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Morphophysiological and biochemical changes in tall fescue (*Festuca arundinacea* Schreb.) under combined salinity and deficit irrigation stresses

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Abstract

Water salinity and drought are the major abiotic stresses limiting turf grass growth. On the other hand, shortage of water resources and salinity of water and soil in the arid and semi-arid zones such as Iran, are the restricting factors in developing lawn turf grasses. An experiment was conducted to evaluate the combined effects of water salinity and deficit irrigation on tall fescue (*Festuca arundinacea* Schreb.). This study was conducted under outdoor conditions in a completely randomized design with factorial arrangements. Treatments included four water salinity levels (0.5, 3, 6 and 9 $dS.m^{-1}$) and three deficit irrigation regimes (50%, 75% and 100% FC) with five replicates under outdoor conditions. Results indicated a rise in the ion leakage, and soluble sugar and proline concentration and a decrease in visual quality, shoot length, leaf area and fresh and dry weights of shoot, leaf relative water content (RWC), leaf chlorophyll content and photosynthetic rate and starch content with an increase in the levels of both stresses. Antioxidant enzymes, superoxide dismutase (SOD, EC 1.15.1.1), and catalase (CAT, EC 1.11.1.6) showed higher activity under moderate drought or water salinity conditions; however, this parameter decreased at higher levels of these salinity stresses. Practically, based on the results of the present study, tall fescue could be grown under moderate levels of the combined stresses of water shortage and salinity without considerable damage to the plant at the physiological and/or biochemical level. This is the first report on applying the combined stresses of water and salinity on an important agricultural crop.

Keywords: Antioxidant; Tall fescue; Deficit irrigation; Turf grass; Water salinity

1. Introduction

Deficiency of the accessible water resources and their high salinity level rank high among the most crucial growth limiting factors for plant species in some arid and semi-arid regions of the world (Levitt, 1972). Awareness of the relative water salinity and drought tolerance among the turf species/cultivars is important for selecting turf grasses that persist during drought and salinity stress. Salt stress imposes a major environmental threat to agriculture and its adverse impacts are creating even more serious problems in regions where saline water is used for irrigation. Therefore, the efforts to increase the salt tolerance of the crop plants are highly significant to produce sustainable agriculture on marginal lands and can potentially improve the overall crop yield. Turf grasses are the most important groundcover-like plants in the world. Tall fescue, considered to be drought resistant, is a cool-season grass, widely used in home lawns and commercial landscapes. However, several studies have shown that the cultivars vary in the degree of drought resistance (Huang and Fry, 1998). The popularity of tall fescue as a turf grass has increased with the introduction of improved cultivars. This is partly due to its good drought resistance (Beard, 1989), as water resources are

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becoming increasingly limited. Tanji and Kielen (2002) collected some data regarding the salinity tolerance of important crops. Based on this data, tall fescue was found to have a threshold value of 3.9 $dS.m^{-1}$ with a 5.3% slope. These values were estimated from the experiments conducted by Bower, Ogata and Tucker, 1970 (FAO 61, irrigation and drainage department). Bower et al., (1970) concluded that tall fescue grass showed 50% yield loss when the average root zone salinity reached to 16.2 $dS.m^{-1}$.

Drought or water salinity tolerance, especially in grasses, depends on their morpho-physiological features (Bahrani *et al*., 2010). Although the general effects of drought and water salinity stresses on plant growth and development have been studied, their effect at the physiological and biochemical levels is yet to be well understood. Water salinity stress may result in the destruction of osmotic balance and relationship as well as ion toxicity (Turkan and Demiral, 2009). The plants that can tolerate saline conditions, may alleviate the adverse effect of the stresses (such as water salinity and drought) by synthesizing the metabolism compatible solutes such as proline, which reduces the rate of turgor changes within the cells, and that in turn will prevent protein (enzymes) degradation (Kamal Uddin *et al*., 2012). Therefore, a limited alteration occurs in the growth rate compared with the sensitive plants (Harivandi *et al*., 1988). On the other hand, some abiotic stresses (such as water salinity and drought) act via the overproduction of the reactive oxygen species (ROS) (Gomez *et al*., 2004), which results in oxidative stress (Mane *et al*., 2011). ROS accumulation may cause the destruction of cell structures, as well as the molecules such as proteins and nucleic acids (Miller, 2010); ROS are also harmful to the cell membranes (Hu *et al*., 2012). To scavenge the ROS, plants have an intrinsic, well-developed, complex, antioxidant defense system, including enzymatic and non-enzymatic antioxidant processes. The antioxidant enzymes include SOD, CAT, POD and APX (Apel and Hirt, 2004). Hu *et al*., (2012) reported that the activity of antioxidant enzymes such as APX, POD, CAT and SOD increased in some *Lolium perenne* L. cultivars after four days' treatment with 250 mM NaCl. DaCosta and Huang (2007) stated that severe drought stress condition resulted in the reduction of the antioxidant enzymes in the *Agrostis* spp.

Knowledge of the relative tolerance of different turf grass species to both heat and drought stresses is important in selecting the turf grasses suitable for hot and dry environments. Recent studies on the effects of water salinity or drought on turf grasses have focused on the growth response mechanisms (Marcum, 1999) and this research was designed to investigate the effects of the combined stresses of drought and water salinity on tall fescue and to study their biochemical and morphophysiological responses under these conditions.

2. Material and methods

2.1. Plant materials and experimental conditions

The experiment was conducted under outdoor conditions in the Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran (52°32_E and 29°36_N, 1810 m asl). Loamy soil was collected from the top 20 cm layer of the Department's research field, and some of the physico-chemical properties of this soil are listed in Table 1. The soil was air-dried and crushed to pass through a 10-mm sieve. Eighty plastic pots, 35 cm in height, 25 cm in diameter, and with a 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. Tall fescue seeds (*Festuca arundinacea* Schreb.) 'Starlett' were weighed and cultured at the rate of 1.0 $g.pot^{-1}$ (about 150 seedlings emerged per pot). Irrigation was performed daily before seed germination and after turf establishment with tap water (EC 0.5 $dS.m^{-1}$). During this period, the soil water content was maintained at field capacity level (the FC and PWP measured were 29% and 19%, respectively) by adding tap water. Deficit irrigation treatments included three irrigation levels W_0, $W_1$and W_2i.e., with 50%, 75% and 100% FC.

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

Physical properties		Chemical properties	
Sand (%)	33	Ca (meq l^{-1})	3.60
Silt (%)	47	Cl (meq l^{-1})	1.90
Clay (%)	20	Na (meql^{-1})	1.40
FC(cm^3 cm^{-3})	0.29	SO_4(meq l^{-1})	1.90
PWP(cm^3 cm^{-3})	0.19	Mg (meq l^{-1})	1.70
		HCO_3(meq l^{-1})	2.40
		EC (dSm^{-1})	1.16

The salinity treatments of the irrigation water were 0.5 (tap water used as control), 3, 6 and 9 $dS.m^{-1}$ (S_0, S_1, S_2, and S_3) obtained by the addition of the same amount of NaCl and $CaCl_2$to the tap water. This study was conducted in a completely randomized design with factorial arrangements. Each treatment included five replications. The water quantity for each irrigation time was determined by weighing the pots. Thirty percent more water was applied as leaching requirement to control salt accumulation in the pots. The chemical analysis of the saline irrigation water is shown in Table 2. The day and night average temperatures were between 30±3 and 21±3°C, respectively. The relative humidity was about 55±5%. The experiment was repeated in two consecutive years (2011 and 2012). Treatments lasted about eight weeks. Data were analyzed using the SAS software (ver 9.1.3) and the means were compared using the least significant difference (LSD) test at $P<0.05$.

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

EC (dSm^{-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. Visual quality and growth parameters

Turf quality was visually rated based on leaf color and the degree of leaf wilting based on a scale of zero (desiccated, brown leaves) to nine (fully turgid, green leaves). Score 9 and 0 represented the highest and lowest quality, respectively. Growth parameters including, shoot length, leaf area, and fresh and dry weights were measured. Dry weights were measured when the samples were oven dried (Memmert 854) at 60°C for 48 h.

2.3. Relative Water Content (RWC) and Electrolyte Leakage (EL)

Relative water content estimation was performed by incubating 0.2 g of a fresh leaf sample in 50 mL of distilled water for 4 h. Next, the turgid weights of the leaf samples were measured. The leaf samples were then oven dried to calculate the dry weight at 70°C for 48 h. The RWC was then calculated using the following equation:

$$\text{RWC}\ \% = \frac{\text{f.w.}-\text{d.w.}}{\text{t.w.}-\text{d.w.}} \times 100$$

where f.w. is fresh-, d.w. is dry- and t.w. is turgid-weight (Sairam *et al*., 2002). Electrolyte leakage in the leaves was measured as described by Saadalla *et al*., (1990), using an electrical conductivity meter (Metrohm 644, Swiss) and calculated by applying the following formula:

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

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

2.4. Chlorophyll content and photosynthetic rate

The chlorophyll content was measured according to the method of Saini *et al*., (2001) using the following formula:

$$\text{mg Chl/g f.w.} = [[(20.2(\text{OD 645 nm}) + (8.02(\text{OD 663 nm})] \times \text{V})/(\text{f.w.} \times 1000)$$

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

2.5. Proline content and reducing sugars

The proline content was determined based on the method described by Bates *et al*., (1973) using a spectrophotometer (Biochrome, UK) at 520 nm wavelength, and appropriate proline standards were included in the calculation of its content in the samples tested. The phenol–sulfuric acid method was used to determine the content of the reducing sugars (Dubois *et al*., 1956). Shoot and root samples were oven dried at 60°C for 48 h, and ground to a powder in an electric mill. The powder samples (0.2 g) were then weighed and centrifuged with 80% ethanol. The final volume was brought up to 25 mL using 80% ethanol. Then, 1 mL of extract was poured into the test tubes and 1 mL of 5% phenol was added. Next, 5 mL of concentrated sulfuric acid was added to tubes and immediately stirred. The light absorption was measured in the wavelength of 490 nm using a spectrophotometer. Glucose solution was used at different concentrations to draw the standard curve.

2.6. Starch content

The starch content in the shoots was measured using the method described by McCready *et al*., (1950). Antron (0.2 g) was mixed in concentrated sulfuric acid and maintained at a temperature of 0◦C. The supernatant in the centrifuge tubes was dissolved in 5 ml distilled water; then, 6.5 ml of perchloric acid 52% was added and stirred for 15 min. Finally, 20 ml of distilled water was added and centrifuged. After removing the upper phase, this step was repeated and then the tubes were placed for 30 min at 0°C temperature. The extract volume was raised to 100 ml. Two-and-a-half ml of the extract was transferred to the tubes and 10 ml of Antron solution was added and the tubes were maintained at 100°C temperature for 7.5 min. Thereafter, the samples were transferred to an ice bath, using a spectrophotometer, the light absorption at wavelength of 630 nm was measured. The starch concentration was calculated using the standard curve of glucose and multiplying it by 0.92.

2.7. Measurement of antioxidant enzyme activity

2.7.1. Enzyme extraction

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

2.7.2. Superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) activity

To extract the antioxidant enzymes, fresh leaf samples were ground to a fine powder with liquid nitrogen, and then homogenized in ice-cold sodium phosphate buffer (50 mM, pH 7.8). The catalase activity was determined according to the method adopted by Dhindsa (1981) and the SOD activity was determined according to the procedure of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of Nitro-Blue Tetrazolium (NBT) in the presence of riboflavin in light.

3. Results

Data collected from the two years of experimentation were not significantly different and, therefore, their mean was used for further analysis. Table 3 indicates the effect of the different levels of deficit irrigation and water salinity and their interaction on the visual quality, shoot length, leaf area, fresh and dry weights. Turf visual quality showed considerable difference when subjected to drought and water salinity stresses. The best and worst visual quality (score 9 and 0 represent the highest and lowest quality, respectively) was observed in the FC, 0.5 $dS.m^{-1}$ and 50%FC, 9 $dS.m^{-1}$ treatments, respectively (Table 3). The saline exposed and irrigated regimes each only showed a significant effect on the shoot length. The highest and lowest shoot lengths were obtained in the FC and 50%FC irrigation treatments, respectively (Table 3). In fact, the highest and lowest shoot lengths were obtained from the 0.5 and 9 $dS.m^{-1}$salinity treatments, respectively. By reducing the irrigation from FC to 50%FC a significant decrease was observed in the fresh and dry weights of the clippings. The interaction between irrigation and salinity resulted in the highest and lowest fresh and dry weights of the clippings' in 0.5$dS.m^{-1}$, FC and 9 $dS.m^{-1}$, and 50%FC treatments. The highest and lowest dry matter was observed in the control and irrigation water of 9 $dS.m^{-1}$, with 50% deficit, respectively (Table 3). The leaf area significantly decreased when the irrigation changed from FC to 50%FC and salinity changed from 0.5 to 9 $dS.m^{-1}$. The highest and lowest leaf areas were observed in the 0.5 $dS.m^{-1}$, FC and 9 $dS.m^{-1}$, and the 50%FC treatments, respectively (Table 3).

Table 4 indicates the effect of the different levels of deficit irrigation and water salinity and their interaction on the leaf chlorophyll content, photosynthetic rate, soluble sugars content, proline concentration and starch content. Data analysis revealed that the drought and salinity treatments exerted significant effects on the shoot starch content. The highest and lowest starch contents were observed in the control and salinity of 9 $dS.m^{-1}$ with 50%FC, respectively (Table 4). A decrease in the content of the reducing sugars simultaneously increased the root starch content at all temperatures and photoperiod regimes tested (Table 4). With the augmentation of the irrigation or salinity levels, the chlorophyll content, photosynthetic rate, starch content, leaf RWC and growth parameters for example, the shoot length,

leaf area, fresh and dry weights, decreased significantly.

Overall, the lowest values for these parameters were obtained from the plants irrigated with 50%FC. This was also true for the plants irrigated with saline water (9dS.m^{-1}). The interaction of water salinity and drought stresses exerted a significant influence on these characteristics, as 85% reduction in the chlorophyll content was observed in plants irrigated with 50%FC, along with the highest saline water. The photosynthetic rate and leaf RWC of the plants irrigated with saline water (9dS.m^{-1}) at 50%FC were the lowest. In the plants treated with drought or water salinity stress and their interaction, the content of the total sugars, proline concentration and EL increased significantly (Tables 4 and 5). A 16% increase in the content of the soluble sugars was observed in plants irrigated at 50%FC, when compared with those irrigated at FC. The proline concentration in the plants treated with 9dS.m^{-1}water salinity was two times higher than that of the control plants. The highest EL percentage was observed in the plants irrigated at 50%FC and treated with the highest level of salinity (Tables 4 and 5). Table 5 indicates the influence of water salinity and deficit irrigation and their interaction on the activity of the free-radical scavenging enzymes. Generally, at moderate water salinity stress levels an increase in the activity of CAT and SOD was detected, although more severe salinity levels reduced the activity of these enzymes; however, an increase in the drought stress raised the activity of these enzymes. The maximum activity of CAT was detected in the plants irrigated at six-day intervals and treated with 6 dS.m^{-1} saline water. The SOD activity was at its maximum when the plants were irrigated with 0.5dS.m^{-1} saline water and irrigated at 50%FC.

Table 3.Visual quality, shoot length, leaf area and clippings fresh and dry weightin tall fescue under different levels of salinity, irrigation intervals and their interactions

Salinity levels (dSm^{-1})	Irrigation intervals (day)			
	2(W_0)	4(W_1)	6(W_2)	Mean
		Visual quality		
ECw 0.5	8.7±0.5a	8.0±0.0b	3.7±0.5d	6.8A
ECw 3	8.7±0.5a	5.5±0.5c	2.5±0.6e	5.5B
ECw 6	5.5±0.5c	3.0±0.0e	1.0±0.0gf	3.1C
ECw 9	1.5±0.5f	1.0±0.0fg	0.5±0.6g	1.0D
Mean	6.1A	4.3B	1.9C	
		Shoot length (cm)		
ECw 0.5	11.4±1.2a	11.0±0.7a	8.9±0.3cd	10.4A
ECw 3	11.1±0.6a	9.0±0.4bc	8.6±0.5cd	9.6B
ECw 6	9.9±0.5b	8.7±0.5cd	6.9±0.6e	8.5C
ECw 9	9.1±0.8bc	8.0±0.6d	6.0±0.5f	7.7D
Mean	10.3A	9.2B	7.6C	
		Leaf area (cm^2/pot)		
ECw 0.5	12.2±1.4a	11.0±0.4ab	7.9±0.3de	10.4A
ECw 3	9.9±1.2bc	9.2±1.5cd	7.3±1.7e	8.8B
ECw 6	8.8±1.7cde	8.0±0.4de	5.4±1.5f	7.4C
ECw 9	5.0±1.0f	5.4±0.7f	4.0±0.8f	4.8D
Mean	9.0A	8.4A	6.2B	
		Clippings fresh weight (g/pot)		
ECw 0.5	20.8±2.0a	16.8±2.0b	12.4±0.6cde	16.4A
ECw 3	16.5±0.7b	13.4±2.4cd	10.7±1.4ef	13.5B
ECw 6	13.9±2.3c	11.4±1.4de	10.3±0.4ef	11.8C
ECw 9	10.3±1.3ef	8.8±1.3fg	7.8±0.7g	9.0D
Mean	15.5A	12.6B	10.3C	
		Clippings dry weight (g/pot)		
ECw 0.5	13.1±1.3a	10.5±2.1bc	8.1±0.9ef	10.6A
ECw 3	11.6±0.3ab	9.2±2.0cde	7.8±1.0ef	9.5B
ECw 6	10.0±1.2bcd	8.6±1.0def	7.6±0.6ef	8.7B
ECw 9	7.6±1.0ef	6.9±1.2fg	5.5±0.7g	6.7C
Mean	10.6A	8.8B	7.2C	

*In each variable, data followed by the same letters±S.D. (small letters for interactions and capital letters for means) are not significantly different at 5% level of probability using LSD test

Table 4. Chlorophyll content, photosynthetic rate, proline content, reducing sugars of shoot and starch of shoot in tall fescue under different levels of salinity, irrigation intervals and their interactions

Salinity levels (dSm^{-1})	Irrigation intervals (day)			
	2(W_0)	4(W_1)	6(W_2)	Mean
Chlorophyll content (mg g^{-1} f.w.)				
ECw 0.5	0.76±0.1a	0.51±0.3bc	0.25±0.1def	0.5A
ECw 3	0.70±0.3ab	0.49±0.2bcd	0.21±0.1ef	0.4AB
ECw 6	0.52±0.3abc	0.36±0.0c-f	0.12±0.0f	0.3AB
ECw 9	0.41±0.1cde	0.29±0.3c-f	0.12±0.0f	0.2C
Mean	0.6A	0.4B	0.1C	
Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})				
ECw 0.5	8.9±0.7a	8.5±1.2bc	5.0±0.8de	7.5A
ECw 3	7.7±0.3ab	7.1±0.3c	4.5±0.4efg	6.4B
ECw 6	5.8±0.7d	4.9±0.9def	4.0±0.4fgh	4.9C
ECw 9	3.6±0.6ghi	3.4±0.6hi	2.9±0.4i	3.3D
Mean	6.5A	6.0B	4.1C	
Proline content (µmol g^{-1} f.w.)				
ECw 0.5	7.1±2.5e	10.4±1.0de	23.4±7.9bc	13.6C
ECw 3	12.2±2.3ed	18.2±6.9cd	33.6±4.9a	21.3B
ECw 6	21.6±2.0bc	26.6±6.9ab	34.4±6.4a	22.6A
ECw 9	22.4±6.5bc	28.1±1.7ab	27.0±1.9ab	25.9AB
Mean	15.8C	20.8B	29.6A	
Reducing sugars ofshoot (mg g^{-1} d.w.)				
ECw 0.5	103.5±18.4c	137.3±22.6abc	171.3±14.7ab	137.4AB
ECw 3	130.0±11.4abc	146.9±29.3abc	185.4±30.3a	154.1A
ECw 6	160.4±19.1abc	161.4±17.2abc	141.0±15.6abc	154.2A
ECw 9	110.1±9.6bc	111.1±16.0bc	108.2±12.9c	109.8B
Mean	126.0A	139.2A	151.5A	
Starch content of shoot (mg g^{-1} d.w.)				
ECw 0.5	122.9±16.8a	115.8±16.6a	82.6±13.0abc	107.1A
ECw 3	106.7±13.6ab	117.6±12.5a	68.8±15.4abc	97.7AB
ECw 6	98.8±17.6abc	95.1±30.4 abc	41.1±16.6bc	78.3AB
ECw 9	63.0±24.5abc	84.7±30.8abc	34.8±24.9c	60.8B
Mean	97.8A	103.3A	56.8B	

*In each variable, data followed by the same letters±S.D. (small letters for interactions and capital letters for means) are not significantly different at 5% level of probability using LSD test.

4. Discussion

The loss of appearance quality was due to the intense drought and toxicity of caused by the sodium and chloride ions. Kramer and Boyer (1985) demonstrated that loss of appearance quality, which occurred under osmotic stress, was due to plant tissue and/or cell water loss, deficient uptake of mineral elements as well as stomatal closure, reduced CO_2 intake and plant weakness. One of the strategies plants employ against stresses is growth reduction and induction of the reproductive phase and completion of the life cycle. Turf species are no exception. When they are exposed to water salinity and drought, their shoot length reduces (Pessarakli, 2007). The reduction in the fresh and dry weights of the clippings occurs due to the restricted water uptake, photosynthesis and lower plant biomass. Atif *et al*., (2010) reported reduced fresh and dry weights of the shoots in some Bermuda grass cultivars. Our results were in accordance with their findings. Also, the limitation of fresh and dry weights of the clippings in our experiment was in accordance with the results of Akram *et al*., (2006). In the present investigation, correlated with the increase in drought and salinity intensity, the leaf area was reduced. Reduction in cell division due to an increase in the ABA (Alves and Setter, 2000) and lack of assimilate supply for leaf growth (DeSouza, 1997) are the most important factors inducing the reduction in the leaf area index, under water salinity and/or drought stress. Besides, Pessarakli (2007) hypothesized that one of the strategies plants employ for drought and/ or water salinity tolerance is leaf area reduction through quick senescence of some of plant leaves, which reduces the plant leaf area and their abscission. Following leaf area reduction, the light absorption decreases and the photosynthesis potential of the plant diminishes, and obviously with the restriction of assimilation under water deficit conditions, the growth and yield of the plants will decrease (Hsia, 1973).

Table 5. Catalase, Superoxide dismutase, relative water contents and electrolyte leakage intall fescueunder different levels of salinity, irrigation intervals and their interactions

Salinity levels (dSm^{-1})	Irrigation intervals (day)			
	2(W_0)	4(W_1)	6(W_2)	Mean
Catalase (CAT) (U mg^{-1} f.w.)				
ECw 0.5	25.2±9.1d	30.7±6.8cd	61.0±12.4a	39.0B
ECw 3	32.9±15.2bcd	58.2±10.9a	57.2±9.2a	49.4A
ECw 6	48.7±8.1ab	44.4±16.8abc	31.9±8.0bcd	41.6AB
ECw 9	29.3±4.5cd	31.1±7.6cd	23.5±5.1d	28.0C
Mean	34.0B	41.1AB	43.4A	
Superoxide dismutase (SOD) (U mg^{-1} f.w.)				
ECw 0.5	113.5±22.8c	155.0±19.5bc	294.0±33.5a	187.5AB
ECw 3	131.5±14.1c	285.5±33.8a	193.0±16.8b	203.3A
ECw 6	192.5±14.6b	148.5±12.9bc	129.7±14.7c	156.9B
ECw 9	121.5±17.9c	131.5±14.2c	110.5±16.9c	121.1C
Mean	139.7B	180.1A	181.8A	
Relative water content (RWC) (%)				
ECw 0.5	83.5±4.7a	77.2±4.6ab	49.0±6.9f	69.9A
ECw 3	75.0±3.8ab	70.7±8.3bc	48.7±5.7f	64.8A
ECw 6	63.3±11.9cd	62.4±14.4cde	37.1±2.7g	53.9B
ECw 9	53.6±11.4def	51.7±8.6ef	36.1±3.8g	47.4B
Mean	68.8A	65.4A	42.7B	
Electrolyte leakage (EL) (%)				
ECw 0.5	15.2±4.9e	22.8±13.4e	58.0±16.9ab	32.0B
ECw 3	17.2±7.1e	28.0±15.0de	61.5±8.5ab	35.6B
ECw 6	33.7±11.3cde	43.7±16.6bcd	69.1±4.2a	48.8A
ECw 9	46.8±9.9bc	57.2±16.8ab	70.6±7.8a	58.2A
Mean	28.2C	37.9B	64.8A	

*In each variable, data followed by the same letters±S.D. (small letters for interactions and capital letters for means) are not significantly different at 5% level of probability using LSD test

Drought and water salinity not only reduce growth and development of the plants, they also induce changes in some metabolic pathways (Wu and Garg, 2003). In our experiment, the leaf chlorophyll content decreased when the adverse effect of water salinity or drought and their interactions were imposed on the plants. Our findings were in accordance with those of the previous studies (Mane *et al*., 2011). A similar report on the reduction in the chlorophyll content of *Paspalum vaginatum* Sw. was found when the plants were grown under saline water (50 $dS.m^{-1}$) (Lee *et al*., 2008). Marcum *et al*., (1999) hypothesized that chlorophyll loss under saline conditions could be due to oxidative stress and overproduction and accumulation of ROS. On the other hand, it might be related to Mg^{2+} deficiency. The photosynthetic responses of the plants to water salinity or drought stresses are highly complex. The intensity and duration of stresses can alter this response (Chaves *et al*., 2009).

As stated in our results, the photosynthetic rate decreased with severity of both the stresses. One reason could be attributed to the chlorophyll degradation which occurred under these conditions. Another reason has been hypothesized by Flowers *et al*., (1997), as salinity stress, which may alter key metabolic processes in a way that results in reducing the photosynthetic rate; water deficiency or salinity can cause the synthesis and accumulation of ABA, which leads to stomatal closure and restriction of the available CO_2 for photosynthesis (Dubey, 2005). Pedrol *et al*., (2000) and Zhu *et al*., (2002) reported a reduction in the RWC during saline or water deficit conditions, and our findings are in accordance with theirs. This reduction might be one of the reasons responsible for the lesser growth in the plants grown under stress conditions. Stresses lead to an increase in the EL percentage. This could be due to the higher membrane permeability or loss of membrane integrity. Our results were in accordance with the findings of Sairam (2002) and Hu *et al*., (2012). In our experiment, the amount of soluble sugars, proline concentration and the activity of the free radical scavenging enzymes increased significantly under stress conditions. Sugars and proline are synthesized by the plants under stress conditions in order to alleviate the adverse impact of stress on the osmotic balance of the plants (Ingram and Bartels, 1996; Ashraf and Foolad, 2007). Plants respond to water salinity and drought stresses by synthesizing compatible solutes like proline. These responses will eventually lead to a restoration of cellular

homeostasis, detoxification and, therefore, survival under stress (Miller *et al*., 2010). Hsiao (1973) stated that the accumulation of sugars under conditions of water stress may stabilize the proteins and membranes. Our findings are in accordance with the results of Ginzberg *et al*., (1998), who in their study, reported a higher amount of soluble sugars in alfalfa (*Medicago sativa* L.) grown under drought conditions, and hypothesized that the degradation of the insoluble carbohydrates might result in the increase in the levels of the total sugars.

Generally, with the increase in the intensity of the salinity stress, the starch content was observed to decrease. The content of soluble sugars may increase under water deficiency and salinity stress conditions because of the degradation of the insoluble carbohydrates and their conversion to soluble sugars (Hsiao, 1973). However, some authors reported that the starch degradation may increase the amount of the monosaccharides (During, 1992). The augmentation of amylase activity under water deficit stress induced by water salinity and/or drought causes starch degradation and the breakdown of this macromolecule into smaller units (Movahhedi Dehnavi, 2004). The increase in free proline is a general method adopted by plants to reduce the severity of most abiotic stresses, although some other amino acids also get accumulated, although not significantly (Gzik, 1996). Our results were in accordance with the findings of Akram *et al*., (2006) and Marcum et al., (1992). Under such conditions as drought or water salinity stress, due to the disturbance of the electron-transfer chains, elevated levels of ROS would accumulate, which could lead to injuries to the bio-membranes and molecules such as proteins (Foyer *et al*., 1994). One of the plant barriers against this situation is the synthesis and accumulation of free radical scavenging enzymes, such as SOD and CAT. The active involvement of these enzymes is related, at least in part, to salt-induced oxidative stress tolerance in Bermuda grass (Mane *et al.*, 2011). SOD is regarded as a key enzyme that maintains the normal physiological processes and enables the plant to cope with oxidative stress by rapidly converting the O^{2-} to O_2 and H_2O_2.

The responses of the antioxidant enzymes to stresses depend on the intensity and duration of the stress and the plant species of the perennial grasses (Bian and Jiang, 2009). The H_2O_2 detoxification is mediated by CAT, mostly localized in the peroxisomes. Several enzymes regulate the H_2O_2 intracellular levels in plants. Catalase, one of these important enzymes, scavenges the H_2O_2 by directly breaking it down to form H_2O and O_2 in the peroxisomes and glyoxysomes (Miller, 2010). Studies indicate that any change in the CAT activity may depend on the plant species, the developmental and metabolic states of the plant, as well as on the duration and intensity of the stress (Chaparzadeh *et al*., 2004). Bian and Jiang (2009) observed an increase in the CAT levels in Kentucky bluegrass (*Poa pratensis* L.) under drought stress conditions. Hu *et al*., (2012) identified similar activity with an increase in the CAT levels. Severe drought or water salinity stress levels may decrease the activity of these enzymes (DaCosta and Huang, 2007), a result which occurred in our study, too. It can be stated that a reduction in the production of antioxidant enzymes under severe stress would negatively affect the other characteristics such as chlorophyll content, photosynthetic rate and content of soluble sugars that will result in lowering the efficiency of the cells under such conditions.

5. Conclusion

Our findings showed that this species is relatively tolerant to the adverse effects of drought and /or water salinity by increasing of the synthesis of the osmoticums such as proline, sugars or the elevation of the activity of the antioxidant enzymes. To the best of our knowledge, there is no other report that has studied the combined effect of drought and water salinity stresses in tall fescue. Practically, based on the results of the present study tall fescue can be grown under moderate combined stresses of drought and water salinity without considerable damage to the plant at a morphophysiological and/or biochemical level. Therefore, tall fescue might be recommended for culture in the semi-arid areas with limited water resources. Further studies are warranted to evaluate the effects of these stresses on the characteristics of tall fescue at the molecular and ultrastructural levels.

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