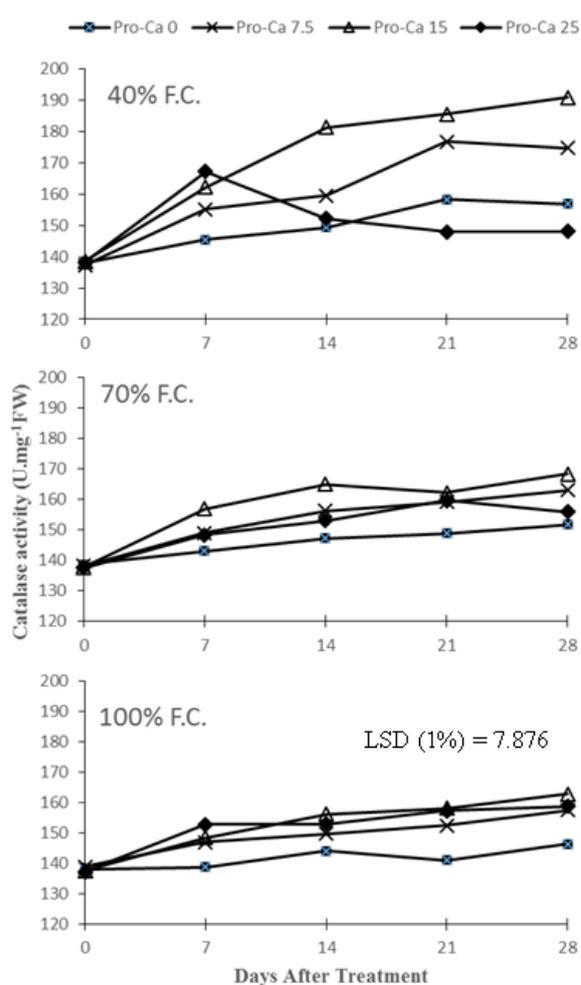


Fig. 7. Catalase activity of kentucky bluegrass treated with prohexadione calcium (Pro-Ca) at 0, 7.5, 15, and 25 mg m⁻² a.i. under given drought conditions.

DISCUSSION

Environmental stresses lead to intensive production of reactive oxygen species (ROS), setting off progressive oxidative damage and finally cell death (Asada, 1999; Reddy *et al.*, 2004; Sharma *et al.*, 2012; Ashraf and Harris, 2013). Drought is a major source of oxidative stress increasing ROS production and further lipid peroxidation, thus cellular homeostasis is disrupted and antioxidant enzymes' production and activity is diminished (Shi *et al.*, 2007). Antioxidant enzymes activity has a beneficial function on plant tolerance in drought stress (Moller *et al.*, 2007; Bian and Jiang, 2009). SOD scavenges O₂⁻ to H₂O₂ (Bowler *et al.*, 1992), and POD, CAT and APX detoxify H₂O₂ to H₂O at different cellular locations (Mittler, 2002). Our results showed that application of Pro-Ca increased the production and activity of SOD, CAT and APX in turfgrass in response to drought stress. Cell membrane stability plays a critical role in maintaining cell turgor and physiological functions, particularly during plant dehydration. Ion leakage as well as MDA content has been widely used to estimate cell membrane stability (Rachmilevitch *et al.*, 2006). Enhanced ion leakage is in part caused by oxidative damage to the biological membranes (Feng *et al.*, 2003; Munne-Bosch and Penuelas, 2003; Farooq *et al.*, 2008). The ROS react with proteins, lipids and DNA, impairing the normal cellular functions (Foyer and Fletcher, 2001). Membrane permeability significantly increased as indicated by increased ion leakage under drought stress. This change indicated a well-marked oxidative stress on the tissues under study. However, Pro-Ca application alleviated drought effects, as revealed by substantially reduced ion leakage and MDA accumulation. This is plausible in view of the fact that Pro-Ca can improve cell-wall elasticity,

acts on the phospholipid bilayer, improves the fluidity of the membrane and ultimately leads to improved plant growth (Leshem and Hamaraty, 1996). ROS in plants are scavenged by a variety of antioxidant enzymes and water-soluble molecules. Of these, antioxidant enzymes are the most effective against oxidative damage (Halliwell and Gutteridge, 1999; Foyer and Fletcher, 2001). Our results showed that Pro-Ca induced drought tolerance is due to profound increases in antioxidant enzymes activity. The increase of SOD, CAT and APX activities reduced the ROS production, which makes it possible to increase osmotic adjustment ability and thus drought tolerance (Zhu, 2002). This enhanced CAT and APX activity could reduce the accumulation of H₂O₂ and alleviate the damage to cell membranes. In conclusion, our results showed that Pro-Ca treatment improved grass drought tolerance via enhancing antioxidant enzymes activity which is associated with higher membrane integrity.

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