

DROUGHT TOLERANCE OF KNOTGRASS (*POLYGONUM MARITIMUM* L.) LEAVES UNDER DIFFERENT DROUGHT TREATMENTS

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Abstract

Drought stress is a major environmental stress factor for plants causing significant adverse effects on crop growth and yield. Therefore, plant resistance against drought should be determined for wild plants to alleviate such impacts. Knotgrass (*Polygonum maritimum* L.) is a wild plant getting rarer every day in sandy coasts. The present study was consulted to determine the responses of knotgrass (*Polygonum maritimum* L.) to drought stress during vegetative growth stages. Plants were drought-stressed by withholding water for (72 hours) and (10 days). Relative water content did not change under drought stress, but the chlorophyll content decreased in both drought treatments. Although, superoxide dismutase (SOD) and catalase (CAT) activity increased more in 10 days drought treatment than 72 hours treatment, ascorbate peroxidase (APX) and glutathione reductase (GR) activity of the treatments were not significantly different. Moreover, both malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents did not change under drought treatments. Such findings indicated *Polygonum maritimum* as a tolerant species to drought stress during vegetative growth stages.

Introduction

Polygonum maritimum L. (knotgrass) is one of the Polygonaceae species and getting rarer day by day in sandy coasts. It is naturally found throughout Europe along the Atlantic, Mediterranean and Black Sea coasts, extending northward to the Channel Islands, England and Belgium. It is defined as a perennial herbaceous plant in the psammophil class. It loves sandy conditions and identified as *Eryngium maritimum*, *Cakile maritima*, *Calystegia soldenella* and *Euphorbia peplis* in vegetation of Sinop Peninsula of Turkey (Kilinc & Karaer, 1995).

All over the world, coastal fore-dunes are regularly subjected to similar environmental stresses such as drought, salinity, low field capacity, high temperatures and high winds (Randall & Scott, 1997). Under such conditions, plants grown over coastal lines develop some kind of defense and adaptation mechanisms against prevailing conditions.

Under drought stress, reactive oxygen species (ROS), including superoxide, hydroxyl and hydrogen peroxide is enhanced in multiple ways. Firstly, CO_2 content of leaves are reduced due to limitation in $NADP^+$ regeneration and over reduction of photosynthetic electron transport chain. Secondly, photorespiratory pathway is induced under drought stress because of the lower CO_2 contents. ROS can oxidize proteins, lipids, carbohydrates and membranes (Smirnov, 1995). The accelerated generation of reactive oxygen species under drought stress induces ROS scavenging enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, 1.8.1.7) (Bowler *et al.*, 1992). Some reports have detected a positive correlation between the induction level of antioxidant enzymes and drought tolerance of the grasses (Abogadallah, 2011; DaCosta & Huang, 2007; Jiang & Huang, 2001).

Drought is one the most important stress factors inhibiting the plant growth and yield. There are many

reports on the mechanisms of perception, transduction and response of plants against drought stress. It is important to clarify the degree of tolerance of wild plants and grasses to drought and to detect the tolerance mechanisms for inhibiting the adverse effects of drought stress and alleviating the stress damage (Zhao *et al.*, 2008). Therefore, in present study, responses of *Polygonum maritimum* leaves to drought stress were studied and relative water content (RWC), lipid peroxidation (MDA), hydrogen peroxide (H_2O_2) and antioxidant enzyme activities (SOD, APX, CAT and GR) were determined under drought stress.

Material and Methods

Plant material and experimental design: *Polygonum maritimum* seeds were collected from wilderness of Akliman in Sinop, Turkey in May 2011 (before the flowering time). The seeds were sown in plastic trays (10 × 14 cm), containing mixture of soil and sand taken from the naturel environment. Following the growth of seedlings in a growth chamber at 25°C, 16h day/8h night photoperiod with, a light intensity of 300 $\mu mol\ m^{-2}\ s^{-1}$, they were watered with Hoagland solution for 14 days (Hoagland & Arnon, 1950).

For drought experiments, the seedlings were divided into two groups. Control groups were watered every day and water was not provided to drought groups. Seedlings were harvested after 72 hours and 10-days of growth. All leaves were stored at -80°C for enzymatic analysis.

Analyses

Relative water content: The relative water content (RWC) was calculated in accordance with Smart and Bingham, (1974). Harvested leaves were weighed to determine their fresh weights (FW). The seedlings were floated on de-ionized water for 5h under low irradiance and then the turgid tissue was quickly blotted to remove excess water and their turgid weights (TW) were determined. Dry weights (DW) were determined after leaves were dried in an oven at 70°C for 72h.

Chlorophyll content: The chlorophyll content of leaves was measured in accordance with the method specified by Lichtenthaler & Wellburn (1983).

Lipid peroxidation: The level of lipid peroxidation in leaf samples was determined in terms of malondialdehyde (MDA) content according to the method of Rao & Sresty, (2000).

Hydrogen peroxide content: Hydrogen peroxide levels were determined in accordance with Velikova *et al.*, (2000).

Antioxidant enzymes: Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by its ability to inhibit photochemical reduction of nitrotetrazolium blue chloride (NBT) at 560 nm (Beauchamp & Fridovich, 1973). CAT (EC 1.11.1.6) activity was estimated according to (Bergmeyer, 1970), which measures the initial rate of disappearance of H₂O₂ at 240 nm. APX (EC 1.11.1.11) activity was measured according to Nakano and Asada, (1981). The assay depends on the decrease in absorbance at 290nm as ascorbate was

oxidized. GR (EC 1.6.4.2) activity was measured according to Foyer & Halliwell (1976).

Statistical analysis: All analyses were carried out in accordance with completely randomized design. The data were subjected to non-parametric Kruskal Wallis test. Each data point was the mean of six replicates (n=6) and significance tests were carried out at p<0.05 level.

Results

In the present study, relative water content (RWC) of *Polygonum* leaves did not change in both drought treatments as compared to control groups. However, significant decreases were observed in chlorophyll content of leaves in both drought stress treatments. Compared to control treatments, such a decrease was 15.28% in 72 h and 33.37% in 10d drought stress. The rate of decrease from 72h to 10d treatments was about 21.35% (Table 1).

Table 1. Changes in relative water content (%), chlorophyll content (mg/ g FW) of *P. maritimum* under drought stress (72h and 10d).

Groups	Relative water content (%)	Chlorophyll content (mg/ g FW)
Control (72hC)	83.12 ± 3.58 ^a	17.47 ± 0.76 ^a
Drought stress (72hD)	83.01 ± 4.34 ^a	14.80 ± 0.40 ^b
Control (10C)	82.85 ± 1.24 ^a	16.46 ± 0.28 ^a
Drought stress (10D)	81.04 ± 5.21 ^a	11.64 ± 0.44 ^c

Different letters indicate significantly different means (p<0.05) values. 72hC: 72hC control; 72hD: 72h drought stress; 10C: 10d control; 10D: 10 d drought stress

According to our results, SOD activity of *Polygonum* leaves was clearly enhanced through drought treatments. Compared to control treatments, SOD activities increased by 22.28 and 76.27% respectively in 72h and 10d drought stresses (Fig. 1a). Moreover, the rate of increase from 72h to 10d drought stress was 44.44%.

In our results, APX enzyme activity did not change under drought treatments of the present study (Fig. 1b). Similar to APX, GR activity also did not change in 72h treatment (Fig. 1d). Nevertheless, it decreased by 19.69% in 10d treatment as compared to control groups. The rate of decrease from 72h to 10d treatment was 24.28%.

Similar to SOD activity, CAT activity also increased in both drought treatments (Fig. 1c). Compared to control treatments, such an increase was 47.72% in 72h treatment and 57.30% in 10d treatment. The rate of increase from 72h to 10d treatment was about 25.84%. Beside this, compared to control treatments, MDA and H₂O₂ contents of the leaves did not change in both drought treatments (Fig. 2a, b).

Discussion

In our results, (RWC) of *Polygonum* leaves did not change in both drought treatments as compared to control groups. Parallel results were also reported by previous researchers (Hernandez *et al.*, 2000; Jurekova *et al.*, 2011, Razzaq *et al.*, 2013). It is well known that leaf water status always interacts with stomatal conductance and a good correlation between leaf water potential and (g_s) always exists, even under stress (Reddy *et al.*, 2004). From this knowledge, it could be suggested drought stress

did not reduce the RWC of leaves by maintaining stomatal conductance.

Plants can lose chlorophyll under drought stress because of the oxidative damage to chloroplasts (Smirnov, 1995). In the present study, significant decreases were observed in chlorophyll content of leaves in both drought stress treatments (Table 1). Similarly, Khan *et al.*, (2009) and Mafakheri *et al.*, (2010) also reported decreasing chlorophyll contents in wheat chickpea cultivars under drought stress respectively. In the present study, malondialdehyde (MDA) contents plant leaves did not change significantly under drought treatments (Fig. 2a). Thus, it can be suggested that the reduction of chlorophyll content was not so much effective for disturbing the photosynthesis regulation and photosystem I (PSI) activity. Otherwise, the vegetative stage can be less sensitive against variations in chlorophyll content.

Under drought conditions, plants can close their stomata to keep the water balance. In this case, CO₂ fixation is limited due to reduced NADP regeneration and over reduction of the photosynthesis electron transport chain through the Calvin cycle (Hernandez *et al.*, 2000). Thus, reactive oxygen species is produced by chloroplasts under such circumstances. Also the photorespiratory pathway is enhanced when RuBP oxygenation is maximal due to limitation on CO₂ fixation (Noctor *et al.*, 2002). Production of this reactive oxygen species cause significant damages on membranes and proteins. To alleviate such damages, plants develop antioxidant defense systems including enzymes as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR).

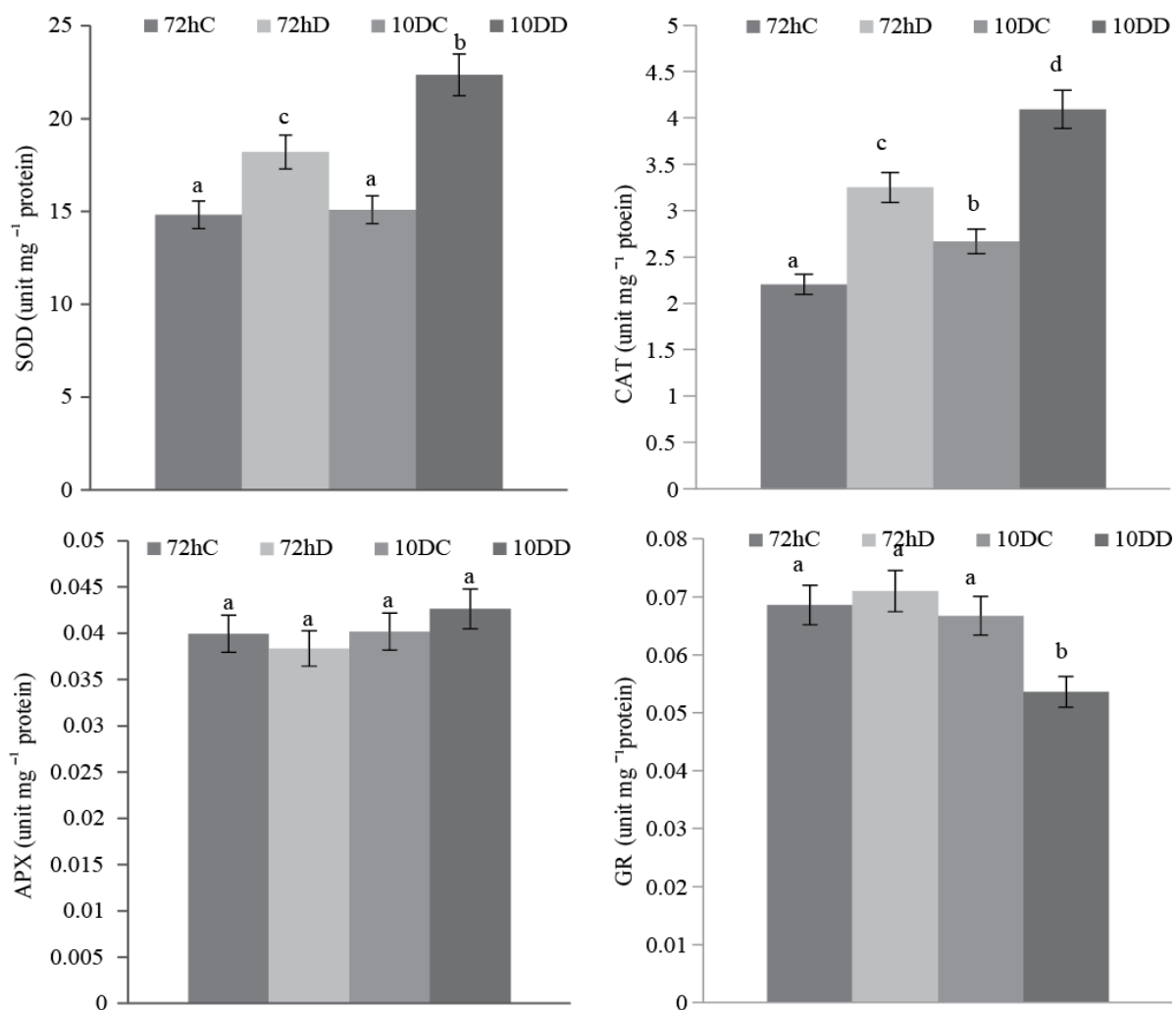


Fig. 1. Changes in a (SOD), b (APX), c (CAT), d (GR) activities of *P. maritimum* leaves under drought stress (72h and 10d). Different letters indicate significantly different means ($p < 0.05$) values. 72hC: 72h control; 72hD: 72hD drought stress; 10DC: 10d control; 10DD: 10d drought stress.

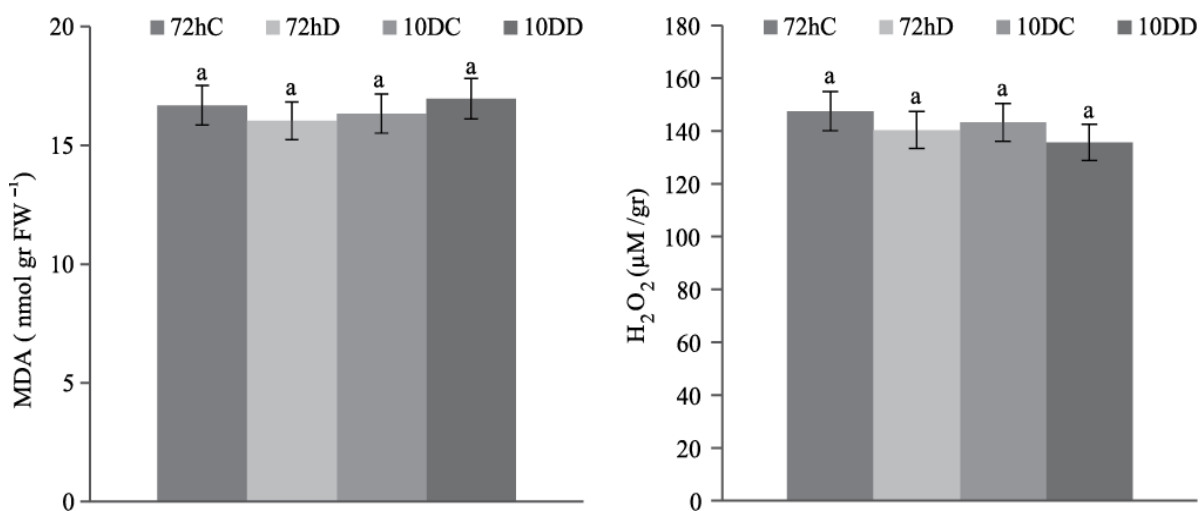


Fig. 2. Changes in a) MDA content (nmol gr FW⁻¹) and b) H₂O₂ (μM/gr) of *P. maritimum* leaves under drought stress (72hD and 10D). Different letters indicate significantly different means ($p < 0.05$). 72hC: 72hC control; 72hD: 72hD drought stress; 10DC: 10d control; 10D: 10d drought stress.

SOD is one of the most important antioxidant enzyme that remove the superoxide radical by catalyzing its dismutation, one superoxide radical being reduced to hydrogen peroxide and another oxidized to oxygen (Gill & Tuteja, 2010). In the present study, SOD activity increased in both treatments but the increase was more remarkable on 10d treatment. In agreement with the current findings, previous researchers also reported increased SOD activities in rice and peas under drought stress (Mittler & Zilinskas, 1994; Sharma & Dubey, 2005).

According to our results, APX enzyme activity did not change under drought treatments of the present study (Fig. 1b). Parallel to current findings, Salekjalali *et al.*, (2012) also reported that APX activity did not change in barley plants under water stress. Although APX exists in every cellular ROS producing compartment, CAT is located principally in peroxisomes which remove the bulk of hydrogen peroxide generated in photorespiration (De Carvalho, 2008). Thus, CAT was found to be more efficient than APX by maintaining the level of hydrogen peroxide of photorespiration. GR is a potential enzyme of the ascorbate glutathione cycle and plays a significant role by sustaining the reduced status of GSH. It catalyzes the reduction of GSH, a molecule involved in many metabolic regulatory and antioxidative processes in plants. In our results, GR activity also did not change in 72h treatment and decreased in 10d (Fig. 1d). Similar results were also reported for wheat by Loggini *et al.*, (1999). It was concluded herein that some of ascorbate-glutathione cycle enzymes (APX, GR) were not efficient in *Polygonum maritimum* plants.

CAT plays an important role in plants through removal of hydrogen peroxide generated in peroxisomes by oxidases involved in β -oxidation of fatty acids, photorespiration and purine catabolism. In the present study, CAT activity also increased in both drought treatments (Fig. 1c). Parallel to current findings, previous researchers also reported increased CAT activities in alfalfa and maize under drought stress (Jiang & Zhang, 2002; Rubio *et al.*, 2002). However, hydrogen peroxide content of the present study did not increase in both treatments (Fig. 2b). Thus, it was concluded that the increase in the CAT activity could be related to maintained hydrogen peroxide content.

Lipid peroxidation is usually used as a marker for oxidative damage in plants (Ashraf *et al.*, 2010). In our results, compared to control treatments, MDA contents of the leaves did not change in both drought treatments (Fig. 2a, b). From this results, it could be suggested that the leaves of *Polygonum maritimum* plant could alleviate the oxidative damage from reactive oxygen species by reducing the MDA and H_2O_2 content with efficient antioxidant enzymes (SOD, CAT).

Conclusion

In general, drought stress inhibited chlorophyll contents of *Polygonum* leaves. But such inhibition was more efficient in 10d drought treatment than 72h treatment. Moreover, it's clear that the increase in CAT activity prevented the increase of MDA and H_2O_2 contents and protected the cells from oxidative damage of

stress treatments. However, the ascorbate-glutathione cycle enzymes (APX, GR) were not found to be efficient to convert hydrogen peroxide to water in the leaves of this plant. Therefore, it was concluded that *Polygonum maritimum* was a tolerant species to drought stress through increasing some of antioxidant enzymes (SOD, CAT). This was the first study reporting the stress tolerance of *Polygonum maritimum*. This plant can be used as a model for increasing the tolerance of plants to drought stress in molecular and biochemical studies. Further studies can be carried out under stress conditions to investigate the tolerance mechanisms and protective strategies (ion regulation and hormonal changes) in roots and leaves of the plant.

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(Received for publication 26 November 2012)