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# The physiological responses of four turfgrass species to drought stress

**Z. Gholamian Jazi (*), N. Etemadi, H. Aalipour**
*Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.*

*Key words*: antioxidant, bermudagrass, cool-season turfgrasses, electrolyte leakage.

**Abstract: Drought stress is one of the most important factors which reduce turfgrass growth and quality in the area with restricted rainfall or irrigation water supply. Using resistant species and varieties can be a useful management program for reducing irrigation requirement in turfgrass. The present study was carried out to examine the physiological changes of four species turfgrass in response to drought stress conditions. The rhizomes of bermudagrass, and seeds of tall fescue, perennial ryegrass and kentucky bluegrass were cultivated at the greenhouse in PVC pots (20 cm in diam., 20 cm long). After four months, when the seedlings well established, drought stress was applied in 100% field capacity (FC), 75% FC, 50% FC and 25% FC. Proline, electrolyte leakage (EL), malondialdehyde (MDA), relative water content (RWC), chlorophyll, catalase (CAT), superoxide dismutase (SOD) and peroxides (POD) was measured. All species showed an ability to tolerate drought stress, but tall fescue exhibited more tolerance, with a higher RWC and proline content. Tall fescue also revealed higher CAT, SOD, POD activities and lowest MDA, EL. This study found that kentucky bluegrass was more vulnerable to severe water stress, and displayed the highest MDA and EL as compared to the other examined species.**



(*) **Corresponding author:**
z.gholamianjazi@gmail.com

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**Data Availability Statement:**
All relevant data are within the paper and its Supporting Information files.

**Competing Interests:**
The authors declare no competing interests.

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## 1. Introduction

Water deficit is the main problem for turf management, especially in arid and semi-arid zones. Turfgrasses play a significant role in the design of urban green spaces, in most cases other plants can not be utilized instead of turfgrasses; therefore it is necessary to find species and cultivars of turfgrass that require little water and are able to maintain their visual quality in drought conditions (Fiorio *et al*., 2012). Increased competition for water has fostered interest in water conservation practices for both warm-season and cool-season turfgrasses. Responses of turfgrass to drought can be viewed in a number of ways. Drought stress will affect visual quality, growth rate and evapotranspiration (ET) (Krishnan *et al*., 2013). Some adaptations and mitigation strategies are necessary to dispose of drought stress. Grass species and cultivars have been found to respond differently to drought stress (Vurukonda *et al*., 2016). Some grass genotype like tall fescue were able to tolerate drought condition in a research it was demonstrated that this kind of grass can be one of the

most suitable plant species to be used for cultivation under arid, semi-arid regions, and areas with limited water supplies or drought conditions (Alam *et al.*, 2018).

Some traits affected by drought stress include relative water contant, electrolyte leakage and some enzyme activities. RWC and EL are indicators for the selection of drought-tolerant plants (Salehi Lisar *et al*., 2012). Reactive oxyen species cause lipid peroxidatin, which leads to damage of cell membrane. Drought stress increased lipid peroxidatin and membrane damage percent in different plants. It is been reported that tall fescue exhibiting a more effective protection mechanism, mitigated oxidative stress and lipid peroxidation by maintaining higher superoxide dismutase (SOD) and catalase (CAT) activities than kentucky bluegrass (Xu *et al*., 2013). There are many reports about increase, decrease, or no change inoxidative enzymes in plants exposed to stress (Fu and Huang, 2001; Ramachandra Reddy *et al*., 2004; Sharma and Dubey, 2005).

Synthesis of compatible solutes such as proline seems to has a central role in osmotic adjustments, preventing or reducing the loss of turgor (Vinocur and Altman, 2005). In fact, studies on several turfgrass species have shown that the free proline concentration increases in leaves with water stress. This phenomenon has been demonstrated in *Festuca arundinacea* Scherb. (Salehi and Salehi, 2012).

Many researches evaluated the physiological adaption or the functional and qualitative response of different cool and warm season grasses to increasing water deficits (Qian and Engelke, 1999; Bastug and Buyuktas, 2003; Fu *et al.*, 2004). Althought all of these studies report that during the drought period, warm-season grasses are more tolerant to drought sress than cool-season grasses, different climatic conditions will influence relative drought tolerance of cool-season and warm-season turfgrass, necessitating regional evaluations. The main goals of this study were to compare the physiological and biochemical responses of cool-season turfgrasses (tall fescue, kentucky bluegrass, perennial ryegrass) and warm-season turfgrasses (bermudagrass) under drought stress condition in Isfahan, iran.

## 2. Materials and Methods

Plant materials and experimental: This reserch was performed during 2015 to 2016 in Department of Horticultural at Isfahan University of Technology, Isfahan, Iran under greenhouse conditions (32°39' N, 51°40' E). Polyvinyl chloride (PVC) pots (20 cm in diam., 20 cm long) filled with sterilized silt-loam soil, which collected from the Isfahan's landscape. For this experiment seeds of *Festuca arundinacea*. 'Astrix', *Lolium perenne*. 'Numan' and *Poa pratensis*. 'Miracle' were sown and rooted rhizomes of *Cynodon dactylon*. 'Tifway' were planted in 48 PVC pots. Irrigation was applied as needed to prevent any visible drought stress during grass establishment. In general, turfs were watered three times weekly to maintain plants under well-watered condition and soil moisture at field capacity. Plants were maintained at a cutting height of 5 cm and moved once a week using a reel-type mower. A fertilizer (urea) was applied at 5 $g.m^{-2}$ rates once every two weeks to provide nutrients and to facilitate plant establishment before initiation of treatments.

*Drought stress treatment and experiment design*

This study was carried out as a factorial experiment based on randomized complete block design (RCBD), with two treatments consisted of four levels of drought stress (100%, 75%, 50%, and 25% field capacity (FC) ), four turfgrass species (tall fescue, bermudagrass, kentucky bluegrass, and perennial ryegrass), with three replications and 16 pots were used for each replication (numbers of pots= 48). The FC was determined by the gravimetric method, which consists on the difference between the wet soil after saturation and free drainage, and the weight of the dry soil (Cleide de Souza *et al*., 2000). The soil water content was kept at gravimetric water capacity (measured 100%, 75%, 50% and 25% FC were 22.3%, 16.72%, 11.15% and 5.57% respectively) by adding tap water. Drought stress was applied for two months and at the end of the experiment, physiological traits were measured.

*Chlorophyll content*

Leaf samples were selected randomly from the plants and homogenized in a mortar in 10 ml of 100% acetone. The extract was centrifuged at 2000 rpm for 10 min. Absorbance of the supernatant was recorded at 663, 645 and 450 nm spectrophotometrically. Chlorophyll (Chl) content was determined following the method of (Lichtenthaler, 1987).

Electrolyte leakage: Leaf electrolyte leakage which is used to assess membrane permeability leakage was assayed base on Lu *et al*. (2008) methods. Leaf samples (0.1 g) was placed into a vial with 20 mL of double distilled water. After incubating the samples at room temperature on a shaker (150 g) for 24h, the

electrical conductivity (EC) of the bathing solution ($EC_1$) was determined. The same samples were then placed in water bath at 100°C for 1h and a second reading ($EC_2$) was determined after cooling solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

*Relative water content*

We determined relative water content (RWC) according to the method developed by Ghoulam *et al.* (2002). About 0.2 g of the fresh leaf sample was cut into smaller pieces and weighed (W1). Then the leaf samples were saturated in 100-ml deionized water for 24 h at 4°C and weighed to determine the turgid weight (W2). Finally leaf samples were dried at 70°C for 24 h, and the dry weight was recorded (W3). RWC was determined using the following equation:

$$RWC\ (\%) = (FW\text{-}DW) / (TW\text{-}DW) \times 100.$$

Where FW, DW, and TW are fresh, dry and turgid weights respectively.

*Proline content*

Proline content measurement was carried out according to a previously described method (Bates *et al*., 1973). Leaves were homogenized in 3% aqueous Sulphosalicylic acid, then centrifuged 5,000 g for 20 min at 4°C. 2 mL of this homogeny solution react acid-ninhdrin and 2 mL of glacial acetic acid in a tube for 1 hour at 100°C and the reaction is torn up in an ice bath and then extracted with 4 mL of toluene. It was kept at room temperature to stabilize. Proline content was measured by spectrophotometer (UV-160A, Shimadzu, Tokyo, Japan) at 520 nm (Bates *et al.*, 1973).

*Malondialdehyde*

In order to determine the content of malondialdehyde (MDA) in the leaves, 0.1 g of leaf tissues was homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) for 10 minutes and then was centrifuged at 5,000 g. 1 ml of conventional solution was mixed with 4 ml of thiobarbituric acid (TBA) (0.5% of TBA in 20%). Then the reaction mixture was placed in a hot bath at 100°C for 15 minutes. Finally the mixture centrifuged at 5,000 g for 10 minutes and the amount of MDA was subsequently read by the spectrophotometer at 450, 532 and 600 nm (Wang *et al*., 2008).

*Enzyme assay*

For enzyme extraction, 0.1 g leaf powder was extracted with 1 ml of sodium phosphates extraction buffer and Triton. The extractions were centrifuged at 12000 × *g* for 30 min at 4°C, and supernatant was collected for enzyme assay. The supernatant was used as a source of SOD enzyme. SOD was measured by a photochemical method (Giannopolitis and Ries, 1977). The reaction mixture (3 ml) contained 0.1 mM EDTA (Ethylenediamine tetra acetate) 0.05 ml HEPES-KOH buffer (pH=7.8), 50 mM $Na_2Co_3$, 13 mM methionine, 63 µM NBT (Nitro blue tetrazolium) 0.05 ml enzyme extract and 1.3 µM riboflavin. The absorbance was read at 560 nm and one unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT. CAT (EC: 1.11.1.6) activity was assayed in a reaction mixture containing 100 mM phosphate buffer (pH 7.0), 15 mM $H_2O_2$ and enzyme 0.05 ml of aliquot. The decomposition of $H_2O_2$ was followed at 240 nm (Aebi, 1984). The catalase (CAT) activity is defined in international unit equals (1 unit) as the amount of catalase necessary to decompose 1 µM of $H_2O_2$ per minute. Activity of peroxides (POD) was determined in a reaction mixture (2.95 ml), which consisted of 75 mM suitable amount of guaiacol, 15 mM $H_2O_2$, 100 mM phosphate buffer and 0.05 ml of enzyme extract. The absorbance of the supernatant at 470 nm was measured (Maehly, 2006). One unit of POD activity was defined as the amount of enzyme necessary to decompose 1 µM of $H_2O_2$ per minute.

*Statistical analyses*

Statistical Analysis System (SAS 9.1) was used for variance analysis and the differences between treatment means were assessed by the least significance difference (LSD) at P= 0.05 probability level.

## 3. Results

*Chlorophyll content*

Results from leaf chlorophyll content measurements showed a significant difference between water stress treatments, species and interaction effects ($P \leq 0.01$) (Table 1). Water stress negatively influenced the Chl in all species. Chl content significantly ($P<0.05$) decreased under water stress condition compared to the well-watered treatment (Fig. 1). As shown in figure 1, Lolium perenne in 25% FC showed the lowest chlorophyll content (12.6 mg/g FW) and chlorophyll content was higher at 100% FC in Poa pratensis (44.24 mg/g FW) compare to other species (Fig. 1).

*Electrolyte leakage*

EL significantly decreased under water stress treatments compared to 100% FC level. The highest EL was manifested under 25% FC level (Table 2). The

Table 1 - Analysis of variance of CHL (chlorophyll content), EL (Electrolyte leakage), RWC (Relative water content), Pr (Proline content), MDA (Malondialdehyde), CAT (catalase), POD (peroxidase), and SOD (superoxide dismutase) activities of turfgrasses species under drought stress

| Effect | | Mean | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DF | CHL | EL | RWC | Pr | MDA | CAT | POD | SOD |
| Block | 2 | 15.00 * | 54.31 ** | 3.93 NS | 0.0001 NS | 0.01 NS | 0.003 NS | 0.01 NS | 0.34 NS |
| Species | 3 | 834.1 ** | 16.08 ** | 354.15 ** | 0.003 ** | 2.09 ** | 0.07 ** | 34.89 ** | 21.18 ** |
| Drought | 3 | 353.86 ** | 29.72 ** | 4618.81 ** | 0.115 ** | 6.70 ** | 0.16 ** | 60.59 ** | 59.101 ** |
| Drought × species | 9 | 29.38 ** | 2.61 NS | 22.33 ** | 0.0007 * | 0.38 ** | 0.01 ** | 4.70 ** | 8.76 ** |
| Error | 30 | 2.84 | 1.44 | 889.86 | 0.0003 | 0.02 | 0.002 | 0.02 | 0.86 |
| Coefficient of variation | | 7.42 | 1.28 | 2.33 | 10.89 | 9.4 | 15.45 | 3.2 | 9.94 |

NS= not significant, *P≤0.05, **P≤0.01.

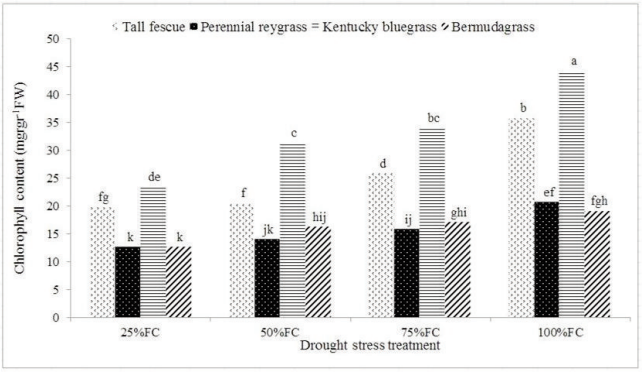


Fig. 1 - Interaction effects of water stress and species on chlorophyll content. Vertical bars (mean ± 1.37) not connected with the same letter represent significant difference between treatments according to LSD test (P<0.05).

EL reached the peak (95.4%) in *P. pratensis*, and the lowest EL (89.3%) was recorded in the *Festuca arundinacea* (Table 2). Interaction of drought stress and species showed no significant effect on electrolyte leakage (Table 1).

*Relative water content*

The results showed that water stress, species, and their interaction had the significant effect on RWC (P ≤0.01) (Table 1). As shown in figure 2, all species showed high value of RWC under 100% FC. Percentage reduction of RWC under 25% FC, were 43.2, 43.9, 51.1 and 59.6 for tall fescue, bermudagrass, perennial ryegrass and kentucky bluegrass respectively, as compared with well-watered control plants (Fig. 2).

*Proline content*

Water stress and species both showed significant effect on proline content (P≤0.01) and their interaction effects were significant at 5% level (Table 1). As shown in figure 3, proline concentrations in four species were all increased under drought stress which indicates osmotic adjustment in turfgrasses. *F. arundinacea* at 25% FC level showed the highest proline content (with 0.3 µmol/g FW) whereas lower proline content was shown for well-watered *Lolium perenne*,

Table 2 - Interaction effects of water stress and species and mean comparison on electrolyte leakage

| Species | Drought stress treatment | Electrolyte leakage | Mean |
|---|---|---|---|
| *Festuca arundinacea* | 100% FC | 89.31 h | 93.08 b |
| | 75% FC | 92.95 defg | |
| | 50% FC | 94.41 bcde | |
| | 25% FC | 95.64 ab | |
| *Lolium perenne* | 100% FC | 92.10 fg | 93.33 b |
| | 75% FC | 92.59 efg | |
| | 50% FC | 93.74 bcdef | |
| | 25% FC | 94.89 bcd | |
| *Poa pratensis* | 100% FC | 94.66 bcd | 95.40 a |
| | 75% FC | 94.17 bcde | |
| | 50% FC | 95.42 abc | |
| | 25% FC | 97.34 a | |
| *Cynodon dactylon* | 100% FC | 91.48 g | 92.92 b |
| | 75% FC | 92.40 efg | |
| | 50% FC | 93.53 cdef | |
| | 25% FC | 94.26 bcde | |

The values represent the mean ± standard error of three replicates. Different letters are showing considerable differents at P≤0.05.

*Poa pratensis* and *Cynodon dactylon* (Fig. 3).

*Malondialdehyde*

According to the results, the MDA content were significantly affected by water stress level, grass species, and their interaction effects (P≤0.01) (Table 1). As the water stress increased, a clear increase in the MDA content has been seen in all species (Fig. 4). As shown in the figure 4, the highest (3.6 µmol $g^{-1}$ FW) and lowest (0.44 µmol $g^{-1}$FW) amounts of MDA were obtained in *P. pratensis* grown under severe water stress (25% FC) and *F. arundinacea* under control treatment, respectively (Fig. 4).

*Enzyme*

The results showed that water stress, species, and interaction effects had significant effect on catalase, peroxidase and superoxide dismutase (P≤0.01) (Table 1). As shown in the figure 5, the maximum CAT activity belonged to *F. arundinacea* at 25% FC whereas the minimum CAT activity belonged to *C. dactylon* at

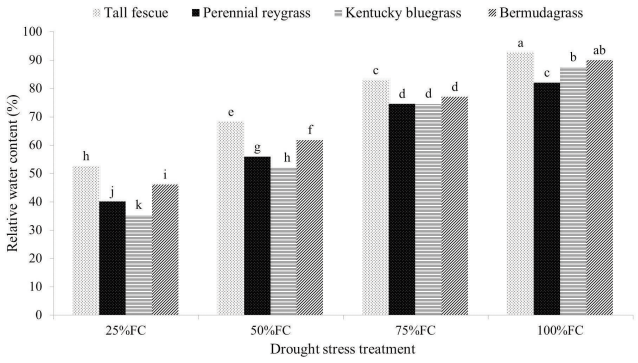


Fig. 2 - Interaction effects of water stress and species on relative water content (RWC). Vertical bars (mean±1.96) not connected with the same letter represent the significant difference between treatments according to LSD test (P<0.05).

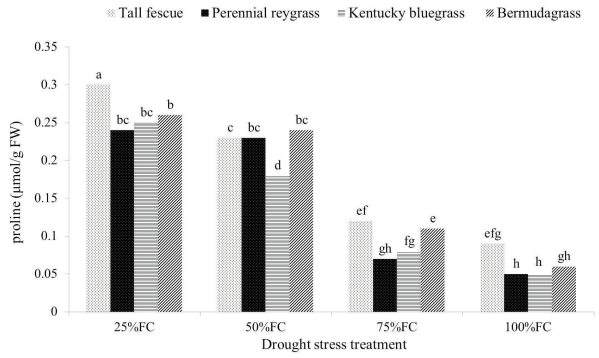


Fig. 3 - Interaction effects of water stress and species on proline. Vertical bars (mean ± 0.01) not connected with the same letter represent significant difference between treatments according to LSD test (P<0.05).

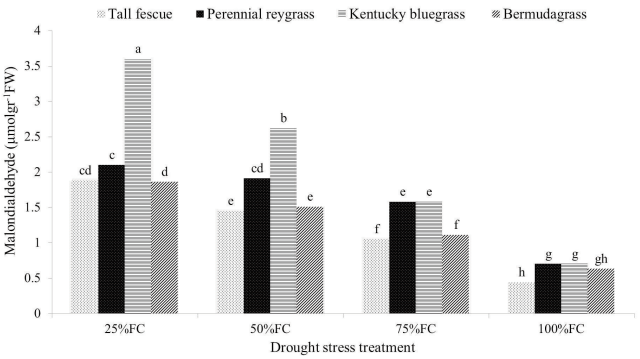


Fig. 4 - Interaction effects of water stress and species on malondialdehyde. Vertical bars (mean ± 0.11) not connected with the same letter represent significant difference between treatments according to LSD test (P<0.05).

100% FC (Fig. 5). Also as shown in figure 6, the highest POD activity was obtained under 50% FC in *F. arundinacea* while the lowest activity was obtained for *Poa pratensis* at 100% FC level., On the whole, POD activities of for species followed a similar pattern under drought stress, which was characterized by a gradual increase until 50% FC level followed by a decline after this treatment (Fig. 6). Moreover, it

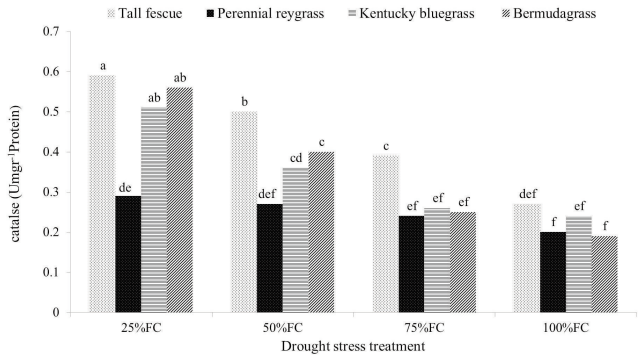


Fig. 5 - Interaction effects of water stress and species on catalase. Vertical bars (mean ± 0.04) not connected with the same letter represent significant difference between treatments according to LSD test (P<0.05).

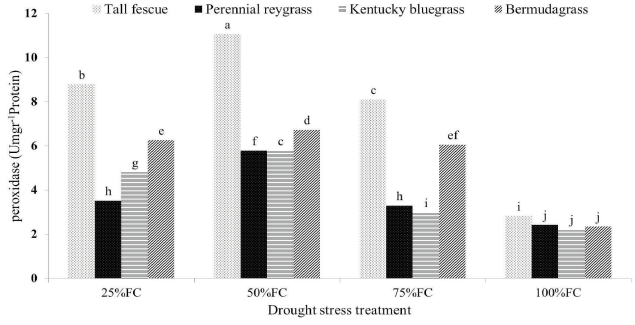


Fig. 6 - Interaction effects of water stress and species on peroxidase. Vertical bars (mean ± 0.12) not connected with the same letter represent significant difference between treatments according to LSD test (P<0.05).

could be obviously seen that SOD activities followed the similar pattern as POD; applying 50% FC in *F. arundinacea* and 100% FC in *L. perenne* scored the highest (15 $Umg^{-1}$Protein) and the lowest (2.5 $Umg^{-1}$Protein) respectively (Fig. 7).

*Traits correlation*

Electrolyte leakage with proline, MDA and CAT showed the significant positive correlation (P≤0.01). Our results showed the significant negative correlation between RWC and EL (P≤0.01). Proline was positively correlated with MDA, CAT, SOD, and POD (P ≤ 0.01). Also, it showed the negative correlation between RWC and chlorophyll at P≤0.01 (Table 3). Our results showed a positive correlation between MDA and CAT (P≤0.01) (Table 3). SOD showed significant positive correlation with CAT and POD (P≤0.01).

## 4. Discussion and Conclusions

In the present study, increasing in water deficit resulted in an decrease in the plant chl content compared with the plants under well-watered conditions. Chlorophyll content (Chl) in live plants is an important factor in determination of photosynthetic capacity. In the present study, leaf Chl content decreased gradually during the stress periods (Fig. 1). Decreased or unchanged Chl content level during drought stress has been reported in other cultivars, depending on drought duration and severity (Zhang and Kirkham, 1996; Jagtap *et al*., 1998). Under the water deficit stress, chloroplast ultra-structures are the first target to be damaged at the cellular levels since it is the major site of reactive oxygen species (ROS) production (Munné-Bosch and Peñuelas, 2003). An enriched ROS in stressed tissues impairs cellular membrane and organelles which affects the integrity of the cell.

In our study, electrolyte leakage gradually increased with increasing water stress in turfgrass species. Increase of EL with development of drought stress has been reported by many researchers (Guo *et al*., 2006; Liu *et al*., 2008). Electrolyte leakage increase is occurred with the increase of cell permeability (Blum and Ebercon, 1981). Results of a study by Abraham *et al*. (2004) showed at higher electrolyte leakage in *Poa pratensis* and its hybrids under drought stress, while low levels of electrolyte leakage (an indicator of cell membrane stability) were observed in the drought-tolerant plants during drought stress. Fu and Huang (2001) reported that EL and MDA increased simultaneously in five species of kentucky bluegrass which indicated the positive relationship between these traits

In this research, drought stress conditions decresed RWC due to reduced leaf water potential, consistent with results reported by Fu and Huang (2001), Farkhondeh *et al*. (2012). RWC as an indicator of plant water status is one of the most reliable indicators for defining water retention in plants. In this study, RWC was affected significantly by water stress

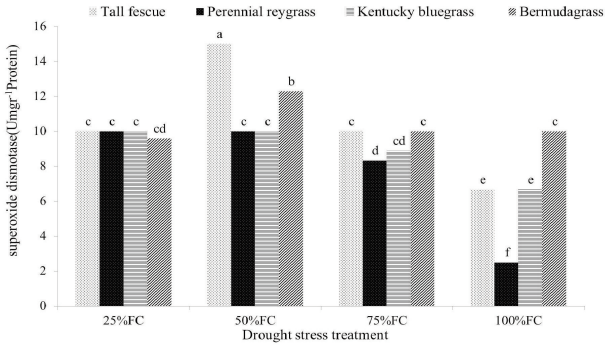


Fig. 7 - Interaction effects of water stress and species on superoxide dismutase. Vertical bars (mean ± 0.75) not connected with the same letter represent significant difference between treatments according to LSD test (P< 0.05).

Table 3 - Pearson correlation coefficients of electrolyte leakage, proline, MDA (Malondialdehyde), SOD (superoxide dismutase), CAT (catalase), chlorophyll, and POD (peroxidase) of turfgrasses species under drought stress

| Traits | Electrolyte leakage | Proline | MDA | SOD | RWC | CAT | Chlorophyll | POD |
|---|---|---|---|---|---|---|---|---|
| Electrolyte leakage | 1 | | | | | | | |
| Proline | 0.454 ** | 1 | | | | | | |
| MDA | 0.59 6** | 0.672 ** | 1 | | | | | |
| SOD | 0.258 NS | 0.575 ** | 0.354 ** | 1 | | | | |
| RWC | -0.576** | -0.855 * | -0.890 ** | -0.374 ** | 1 | | | |
| CAT | 0.422 ** | 0.760 ** | 0.510 ** | 0.491 ** | -0.587 ** | 1 | | |
| Chlorophyll | -0.029 NS | -0.475 ** | -0.242 NS | -0.308 * | 0.455 ** | -0.205 NS | 1 | |
| POD | 0.273 NS | 0.627 ** | 0.311 ** | 0.690 ** | 0.364 ** | 0.694 ** | -0.274 NS | 1 |

NS= not significant, *P≤0.05, **P≤0.01.

in all species. However, reduction was less pronounced for *F. arundinacea*, which maintained a higher RWC than other species. The amount of RWC in plant with high resistance to drought stress is higher than that of susceptible plants. In other words, plant having higher yields under drought stress should have higher RWC (Liu *et al*., 2002). Under water deficit, the cell membrane is subjected to changes such as penetrability and decrease in sustainability (Blokhina *et al*., 2003). Results of a study by Wang and Huang (2003) showed a decline in the RWC under drought stress, especially in; susceptible cultivars (Wang and Huang, 2003). Bian and Jiang (2009) showed that RWC in *P. pratensis* decreaed during drought stress.

The initial physiological response of plants to drought stress is osmoregulation which decreases water potential and maintains turgor to hold water inside of tissues and absorbing moisture from the environment at the same time, thus finally maintain other physiological activities of the cell (Li *et al*., 2015). The results of the present study clearly showed that proline content increased in all turfgrass species under water deficit compared to the well-watered conditions. Increase of proline under drought stress has been reported by many researchers (Turkan *et al*., 2005; Wang *et al*., 2008). Proline content of *F. arundinacea* under drought stress increased dramatically, which could be a significant factor for maintaining the relative water content. The correlation between proline and antioxidant enzymes had been reported by Morot-Gaudry *et al.* (2001). Since proline can act as a scavenger or reducer of superoxide production, it is normal to find a significant positive correlation seems between proline and antioxidant enzymes. Bian *et al*. (2009) concluded that proline content increased in creeping bentgrass (*Agrostis stolonifera* L.) under drought stress.

Lipid peroxidation has been associated with damages provoked by some environmental stresses (Jaleel *et al*., 2008). The rise in MDA content under different stress conditions showed that drought could induce membrane lipid peroxidation by means of ROS (Moussa and Abdel-Aziz, 2008). In this condition, low concentration of MDA has been associated with drought-tolerant plants (DaCosta and Huang, 2007; Hassan *et al*., 2015). In our experiment, *F. arundinacea*, with low concentration of MDA in different levels of drought stress treatment, showed more tolerance to drought stress. According to other studies (e.g. Sharma and Dubey, 2005; Pan *et al*., 2006; Zlatev *et al*., 2006), drought stress increased MDA concentrations in leaves. Positive correlation between MDA and CAT indicates that the antioxidant enzymes act as a first defensive line to counter oxidative stress in plants. Oxidative stress occurs when the antioxidant defense decreases or the formation of free oxygen radicals increases (Matés *et al*., 1999).

To cope with detrimental effects of oxidative stresses under extremely adverse conditions, plants have developed an antioxidant defense system which includes the antioxidant enzymes SOD, APX, POD, and CAT. The levels of antioxidant enzymes are higher in tolerant cultivars than sensitive ones under various environmental stresses (Wang *et al*., 2009). Accordingly, we observed higher SOD activity in *F. arundinacea* at 50% FC level compared to other species, which suggest that this drought-tolerant grass possess a better reactive oxygen scavenging ability. However, in the 25% FC this trend changed considerably and the amount of SOD decreased. Previous studies have shown that responses of SOD activity to water deficit have varied with drought severity, duration, and species. Zhang and Kirkham (1996) suggested that water stress did not influence SOD activity under moderate stress in sorghum [*Sorghum bicolor* (L.) Moench]. In wheat (*Triticum aestivum* L.), SOD activity increased or remained unchanged in the early phase of drought and then decreased with further water stress (Zhang *et al*., 1995). This reduction in SOD activity could be associated with reduced synthesis or enhanced degradation of the enzyme. SOD converts the toxic $O_2^-$ radicals to $H_2O_2$ which must be scavenged to $O_2$ and water by the antioxidant enzyme such as CAT, POD, and APX (Ozkur *et al*., 2009).

Increase in POD activity under various stress conditions has been linked with protection from oxidative damage, lignification, and cross-linking of the cell wall to cope with such adverse conditions (Moussa and Abdel-Aziz, 2008). In our study, drought-induced POD activity in shoot of four species. However, activity of this enzyme in *F. arundinacea* under both control and stress conditions was higher than others species, suggesting a better antioxidant system for removing $H_2O_2$ by POD. In Kentucky bluegrasss this enzyme activity initially increased and then decreased with development of drought (Fu and Huang, 2001), similar to changes of this enzyme's activity founding *P. pratensis* in the response to drought stress. The activity of POD increased during initial periods of drought stress and decreased as stress intensity increased. The similar trend of POD activity during water stress has been reported in *Poa pratensis*

(Farkhondeh *et al*., 2012).

Catalase is another antioxidant enzyme that scavenges $H_2O_2$ in cells (Shao *et al*., 2007). High activity of CAT indicated drought tolerance in Chrismas tree (Sharma and Dubey, 2005) and wheat (Simova-Stoilova *et al*., 2010). Fu and Huang (2001) reported that CAT activity was decreased in all studied *Poa pratensis* and *F. arundinacea* cultivars under water stress condition. Accordingly, they concluded that the reduction of CAT activity was supposedly due to inhibition of enzyme synthesis, change in the inhibition of enzyme precursor, or protein degradation under drought stress. In the present research, CAT activity in *F. arundinacea* was higher than other species in different levels of water stress treatment. The high activity of CAT in *F. arundinacea* during drought stresses demonstrated more ability of these species to decomposition of $H_2O_2$ in stress condition. CAT showed the positive correlation with POD. The correlation between antioxidant enzymes reported in mature leaves of Arabidopsis under drought stress (Jung, 2004). Mercado *et al.* (2004) reported the significant positive correlation between POD activity and RWC content which are in agreement with our results.

According to the results presented here, it can be concluded that among the evaluated species, tall fescue has good tolerance to drought stress. The higher tolerance induced by tall fescue was associated with more efficient osmotic adjustment, which was reflected by the smaller reduction in RWC and cell membrane stability. The identification of these indices is valuable because they can be rapidly assessed and can be used in the early stages of breeding turfgrasses for screening drought tolerant species; however, for efficient selection and better understanding of the mechanisms involved in drought tolerance, biochemical and molecular markers must also be included.

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