

Morpho-physiological alteration in common bermudagrass [*Cynodon dactylon* (L.) Pers.] subjected to limited irrigation and light condition

N. Adamipour (*), H. Salehi, M. Khosh-khui

Department of Horticulture Science, College of Agriculture, University of Shiraz, Shiraz, Iran.

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Abstract: Bermudagrass (*Cynodon* spp.) is the most popular warm-season turfgrass used in warm climatic regions of the world due to its recuperative ability, high traffic tolerance, heat tolerance, and relative drought and salt tolerance. However, shade is a microenvironment in which bermudagrass performs poorly. In order to evaluate the interaction of photoperiod and irrigation on [*Cynodon dactylon* (L.) Pers. California Origin], a greenhouse experiment was conducted at the Research Greenhouse of the Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran. The experiment was conducted with four field capacity regimes (25%, 50%, 75% and 100%) and three light durations (8, 12 and 16 h) in a completely randomized design factorial arrangements with four replications. Results showed that decreasing field capacity and photoperiod decreased fresh and dry weights shoot and root, chlorophyll and starch contents and superoxide dismutase, catalase and ascorbate peroxidase activities. Decreasing the field capacity and light duration increased proline content. Reducing sugars and peroxidase enzyme in leaves increased with decreasing field capacity. Shoot height and leaf area increased by shortening the photoperiod. In overall, results showed that, the increase in irrigation alleviates the destructive effects of reduced day lengths and vice versa. Further studies are needed to clarify more the interaction between irrigation and light treatments at structural and ultrastructural levels, in common bermudagrass.

1. Introduction

Bermudagrass is a warm-season, C4, perennial grass. It has short, grey-green blades with rough edges, stems of 1 to 30 cm in length and a deep root system that can penetrate 2 m into the ground; however, most of the root mass is less than 60 cm deep (Xu *et al.*, 2011). Among the many advantages of turfgrass areas are erosion and dust control, aquifer recharge and protection from pollutants, heat reduction in urban environments, reduction of noise and pollution, and providing human health and aesthetic benefits (Stier *et al.*, 2013). Water scarcity is an increasing challenge to the turfgrass industry and may result in irrigation restrictions being imposed without regard for damage to turfgrass (Beard and Kenna, 2008). For turf managers, thriving in an indus-

try where turf quality is of utmost importance is difficult when water is limiting. Therefore, researches investigating turfgrass resistance to drought stress have become increasingly important (Fry and Huang, 2004). Fu and Huang (2001) investigated the effects of drought stress on two cool-season turfgrasses and found that moderate drought stress had not effects on morphological and physiological characteristics, however in intensive drought stress, antioxidant enzyme activities, chlorophyll content, relative water content and shoot dry weight were decreased. In addition to limited amounts of water, turfgrasses are impacted by low-light environments. Shade is more problematic for warm-season turfgrasses to maintain quality given their higher light saturation point compared to cool-season turfgrasses (Fry and Huang, 2004). Turfgrasses perform poorly in reduced light environments due to high traffic rate, daily mowing, and reduced photosynthesis. In shade, increased disease presence adversely affects cool-season turfgrass development, while morphological limitations, such

(*) Corresponding author: adamipournader@yahoo.com

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as reduced lateral stem growth, inhibits warm-season turfgrass development (Beard, 1972). Variations of shade responses among species and cultivars (Jiang *et al.*, 2004; Trenholm and Nagata, 2005; Sladek *et al.*, 2009) make it possible to select turfgrasses with superior shade tolerance. Identifying morphological characteristics that are associated with superior shade performance based on genetic variation would add value to germplasm screening for shade tolerant species and cultivars. Esmaili and Salehi (2012) noted in bermudagrass that were treated with short photoperiod duration, verdure fresh and dry weight, shoot height, tiller density, leaf area and chlorophyll and relative water contents were decreased, however electrolyte leakage and proline content were increased. Although bermudagrass, the most widely grown C4 turfgrass on an international basis (Shearman, 2006), has been extensively studied, many challenges and questions still remain when light is a limiting growth factor. The main objective of the present study was to investigate the effects of both irrigation interval and light duration on growth and quality of common bermudagrass.

2. Materials and Methods

Plant material and experimental conditions

This experiment was conducted at the Research Greenhouse of the Department of Horticultural Sciences, College of Agriculture, Shiraz University, Shiraz, Iran (52°32'E and 29°36'N, 1810 m asl). Seeds of common bermudagrass (*Cynodon dactylon* [L.] Pers. California Origin) were weighed and cultured in plastic pots with 19 cm in diameter and 25 cm in height, without drainage (0.25 g pot⁻¹) filled with 4 kg clay-loam soil with permanent wilting point (PWP) of 19% and field capacity (FC) 29%. Watering was carried out daily prior to beginning of treatments. Plants were kept in a greenhouse with 31/25°C (day/night) temperature and 35% relative humidity for one month before the beginning of treatments. Treatments were conducted at four irrigation levels (25%, 50%, 75% and 100% FC) and three photoperiod duration [8, 12 and 16 h as short day length (SDL), intermediate day length (IDL) and long day length (LDL)]. Watering was carried out daily before seed germination and after turf establishment. Then, the turves were watered equally when required. Established turves were clipped from 3 cm above soil by a hand mower and were transferred to a covered

frame which temperature, light (intensity and length) and relative humidity were controlled with digital sensors. The environmental condition of covered frame was 31°C, white and creamy fluorescent lamps one m above the pots with a constant light intensity of 3000 lux, and 35% relative humidity for applying simultaneous irrigation and photoperiod treatments. Pots were weighed daily and set to different irrigation treatments (25, 50, 75 and 100% FC), during the whole of experiment. After three months, plants were harvested in order to measure morphological and biochemical traits.

Growth parameters

Growth parameters including, shoot height (cm), leaf area (cm²) and fresh and dry weights of shoot and root (g) were measured. Dry weights were measured when the materials dried at 60°C for 48 h.

Chlorophyll content

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

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

Where: OD is optical density, V is the final solution volume in ml and f.w. is tissue fresh weight in mg.

Proline content

Proline was determined according to the method described by Bates *et al.* (1973). Using spectrophotometer (Biowave II, England) at 520 nm wavelength, appropriate proline standards were included in calculation of its content in samples.

Total soluble sugars and starch analysis

The total soluble sugars were measured using the method as previously described by Dubois *et al.* (1956). The total soluble sugar content of samples was measured at 490 nm of absorbance and glucose solution was used at different concentrations for standard curve drawing. The starch content was quantified using the Bradford method (McCready *et al.*, 1950). The starch content was measured at absorbance of 630 nm and calculated using the standard curve of glucose and multiplying it by 0.92.

Antioxidant analysis

Fresh samples were homogenized in extraction buffer (0.1 M phosphate buffer pH 6.8) with mortar and pestle on ice. The homogenate was then centrifuged at 12,000 g for 15 min at 4°C and the supernatant was used as the crude extract for the superox-

ide dismutase (SOD), guaiacol peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT). The SOD, POD, APX and CAT enzymes were estimated using the methods previously described by Beauchamp and Fridovich (1971), Chance and Maehly (1995), Nakano and Asada (1981) and Dhindsa et al. (1981), respectively.

Experimental design and data analysis

This study was conducted in a completely randomized design with factorial arrangements and two factors: field capacity and photoperiod with four replicates. Data were analyzed using statistical software (SAS Software) and mean comparisons were performed using LSD test at 5% level.

3. Results and Discussion

Results of analysis of variance (Tables 1 and 2) showed that photoperiod (except for soluble sugar) and irrigation had significantly influenced the measured traits and also the interaction of photoperiod and irrigation had a significant effect on fresh and dry weights of shoot, proline content and the level of activity of superoxide dismutase.

Shoot height and leaf area

Shoot height and leaf area significantly declined by decreasing field capacity from 100% to 25% (Table 3). Shoot height and leaf area decreased (47.09% and 27.77%, respectively) at 25% FC compared to 100% FC (Table 3). Ryan (2011) reported that growth can be reduced through impairment of cell division and

cell expansion which occurs at a lower water stress threshold rather than photosynthetic inhibition. Fu and Huang (2001) reported that shoot growth of both

Table 3 - Effect of field capacity and photoperiod and their interaction on shoot length, leaf area, shoot fresh and dry weight, root fresh and dry weight and chlorophyll content

Variables	Photo-period	Field capacity (%)				Mean
		100%	75%	50%	25%	
Shoot length (cm)	LDL	22.25 d*	21.27 e	16.67 h	11.05 k	17.81 C
	IDL	23.75 c	22.77 d	18.17 g	12.55 j	19.31 B
	SDL	25.35 a	24.37 b	19.77 f	14.15 i	20.91 A
	Mean	23.78 A	22.80 B	18.20 C	12.58 D	
	LDL	1.22 bc	1.22 bc	1.17 c	0.87 e	1.12 B
Leaf area (cm ²)	IDL	1.23 bc	1.23 bc	1.18 c	0.88 e	1.13 B
	SDL	1.32 a	1.32 a	1.27 ab	0.97 d	1.22 A
	Mean	1.26 A	1.25 A	1.20 B	0.91 C	
	LDL	20.30 a	20.26 a	16.16 b	14.06 c	17.69 A
	IDL	16.32 b	16.26 b	12.81 d	9.13 e	13.63 B
Shoot fresh weight (g)	SDL	13.85 c	12.83 d	9.69 e	5.34 f	10.42 C
	Mean	16.82 A	16.45 A	12.88 B	9.51 C	
	LDL	11.30 a	11.26 a	7.16 d	5.06 e	8.69 A
	IDL	10.32 b	10.26 b	6.81 d	3.13 f	7.63 B
	SDL	9.85 b	8.83 c	5.69 e	1.34 g	6.42 C
Shoot dry weight (g)	Mean	10.49 A	10.11 A	6.55 B	3.17 C	
	LDL	39.78 a	38.69 b	29.54 g	16.99 j	31.25 A
	IDL	37.80 c	36.71 e	27.56 h	15.01 k	29.27 B
	SDL	36.81 d	35.72 f	26.57 i	14.02 l	28.28 C
	Mean	38.13 A	37.04 B	27.89 C	15.34 D	
Root fresh weight (g)	LDL	19.78 a	18.69 b	13.46 d	8.54 f	15.12 A
	IDL	19.69 a	18.60 b	13.37 d	8.45 f	15.03 A
	SDL	18.82 b	17.73 c	12.50 e	7.43 g	14.12 B
	Mean	19.43 A	18.34 B	13.11 C	8.14 D	
	LDL	5.57 i*	7.06 g	14.35 f	23.25 c	12.56 C
Proline content (mol g ⁻¹ f.w.)	IDL	5.63 i	6.51 h	15.19 e	24.20 b	12.88 B
	SDL	5.66 i	6.89 gh	17.29 d	24.74 a	13.65 A
	Mean	5.62 D	6.82 C	15.61 B	24.07 A	

*In each variable, data followed by the same letters (small letters for interactions and capital letters for means) are not significantly different using LSD at 5% level.
LDL= long day length.
IDL= intermediate day length
SDL= short day length.

Table 1 - Analysis of variance of photoperiod, field capacity and interaction between photoperiod and field capacity measured traits

Source of variability	df	Shoot height (cm)	Leaf area (cm ²)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Sugars of shoot (mg g ⁻¹ d.w.)
Photoperiod	2	38.45 **	0.04**	212.24**	20.59**	36.59**	4.87**	5.77 NS
Field capacity	3	314.82**	0.33**	141.40**	141.40**	1337.54**	324.86**	79732.82**
Photoperiod * field capacity	6	0.0 NS	0.0 NS	1.25**	1.25**	0.0 NS	0.0 NS	0.0 NS
Error	33	0.17	0.002	0.24	0.24	0.00	0.08	7.01
CV	-	2.18	4.32	3.58	6.57	0.00	2.02	1.96

** and NS significant at the 0.01 level and not significant respectively.

Table 2 - Analysis of variance of photoperiod, field capacity and interaction between photoperiod and field capacity measured traits

Source of variability	df	Superoxide dismutase (Ug ⁻¹ FW)	Catalase (Ug ⁻¹ FW)	Peroxidase (Ug ⁻¹ FW)	Ascorbate peroxidase (Ug ⁻¹ FW)	Chlorophyll (mg g ⁻¹ FW)	Proline (µmol g ⁻¹ FW)	Starch content (mg g ⁻¹ DW)
Photoperiod	2	4044.08**	97.06**	969.12**	28933.33**	0.59**	4.99**	3.33**
Field capacity	3	55360.44**	234.71**	5248.15**	257973.85**	1.74**	7238.96**	105235.35**
Photoperiod * Field capacity	6	2.52 NS	0.0 NS	0.0 NS	0.0 NS	0.00 NS	2.27**	0.00 NS
Error	33	235,20	3.26	27.65	260.03	0.00	0.12	0.00
CV	-	9.91	5.65	6.67	1.79	0.0	2.68	0.00

** and NS significant at the 0.01 level and not significant respectively.

kentucky bluegrass and tall fescue generally were not affected by surface soil drying but under full drying, shoot growth declined for both species. The reduced leaf area is a modification to avoid evapo-transpiration loss and to increase water use efficiency in grasses which helps to tolerate water stress. Low leaf surface area would reduce transpiration rate also by lowering stomatal activity (Riaz *et al.*, 2010). Turf shoot height showed considerable difference in LDL treatments compared to SDL treatments. Reducing photoperiod significantly increased the shoot height and leaf area (Table 3). Shoot height and leaf area increased significantly with shortening day length that its maximum and minimum decreased (14.82% and 8.19%, respectively) was observed at SLD compared to LDL (Table 3). Similar results have reported on bermudagrass (Tegg and Lane, 2004) and zoysiagrass (Qian and Engelke, 1999).

Shoot fresh weight

Reducing field capacity from 100% FC to 25% FC significantly decreased the shoot fresh weight to 43.46% at 25% FC compared to 100% FC (Table 3). Riaz *et al.* (2010) demonstrated that, water deficit conditions had a significant inhibitory effect on shoot fresh and dry weights of three bermudagrass cultivars. The extended photoperiod (16 h) significantly increased fresh weight compared to shorter photoperiods (12 h and 8 h). Shoot fresh weight increased 41.09% under LDL compared to the SDL condition (Table 3). Sinclair *et al.* (2004) demonstrated that the extended photoperiod increased biomass accumulation of four grasses ('Pensacola' bahiagrass, *Paspalum notatum* Flugge var. *Saurde* Parodi; 'Tifton 85' bermudagrass, *Cynodon* spp. L. Pers.; 'Florakirk' bermudagrass; and 'Florona' stargrass, *Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) compared to short day condition. Interaction between field capacity and photoperiod resulted in the highest and lowest fresh weight in 100% FC-LDL and 25% FC-SDL treatments (Table 3).

Shoot dry weight

Different percentages of field capacity and photoperiod had significant effects on dry weight (Table 3). Reducing field capacity and photoperiod significantly decreased the dry weight. The shoot dry weight in 100% FC conditions decreased 69.78% compared to 25% FC condition (Table 3). Similar results have been reported on creeping bentgrass (*Agrostis stolonifera* L.), rough bluegrass (*Poa trivialis* L.), and perennial ryegrass (*Lolium perenne* L.) (Pessaraki and Kopec, 2008), bermudagrass

(*Cynodon dactylon* L.) (Riaz *et al.*, 2010). The highest and lowest dry weight was observed in 100% FC-LDL and 25% FC-SDL treatments, respectively (Table 3). Burton *et al.* (1988) stated that day length was highly correlated with yield of 'Coastal' bermudagrass, with yield reduction occurring in day lengths under 13 h. Therefore, photoperiod influenced dry matter production of forage grasses. Extended photoperiod throughout the cool-season in short-day length conditions substantially decreased forage yield (Sinclair *et al.*, 1997, 2001, 2003).

Root fresh weight

Root fresh weight significantly declined by decreasing field capacity from 100% to 25%. Root fresh weight decreased (59.76%) at 25% FC compared to 100% FC (Table 3). The impact of partially closing stomata limits CO₂ availability and reduces photosynthesis, which is vital to produce and translocate carbohydrates to roots to explore deeper moisture (Huang, 2006). Huang and Gao (2000) found that severe leakage of organic solutes from roots in drying soil gives evidence that root death of tall fescue cultivars during drought stress may correlate with root desiccation. There was a significant difference between LDL, IDL and SDL treatments and the highest and lowest root fresh weights were obtained in LDL and SDL treatments, respectively. Root fresh weight decreased 9.50% at SDL compared to LDL (Table 3). This is in agreement with Wang *et al.* (2004) who reported that an increase in root growth is associated with extended light duration and is related to increase in internal cytokinin concentration and its increased activity in root tips.

Root dry weight

As shown in Table 3, reduction in field capacity decreased root dry weight of plants. The highest and lowest root dry weights were observed in 100% FC and 25% FC treatments, respectively and in 25% FC decreased 58.10% compared to 100% FC. Pessaraki and Kopec (2008) demonstrated that, water deficit conditions showed a significant decrease in root dry weight of three turfgrass species. The highest and lowest root dry weight was obtained in LDL and SDL treatments, respectively (Table 3). Root dry weight decreased 6.61% at SDL compared to LDL (Table 3). Beard (1972) reviewed the morphological responses of turfgrasses under shade based on the research conducted before 1995, and found alterations such as: reduced tillering and shoot density, longer internodes with a reduced stem diameter, increased leaf length, decreased leaf width, thinner leaves, more

vertical leaf orientation, and fewer roots (McBee and Holt, 1966; Almodares, 1980; Dudeck and Peacock, 1992). A shift in allocation of dry matter occurs in response to shade, resulting in more dry matter partitioning into shoots rather than roots (Allard *et al.*, 1991; Dias-Filho, 2000). In response to lower irradiance, accelerated leaf elongation and decrease in partitioning to root dry matter are adaptive strategies to enhance light capture (Semchenko *et al.*, 2012).

Proline content

Reducing field capacity and photoperiod significantly increased proline content in all plants. The highest amount of proline content was obtained in 25% FC and the lowest one was obtained in 100% FC treatment (Table 3). This is in agreement with (Etemadi *et al.*, 2005) who demonstrated that the increase in drought increased proline content in bermudagrass (*Cynodon dactylon* L.). During drought stress, plants respond to different stresses with changes they create in their physiological features. Accumulation of soluble material in response to drought is a way to maintain turgor. It seems that the accumulation of free proline in plants is the general reaction to the stress. However several other amino acids increase under drought and salinity stress. But the degree of changes is not comparable with proline accumulation (Gzik, 1996). In a comparative study between perennial ryegrass and red fescue for the amount of resistance to the drought, it was seen that the amount of proline in red fescue was more than perennial ryegrass (Bandurska and Jozwiak, 2010). The highest and lowest proline content was obtained in SDL and LDL treatments, respectively (Table 3). This is in agreement to the findings reported on the effects of decreased photoperiod on bermudagrass (Esmaili and Salehi, 2012). Interaction between field capacity and photoperiod resulted in the highest and lowest proline content in 25% FC-SDL and 100% FC-LDL treatments (Table 3).

Chlorophyll content

Field capacity and light durations had significant effects on leaf chlorophyll content. The highest and lowest chlorophyll content, were observed in 100% FC and 25% FC treatments, respectively (Table 4). Induction of drought has caused a reduction of electron carrier in photosynthesis and a reduction in chlorophyll content which has been reported by (Zuily *et al.*, 1990; Moran *et al.*, 1994). Prolonged drought, heat, and the combined stresses could lead to loss of chlorophyll and lipid peroxidation, resulting

Table 4 - Effect of field capacity and photoperiod and their interaction on proline, sugars and starch contents, activity of Superoxidase dismutase, Catalase, Peroxidase, and Ascorbate peroxidase enzymes

Variables	Photo-period	Field capacity (%)				Mean
		100%	75%	50%	25%	
Chlorophyll content (mg Chl g ⁻¹ f.w.)	LDL	1.81 a	1.78 b	1.48 e	0.99 j	1.52 A
	IDL	1.74 c	1.71 d	1.41 h	0.92 k	1.44 B
	SDL	1.45 f	1.42 g	1.12 i	0.63 l	1.15 C
	Mean	1.67 A	1.64 B	1.33 C	0.85 D	
Sugars of shoot (mg g ⁻¹ d.w.)	LDL	62.63 de	67.19 c	199.39 b	212.15 a	135.34 A
	IDL	61.81 e	66.37 cd	198.22 b	211.33 a	134.52 A
	SDL	61.45 e	66.02 cd	198.22 b	210.98 a	134.17 A
	Mean	61.96 D	66.53 C	198.72 B	211.49 A	
Starch content (mg g ⁻¹ d.w.)	LDL	225.30 a	224.10 d	91.50 g	41.30 j	145.50 A
	IDL	224.90 b	223.70 e	91.10 h	40.90 k	145.10 B
	SDL	224.30 c	223.20 f	90.60 i	40.40 l	144.60 C
	Mean	224.80 A	223.70 B	91.10 C	40.90 D	
Superoxide dismutase (Ug ⁻¹ FW)	LDL	136.00 cd	148.50 c	266.00 a	116.00 def	166.62 A
	IDL	128.50 cde	143.50 c	261.50 a	109.50 ef	160.75 A
	SDL	106.00 fg	118.50 def	236.00 b	86.00 g	136.62 B
	Mean	123.50 C	136.83 B	254.50 A	103.83 D	
Catalase (Ug ⁻¹ f.w.)	LDL	31.48 ef	34.45 cd	40.71 a	31.18 efg	34.45 A
	IDL	28.89 fgh	31.86 de	38.12 ab	28.59 ghi	31.86 B
	SDL	29.53 efg	29.53 efg	35.78 bc	26.25 i	29.53 C
	Mean	28.98 C	31.95 B	38.20 A	28.67 C	
Peroxidase (Ug ⁻¹ f.w.)	LDL	68.40 fg	70.75 f	94.58 c	112.40 a	86.53 A
	IDL	60.80 hi	63.15 hi	86.99 d	104.81 b	78.94 B
	SDL	52.83 j	55.18 ji	79.02 e	96.84 c	70.97 C
	Mean	60.68 C	63.03 C	86.86 B	104.68 A	
Ascorbate peroxidase (Ug ⁻¹ f.w.)	LDL	869.64 de	879.64 d	1160.36 a	854.29 ef	940.98 A
	IDL	829.64 gh	839.64 fg	1120.36 b	814.29 hi	900.98 B
	SDL	784.64 kj	794.64 ij	1075.36 c	769.29 k	855.98 C
	Mean	827.97 B	837.97 B	1118.69 A	812.61 C	

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

LDL= long day length.

IDL= intermediate day length

SDL= short day length.

in further turf quality decline (Jiang and Huang, 2001). Water is required to facilitate photosynthesis in plants. Low energy electrons are extracted from water and are energized through light energy captured by chlorophyll. These energized electrons enable the production of NADPH and ATP which are then used to reduce CO₂. CO₂ is taken up from the atmosphere through stomata. Stomata are very sensitive to external environmental factors such as light, CO₂, water status, and temperature (Hopkins and Hüner, 2004). The loss of chlorophyll by the plant in an intense stress can be associated with photo oxidation and consequently oxidative stress (Kato and Shimizu, 1985). Kaiser (1987) indicated that an irreversible decrease in plant photosynthetic capacity occurs as RWC declines below 30%, leading to cell death from membrane damage in chloroplasts.

Detrimental effects on chloroplast biochemistry or chlorophyll fluorescence occur when RWC drops below 60% in tall fescue (Huang *et al.*, 1998). Surface drying had no effects on chlorophyll content in kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* Schreb.) while under full drying, chlorophyll content decreased in both grasses (Fu and Huang, 2001). Our findings were in agreement are (Fu and Huang, 2001) who reported that amount of chlorophyll in bermudagrass under moderate stress is not reduced, but it will be reduced in the severe drought. Chlorophyll content decreased with decreasing day length and the highest and lowest ones were observed in LDL and SDL treatments, respectively (Table 4). Shorting photoperiod caused decrease in chlorophyll content. In a research, the resistances to low light stress in both bermudagrass and paspalum have been examined and it was concluded that resistance to low light stress in the paspalum is more than bermudagrass (Jiang *et al.*, 2004). Baldwin *et al.* (2008) reported that bermudagrass showed significant decrease in chlorophyll content in response to short day length condition.

Total soluble sugars and starch content

Regardless of photoperiod, decrease in field capacity significantly increased total soluble sugars in the shoot (Table 4). Starch content declined by decreasing field capacity from 100% to 25% (Table 4). Shoot starch content, were highest and lowest in 100% and 25% FC treatments, respectively (Table 4). On the other hand, total soluble sugars during the drought can increase making these compounds non-photosynthetic routes and growth stopping due to the destruction of in soluble sugars and their change to soluble sugars (Hissao, 1973). Although some researchers have suggested that the destruction of starch can also increase monosaccharaides (Düring, 1992). The researchers stated that an increase of amylase in water stress causes starch degradation and the conversion of this large molecule into smaller units (Movahhedi-Dehnavi *et al.*, 2004). Different photoperiod had no significant effects on total soluble sugars (Table 4). Shoot starch content decreased by different light durations and the highest and lowest one was observed in LDL and SDL treatments, respectively (Table 4). Starch content decreased in response to shortening the photoperiod. Some researchers have reported that prolonging photoperiod increases carbohydrates (Hay and Pederson, 1986; Solhoug, 1991; Wang *et al.*, 1998). Other researchers reported that the photoperiod had no

effect on carbohydrates production (Sicher *et al.*, 1982; Logendra and Janes, 1992).

Antioxidant enzyme activities

APX, POD, CAT and SOD enzymes activities showed significant differences among field capacity and photoperiod treatments. The activities of APX were not significantly different between 100% FC and 75% FC treatments while were significantly increased in 50% FC treatment and minimum APX activity was observed at 25% FC treatment (Table 4). Bian and Jiang (2009) investigated the accumulation of reactive species of oxygen and antioxidants activity and the pattern of gene expression of antioxidant enzymes in the kentucky bluegrass in the drought condition. They observed that drought stress increased the activity of APX and CAT and decreased SOD and they stated that antioxidant enzymes and their gene expression might be different or occur in the immune system of kentucky bluegrass roots and leaves. POD enzyme activities increased with decrease in field capacity levels. Differences in leaf POD enzyme activities were not detected between 100% FC and 75% FC treatments. The maximum and minimum POD activity was obtained in 25% FC and 100% FC treatments, respectively (Table 4). In a research on drought tolerance of three cultivars of creeping bentgrass, it was observed that long-term drought stress reduced the activity of antioxidants such as POD and increased lipid peroxidation and the 'Greenwich' showed high resistance to drought (DaCosta and Huang, 2007). CAT and SOD enzymes activities significantly increased with decreasing field capacity from 100% to 50% then, declined in 25% FC treatment (Table 4). Shao *et al.* (2005) reported that in the of drought stress, the production amount of three enzymes, CAT, SOD and POD in resistant bermudagrass varieties have been significantly more than drought-sensitive ones. General declines in antioxidants, including CAT were reported in the response of three species of creeping bentgrass to drought stress. Moreover, they found that the species *Agrostis canina* L. was the most resistant species to drought (DaCosta and Huang, 2007). Liu *et al.* (2008) in a research, physiologically and morphologically investigated the five cultivars of kentucky bluegrass under drought and heat stress and observed that drought and heat stress simultaneously reduces SOD enzyme in all cultivars and stated that an increase of SOD enzyme activity cannot inhibit stress and would only delay free radicals accumulation. Results of present study indicated that regard-

less of field capacity treatments, APX, POD, CAT and SOD enzymes activities significantly decreased in response to decreasing day length therefore, the maximum and minimum enzymes activity was observed in LDL and SDL treatments (Table 4). Similar findings have been previously reported by (Burritt and Mackenzie, 2003) who stated that when the begonia plant is transferred from low light to bright light, CAT activity increases. Also, they stated that when the (*Picea abies* L.) seedlings are transferred from low light to high light, the activity of CAT enzyme decreases. Xu *et al.* (2010) investigated the effect of nitric oxide and sodium nitroprusside in tall fescue under high light stress and concluded that using sodium nitroprusside reduces enzyme activity of SOD, CAT and APX, but using nitric oxide increases the activity of mentioned enzymes. Jiang *et al.* (2005) demonstrated that, low light conditions showed a significant decrease in activity APX and CAT of bermudagrass and paspalum. Grace and Logan (1996) reported that the CAT enzyme activity varies depending on light intensity. The CAT enzyme activity in Schefflera [*Schefflera arboricola* (Hayata) Merrill] and Vinca (*Vinca major* L.) plants did not change with a change in light intensity, but in Mahonia (*Mahonia repens* (Lindley) Don.), CAT enzyme activity increased with an increase of light intensity. Interaction between field capacity and photoperiod resulted in the highest and lowest SOD enzyme activities in 50% FC-LDL and 25% FC-SDL treatments (Table 4).

4. Conclusions

The results proved that the reduction in photoperiod led to a progressive increase in shoot height and leaf area, however, the increase in irrigation inhibited their progressive growths. Additionally, the reduction in photoperiod caused a decrease in fresh and dry weight of root and shoot. However, the increase in irrigation led to alleviation of these negative effects during the day-time and thus increased the fresh and dry weight of root and shoot. Therefore, it appears as though the increased irrigation might have contributed to the enlargement and flexibility of cells, which, in turn helped increasing the dry and fresh weight of root and shoot. The reduced photoperiod led to a reduction in chlorophyll and starch contents and enzymes activities, and the increased irrigation compensated this reduction to some extent. This phenomenon might be, at least in part,

explained by the fact that irrigation reduced ABA production, inhibited ROS production and thus inhibited the closure of stomata. In overall, the increase in irrigation caused the destructive effects of reduced photoperiod to diminish, and vice versa. It seems that the interaction of photoperiod and irrigation treatments has superior effects on alleviating of the symptoms of stressed plants, than their separate. Further studies are needed to clarify more the interaction between irrigation and light treatments at structural and ultrastructural levels, in common bermudagrass.

References

- ALLARD G., NELSON C.J., PALLARDY S.G., 1991 - *Shade effects on growth of tall fescue: I. Leaf anatomy and dry matter partitioning.* - Crop Sci., 31: 163-167.
- ALMODARES A., 1980 - *The adaptation of Stenotaphrum secundatum (Walt.) Kuntze and Festuca arundinacea Schreb. to tree shade environments as affected by mowing heights.* - Texas A&M University, College Station, Texas, USA.
- BALDWIN C.M., LIU H., McCARTY L.B., 2008 - *Diversity of 42 bermudagrass cultivars in a reduced light environment. II International Conference on Turfgrass Science and Management for Sports Fields.* - Acta Horticulturae, 783: 147-158.
- BANDURSKA H., JOZWIAK W., 2010 - *A comparison of the effects of drought on proline accumulation and peroxidases activity in leaves of Festuca rubra L. and Lolium perenne L.* - Plant. Physiol., 79: 111-116.
- BATES L.S., WALDREN R.P., TEARE I.D., 1973 - *Rapid determination of free proline for water stress studies.* - Plant Soil., 39: 107-205.
- BEARD J.B., 1972 - *Turfgrass: science and culture.* - Prentice-Hall, Englewood Cliffs, NJ, USA, pp. 658.
- BEARD J.B., KENNA M.P., 2008 - *Water quality and quantity issues for turfgrasses in urban landscapes.* - Council for Agriculture Science and Technology, Ames, IA, USA.
- BEAUCHAMP C., FRIDOVICH I., 1971 - *Superoxide dismutases: improved assays and an assay predictable to acrylamide gels.* - Anal. Biochem., 44: 276-287.
- BIAN S., JIANG Y., 2009 - *Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery.* - Sci. Hort., 120: 264-270.
- BURRITT D.J., MACKENZIE S., 2003 - *Antioxidant metabolism during acclimation of Begonia erythrophylla to high light levels.* - Ann. Bot., 91: 783-794.
- BURTON G.W., HOOK J.E., BUTLER J.L., HELLWIG R.E., 1988 - *Effect of temperature, daylength, and solar radiation on production of Coastal bermudagrass.* - Agron. J., 80:

- 557-560.
- CHANCE B., MAEHLY A.C., 1955 - *Assay of catalase and peroxidase*. - *Methods Enzymol.*, 2: 764-775.
- DACOSTA M., HUANG B., 2007 - *Changes in antioxidant enzyme activities and lipid peroxidation for bentgrass species in response to drought stress*. - *J. Am. Soc. Hort. Sci.*, 132: 319-326.
- DHINDSA R.S., PLUMB-DHINDSA P., THORPE T.A., 1981 - *Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase*. - *J. Exp. Bot.*, 32: 93-101.
- DIAS-FILHO M.B., 2000 - *Growth and biomass allocation of the C4 grasses Brachiaria brizantha and B. humidicola under shade*. - *Pesqui. Agropecu. Bras.*, 35: 2335-2341.
- DUBOIS M., GILLES K.A., HAMILTON J.K., REBERS P.A., SMITH F., 1956 - *Colorimetric method for determination of sugar and related substances*. - *J. Anal. Chem.*, 28: 350-356.
- DUDECK A.E., PEACOCK C.H., 1992 - *Shade and turfgrass culture*, pp. 269-284. - In: WADDINGTON D.V., R.N. CARROW, and R.C. SHEARMAN (eds.) *Turfgrass*. American Society of Agronomy: Madison, Wisconsin, USA.
- DÜRING H., 1992 - *Evidence for osmotic adjustment to drought in grapevines (Vitis vinifera L.)*. - *Vitis*, 23: 1-10.
- ESMAILI S., SALEHI H., 2012 - *Effects of temperature and photoperiod on postponing bermudagrass (Cynodon dactylon [L.] Pers.) turf dormancy*. - *J. Plant. Physiol.*, 169: 851-858.
- ETEMADI N., KHALIGHI A., RAZMJOO K.H., LESSANI H., ZAMANI Z., 2005 - *Drought resistance of selected bermudagrass [Cynodon dactylon (L.) Pers.] accessions*. - *Int. J. Agric. Bio.*, 4: 612-615.
- FRY J., HUANG B., 2004 - *Applied turfgrass science and physiology*. - John Wiley & Sons, Inc, Hoboken, NJ, USA, pp. 320.
- FU J., HUANG B., 2001 - *Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress*. - *Environ. Exp. Bot.*, 45: 105-114.
- GRACE S.C., LOGAN B.A., 1996 - *Acclimation of foliar antioxidant systems to growth irradiance in three broadleaved evergreen species*. - *J. Plant. Physiol.*, 112: 1631-1640.
- GZIK A., 1996 - *Accumulation of proline and pattern of α -amino acids in sugar beet plants in response to osmotic, water and salt stress*. - *Environ. Exp. Bot.*, 36: 29-38.
- HAY R.K.M., PEDERSON K., 1986 - *Influence of long photoperiod on the growth of timothy (Phleum pratense L.) varieties from different latitudes in northern Europe*. - *Grass Forage Sci.*, 41(4): 311-317.
- HISSAO T., 1973 - *Plant responses to water stress*. - *Annu. Rev. Plant. Biol.*, 24: 519-570.
- HOPKINS W.G., HÜNER N.P.A., 2004 - *Introduction to plant physiology*. - John Wiley & Sons, New Jersey, USA, pp. 528.
- HUANG B., 2006 - *Plant environment interactions*. - CRC Press/Taylor and Francis, Boca Raton, FL, USA.
- HUANG B., FRY J., WANG B., 1998 - *Water relations and canopy characteristics of tall fescue cultivars during and after drought stress*. - *HortScience*, 33: 837-840.
- HUANG B., GAO H., 2000 - *Root physiological characteristics associated with drought resistance in tall fescue cultivars*. - *Crop Sci.*, 40: 196-203.
- JIANG Y., CARROW R.N., DUNCAN R.R., 2005 - *Physiological acclimation of seashore paspalum and bermudagrass to low light*. - *Sci. Hort.*, 105: 101-115.
- JIANG Y., DUNCAN R.R., CARROW R.N., 2004 - *Assessment of low light tolerance of seashore paspalum and bermudagrass*. - *Crop Sci.*, 44: 587-594.
- JIANG Y., HUANG B., 2001 - *Physiological responses to heat stress alone or in combination with drought: a comparison between tall fescue and perennial ryegrass*. - *HortScience*, 36: 682-686.
- KAISER W.M., 1987 - *Effects of water deficit on photosynthetic capacity*. - *Physiol. Plant.*, 71: 142-149.
- KATO M., SHIMIZU S., 1985 - *Chlorophyll metabolism in higher plants. VI. Involvement of peroxidase in chlorophyll degradation*. - *Plant. Cell Physiol.*, 26: 1291-1301.
- LIU J., XIE X., DU J., SUN J., BAI X., 2008 - *Effects of simultaneous drought and heat stress on kentucky bluegrass*. - *Sci. Hort.*, 115: 190-195.
- LOGENDRA S., JANES H.W., 1992 - *Light duration effects on carbon partitioning and translocation in tomato*. - *Sci. Hort.*, 52: 19-25.
- McBEE G.G., HOLT E.C., 1966 - *Shade tolerance studies on bermudagrass and other turfgrasses*. - *Agron. J.*, 58: 523-525.
- McCREADY R.M., GUGGLOZ J., SILVIERA V., OWENS H.S., 1950 - *Determination of starch and amylose in vegetables*. - *Anal. Chem.*, 22: 1156-1158.
- MORAN J.F., BECANA M., ITURBE-ORMAETXE I., FRECHILLA S., KLUCAS R.V., APARICIO-TEJO P., 1994 - *Drought induces oxidative stress in pea plants*. - *Planta*, 194: 346-352.
- MOVAHHEDI-DEHNAVI M., MODARRES A.M., SANAVI-SOROUSH-ZADE A., JALALI M., 2004 - *Changes of proline, total soluble sugars, chlorophyll (SPAD) content and chlorophyll fluorescence in safflower varieties under drought stress and foliar application of zinc and manganese*. - *Biaban.*, 9: 93-110.
- NAKANO Y., ASADA K., 1981 - *Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts*. - *Plant Cell Physiol.*, 22: 867-880.
- PESSARAKLI M., KOPEC D.M., 2008 - *Comparing growth responses of selected cool-season turfgrasses under salinity and drought stresses*. - *Acta Horticulturae*, 783: 169-174.
- QIAN Y.L., ENGELKE M.C., 1999 - *Influence of trinexapac-ethyl on diamond zoysiagrass in a shaded environment*. - *Crop Sci.*, 39: 202-208.
- RIAZ A., YOUNIS A., HAMEED M., KIRAN S., 2010 - *Morphological and biochemical responses of turfgrass-*

- es to water deficit conditions - Pakistan. J. Bot., 42: 3441-3448.
- RYAN M.G., 2011 - *Tree responses to drought*. - Tree Physiol., 31: 237-239.
- SAINI R.S., SHARME K.D., DHANKHAR O.P., KAUSHIK R.A., 2001 - *Laboratory manual of analytical techniques in horticulture*. - Agrobios, Jodhpur, India, pp. 49-50.
- SEMCHENKO M., LEPIK M., GÖTZENBERGER L., ZOBEL K., 2012 - *Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate?* - J. Ecol., 100: 459-466.
- SHAO H.B., LIANG Z.S., SHAO M.A., WANG B.C., 2005 - *Changes of some physiological and biochemical indices for soil water deficits among 10 wheat genotypes at seedling stage*. - Colloids, Surf. B., 42: 107-113.
- SHEARMAN R.C., 2006 - *Fifty years of splendor in the grass*. - Crop Sci., 46: 2218-2229.
- SICHER R.C., KREMER W.G., CHATTERTON N.J., 1982 - *Effects of shortened day length upon translocation and starch accumulation by maize, wheat and pangola grass leaves*. - Can. J. Bot., 60: 1304-1309.
- SINCLAIR T.R., BENNET J.M., RAY J.D., 1997 - *Environmental limitation to potential forage production during the winter in Florida*. - Soil. Crop Sci. Soc. Flo. Proc., 56: 58-63.
- SINCLAIR T.R., MISLEVY P., RAY J.D., 2001 - *Short photoperiod inhibits winter growth of subtropical grasses*. - Planta, 213: 488-491.
- SINCLAIR T.R., RAY J.D., MISLEVY P., PREMAZZI L.M., 2003 - *Growth of subtropical forage grasses under extended photoperiod during short day length months*. - Crop Sci., 43: 618-623.
- SINCLAIR T.R., RAY J.D., PERMAZZI L.M., MISLEVY P., 2004 - *Photosynthetic photon flux density influences grass responses to extended photoperiod*. - Environ. Exp. Bot., 51: 69-74.
- SLADEK B.S., HENRY G.M., AULD D.L., 2009 - *Evaluation of zoysiagrass genotypes for shade tolerance*. - HortScience, 44: 1447-1451.
- SOLHOUG K.A., 1991 - *Effects of photoperiod and temperature on sugars and fructans in leaf blades, leaf sheaths and stem, and roots in relation to growth of Poa pratensis*. - J. Plant. Physiol., 82: 171-178.
- STIER J.C., STEINKE K., ERVIN E.H., HIGGINSON F.R., MCMAUGH P.E., 2013 - *Turfgrass and Issues*, pp. 105-145. - In: STIER J.C., P.B. HORGAN, and A.S. BONOS (eds.) *Turfgrass: Biology, use, and management*. American Society of Agronomy, Madison, WI, USA.
- TEGG R.S., LANE P.A., 2004 - *A comparison of the performance and growth of a range of turfgrass species under shade*. - Aust. J. Exp. Agric., 44: 353-358.
- TRENHOLM L.E., NAGATA R.T., 2005 - *Shade tolerance of St. Augustine grass cultivars*. - HortTechnology, 15: 267-271.
- WANG Z., YUAN Z., QUEBEDDEAUX B., 1998 - *Photoperiod alters partitioning of newly-fixed ¹⁴C and reserve carbon into sorbitol sucrose and starch in apple leaves, stems and roots*. - Aust. J. Plant. Physiol., 25: 503-506.
- WANG Z.H., XU Q., HUANG B., 2004 - *Endogenous cytokinin level and growth responses to extended photoperiods for creeping bentgrass under heat stress*. - Crop Sci., 44: 209-213.
- XU J., WANG Z., CHENG J.J., 2011 - *Bermudagrass as feedstock for biofuel production: a review*. - Bioresour. Technol., 102: 7613-7620.
- XU Y.F., SUN X.L., JIN J.W., ZHOU H., 2010 - *Protective roles of nitric oxide on antioxidant systems in tall fescue leaves under high-light stress*. - Afr. J. Biol., 9: 300-306.
- ZUILY F.Y., VAZQUEZ T.A., VIEIRA D.J., 1990 - *Effect of water deficit on cell permeability and on chloroplast integrity*. - Bulletin de la Société Botanique de France, Actual. Bot., 137: 115-123.

