South Western Journal of Horticulture, Biology and Environment
P-Issn: 2067- 9874, E-Issn: 2068-7958

Vol.8, No.1, 2017
pp.41-54
Art.no. e17104

EFFECT OF PHOTOPERIOD AND IRRIGATION REGIME ON GROWTH AND PHYSIOLOGICAL INDICES OF TALL FESCUE (*Festuca arundinacea* Schreb.)

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ABSTRACT. *Drought and light are two major factors limiting the growth of cool-season turfgrasses in many areas. The main objective of the present study was to investigate the effects of both irrigation levels and light duration on growth and quality of tall fescue. In order to, a greenhouse experiment was conducted to evaluate the interaction of photoperiod and irrigation on Festuca* arundinacea *Schreb. 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 of shoot and root, chlorophyll contents and superoxide dismutase, catalase and ascorbate peroxidase activities. Decreasing the field capacity increased proline content and peroxidase enzyme activity. In overall, results showed that, the increase in day lengths alleviates the destructive effects of reduced irrigation and vice versa.*

KEY WORDS*: Field Capacity, Photoperiod, Turfgrass, Antioxidative Enzymes*

INTRODUCTION

The availability of water is a major factor limiting distribution, growth, and productivity of both cool-season and warm-season turfgrass species (Nilsen & Orcutt 1996). Water availability for irrigation of turfgrasses is becoming increasingly limited, making water conservation a prime concern of turfgrass growers and managers across many areas of the country. Knowledge of water use requirements of various grass species is important for identifying grasses that persist with reduced water inputs and also for developing efficient irrigation management practices (Fu et al. 2004). Tall fescue (*Festuca arundinacea* Schreb.) was cool-season grass which was widely used for turfgrass on home lawns, sports fields and golf courses in cool climatic regions. The growth of cool-season turfgrass is often limited by drought stress during summer months in warm climates (Lu et al. 2008). Hatamzadeh et al. (2015) investigated the effects of drought stress on two cool-season turfgrasses and found that intensive drought stress, antioxidant enzyme activities, chlorophyll content, relative water content and ion leakage were decreased. Turfgrasses require a minimum daily duration of light for growth. Duration is affected by the time of year (the sun's angle), latitude, and size and location of the tree or structure creating shade (Fry & Huang 2004). Most turfgrasses require four to five hours of full sun per day or an entire day of filtered light. Prolonging light duration promotes leaf growth, tiller production and dry matter accumulation and may increase environmental stress resistance (Aamlid 1992; Hay & Heide 1983; Hay & Pedersen 1986). Xu & Huang (2004) reported that extending the light duration from 14 to 22 hours significantly increased root length and root number for creeping bentgrass (*Agrostis stolonifera* L.) exposed to heat stress. Improved turf performance associated with extended light duration was related to increases in carbohydrate content. Supplemental lighting has been used to increase light duration on creeping bentgrass putting greens at some U.S. golf courses, including August National's hole 12 and Riviera Country Club's hole 6, which are situated in shaded areas (Fry & Huang 2004). According to the shortage of water resources and possibility of increasing light duration in some places like parks and stadiums, the objective of the present study was to investigate the effects of raising light duration on alleviating the adverse effects of water stress. The other aim was to study the effects of increasing watering amount on alleviating the adverse effects of shade stress in the areas like near the buildings or under the trees canopy.

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 tall fescue were weighed and cultured in plastic pots with 19 cm in diameter and 25 cm in height, without drainage (1.66 $g.pot^{-1}$) 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 ºC (day/night) temperature and 35% relative humidity for one month before the beginning of treatments. Treatments were conducted at four irrigation treatments (25%, 50%, 75% and 100%FC) and three photoperiod levels [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. Established turfs 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 31ºC, white and creamy fluorescent lamps 1 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 two months, to determine morphological and biochemical characteristics one sample from each replication was analyzed.

Growth parameters. Growth parameters including, fresh and dry weights of shoot and root (g) were measured. Dry weights were measured when the samples were oven dried (Memmert 854) 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:

Chlorophyll (mg/g f.w.) = [20.2(OD 645 nm) + 8.02(OD 663 nm) × V/(f.w.×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.

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 12,000g for 15 min at 4 ◦C and the supernatant was used as the crude extract for the superoxide 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 & Fridovich 1971, Chance & Maehly 1955, Dhindsa et al. 1981, Nakano & Asada 1981), respectively.

Statistical analyses. The data were analyzed using one-way analysis of variance at $P < 0.05$ significance with SAS version 9.1 software (SAS Institute Inc., Cary, NC). LSD test was conducted to determine the statistical differences among different treatments.

RESULTS AND DISCUSSION

Shoot fresh weight

Reducing field capacity from 100%FC to 25%FC significantly decreased the shoot fresh weight to 49.63% at 25%FC compared to 100%FC (Table 1). Shoot growth is usually more sensitive to drought stress than root growth. The differences in sensitivity between shoots and roots to drought stress may also be related to the effects of abscisic acid (ABA), which serves to inhibit shoot growth while maintaining root growth (Hsiao & Xu 2000). During drought stress, there is increased carbon allocation to roots relative to shoots (Huang & Fu 2000). Similar results have been reported on tall fescue Manuchehri & Salehi (2015), bermudagrass (*Cynodon dactylon* [L.] Pers.) Riaz et al. (2010), and kentucky bluegrass (*Poa pratensis* L.) (Fu & Huang 2001). The extended photoperiod (16 h) significantly increased fresh weight compared to shorter photoperiods (12 h and 8 h). Shoot fresh weight increased 17.36% under LDL compared to the SDL 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 1). Jiang et al. (2004) Turf density decrease in shade is likely due to decreased tiller or leaf area. 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.

Shoot dry weight

Different percentages of field capacity and photoperiod had significant effects on dry weight (Table 1). Reducing field capacity and photoperiod significantly decreased the dry weight. The shoot dry weight in 100%FC conditions decreased 139.71% compared to 25%FC condition. Many

Table 1. Effect of field capacity and photoperiod and their interaction on shoot fresh and dry weight, root fresh and dry weight and chlorophyll content.

Variables	Photoperiod	Field Capacity (%)				Mean
		100%	75%	50%	25%	
Shoot fresh weight (g)	LDL	20.59±0.48a*	18.79±0.48b	14.75±0.21f	10.74±0.21h	16.22±3.94A
	IDL	18.75±0.20b	18.58±0.20b	15.58±0.30e	10.66±0.30h	15.89±3.38B
	SDL	17.97±0.49c	16.23±0.20d	13.64±0.29g	7.46±0.30i	13.82±4.12C
	Mean	19.10±1.21A	17.87±1.24B	14.66±0.86C	9.62±1.61D	
Shoot dry weight (g)	LDL	13.79±0.48a	12.49±0.48c	8.95±0.21g	5.94±0.21i	10.89±3.19A
	IDL	13.15±0.20b	13.08±0.20b	10.68±0.30e	6.66±0.30h	10.30±2.73B
	SDL	13.27±0.49b	11.63±0.20d	9.64±0.29f	4.16±0.30j	9.67±3.55C
	Mean	13.40±0.47A	12.40±0.68B	9.76±0.78C	5.59±1.12D	
Root fresh weight (g)	LDL	43.31±1.99ab	42.22±1.99b	33.07±1.99c	20.52±1.99e	34.78±9.61A
	IDL	45.39±2.55a	44.30±2.55ab	27.06±2.55d	15.92±2.55f	33.16±12.93B
	SDL	44.31±2.55ab	43.22±2.55ab	25.98±2.55d	14.84±2.55f	32.08±12.93B
	Mean	44.33±2.33A	43.24±2.33A	28.70±3.90B	17.09±3.35C	
Root dry weight (g)	LDL	22.93±1.99a	20.94±1.99d	18.03±1.99g	8.05±1.99j	17.49±6.17A
	IDL	22.85±1.99b	20.86±1.99e	17.95±1.99h	7.97±1.99k	17.41±6.17B
	SDL	22.77±1.99c	20.78±1.99f	17.87±1.99i	7.89±1.99l	17.32±6.17C
	Mean	22.85±1.80A	20.86±1.80B	17.95±1.80C	7.97±1.80D	
Chlorophyll content (mg Chl g^{-1}f.w.)	LDL	2.09±0.01a	2.06±0.01c	1.76±0.01g	1.31±0.01j	1.81±0.32A
	IDL	2.07±0.01b	2.04±0.01d	1.75±0.01h	1.29±0.01k	1.79±0.32B
	SDL	1.81±0.01e	1.79±0.01f	1.49±0.01i	1.03±0.01l	1.53±0.32C
	Mean	1.99±0.13A	1.96±0.13B	1.67±0.13C	1.21±0.13D	

*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. Data represent the mean value of four replicates ± SD.

physiological processes in the turfgrass plant are interrupted during drought stress, including photosynthesis, respiration, hormone synthesis, and water and nutrient uptake (Huang & Gao 1999). Stomatal regulation is often thought to be the first line of defense against drought stress, for it controls transpiration and carbon fixation in photosynthesis. Water deficits, as manifested by the decrease in leaf water status, can directly limit growth and cause stomatal closure (Kramer & Boyer 1995). Similar results have been reported on Creeping bentgrass, Rough bluegrass (*Poa trivialis* L.) and Perennial ryegrass (*Lolium perenne* L.) (Pessarakli & Kopec 2008). Shoot dry weight decreased with decreasing day length and the highest and lowest ones were observed in LDL and SDL treatments, respectively. Interaction between field capacity and photoperiod resulted in the highest and lowest shoot dry weight in 100%FC-LDL and 25%FC-SDL treatments (Table 1). A reduced respiration rate is often considered a major physiological adaptation, allowing plants to conserve carbohydrates in shade. Reduced leaf respiration at low light levels has been well documented (Givnish 1988). The photosynthetic-respiratory balance is a critical factor in shade tolerance, and a positive CO_2 balance contributed to shade adaptation in red fescue (Wilkinson et al. 1975). A low ratio of photosynthesis to respiration in response to stress condition may result in a reduced total nonstructural carbohydrate content and inferior turf recovery from stress (Fry & Huang 2004). Extended photoperiod throughout the cool-season in short-day length conditions substantially increased forage yield (Sinclair et al. 1997, Sinclair et al. 2001, Sinclair et al. 2003).

Root fresh weight

Root fresh weight significantly declined by decreasing field capacity from 100% to 25% (Table 1). Root fresh weight decreased (61.44%) at 25%FC compared to 100%FC. Fu & 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, root fresh weight was decreased. 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 (Table 1). The highest and lowest root fresh weights were observed in 100%FC-LDL and 25%FC-SDL treatments, respectively (Table 1). Root fresh weight decreased 7.76% at SDL compared to LDL. 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 1, 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 65.12% compared to 100%FC. Deep extensive rooting allows plants to exploit a larger soil volume and increase water absorption. However, plants must invest significant amounts of carbon in developing and maintaining large root systems. Among all root characteristics root viability is the most important factor for efficient water uptake and drought resistance of tall fescue was more closely related to root viability than to total root mass or length (Huang & Carrow 1997). During drought, turfgrass root metabolism and carbohydrate allocation levels change in response to the plant's effort to cope with the stress. Drought increases carbon allocation to roots, which can have tremendous positive effects on whole plant growth sometimes even more so than the photosynthesis rate (Fry & Huang 2004). Pessarakli & 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. Root dry weight decreased 0.97% at SDL compared to LDL. Interaction between field capacity and photoperiod resulted in the highest and lowest root dry weight in 100%FC-LDL and 25%FC-SDL treatments (Table 1). Xu & Huang (2004) reported that extending the light duration from 14 to 22 hours significantly increased root length and root number for creeping bentgrass exposed to heat stress. Improved turf performance associated with extended light duration was related to increases in carbohydrate content.

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 1). Induction of drought has caused a reduction of electron carrier in photosynthesis and a reduction in chlorophyll content which has been reported by Moran et al. (1994). Prolonged drought, heat, and the combined stresses could lead to loss of chlorophyll and lipid peroxidation, resulting in further turf quality decline (Jiang & Huang 2001). Our findings were in agreement are Fu & Huang (2001) who reported that amount of chlorophyll in two cool-season grasses 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 1). The interaction between field capacity and photoperiod regimes showed that the highest and lowest chlorophyll content were obtained in 100%FC-LDL and 25%FC-SDL treatments, respectively (Table 1). Baldwin et al. (2007) reported that bermudagrass showed significant decrease in chlorophyll content in response to short day length condition.

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 2). Drought tolerance can be defined as a plant's ability to maintain physiological functions when very little or no water is available to the plant. One of the important factors controlling cell tolerance to desiccation or dehydration is the cell's capability to maintain adequate turgor pressure during drought stress. Generally, this is done by increasing the concentration of compatible solutes within the cell. Compatible solutes or osmoregulants include inorganic solutes such as potassium, calcium and sodium and organic solutes such as soluble sugars sucrose and mannitol, and nonprotein amino acids (proline) (Nilsen & Orcutt 1996). DaCosta & Huang (2006) have shown the importance of osmotic adjustment in creeping bentgrass and velvet bentgrass (*Agrostis canina* L.). Compared with creeping bentgrass, velvet bentgrass showed a 50–60% higher magnitude of osmotic adjustment under water deficit. The highest and lowest proline content was obtained in SDL and LDL treatments, respectively (Table 2). Interaction between field capacity and photoperiod resulted in the highest and lowest proline content in 25%FC-IDL and 100%FC-LDL treatments (Table 2). Esmaili & Salehi (2012) noted in bermudagrass that were treated with short photoperiod duration, proline content was increased.

Table 1. Effect of field capacity and photoperiod and their interaction on proline contents, activity of SOD, CAT, POD, and APX enzymes.

Variables	Photoperiod	Field Capacity (%)				Mean
		100%	75%	50%	25%	
Proline content (μmol g^{-1}f.w.)	LDL	6.61±0.27g*	8.10±0.27e	15.39±0.27cd	24.29±0.27b	13.60±7.24AB
	IDL	6.67±0.32g	7.55±0.32efg	16.23±0.32c	25.78±0.32a	14.06±7.99A
	SDL	6.70±0.27fg	7.93±0.27ef	14.28±0.35d	24.79±2.91ab	13.41±7.50B
	Mean	6.66±0.26D	7.86±0.35C	15.30±0.88B	24.94±1.67A	
SOD (Ug^{-1} f.w.)	LDL	126.00±19.25cd	138.50±13c	256.00±10.32a	106.00±14.87def	156.62±61.87A
	IDL	118.50±16.84cde	133.50±8.22c	251.50±17.31a	99.50±20.42ef	150.75±63.06A
	SDL	96.00±19.25fg	108.50d±13ef	226.00±10.32b	76.00±14.87g	126.22±61.87B
	Mean	113.50±21.7C	126.83±17.27B	244.50±18.17A	93.83±20.38D	
CAT (Ug^{-1} f.w.)	LDL	31.28±1.57ef	34.25±1.12cd	40.50±1.25a	30.97±2.95efg	34.25±4.29A
	IDL	28.69±1757fgh	31.66±1.12de	37.91±1.25ab	28.38±2.95ghi	31.66±4.29B
	SDL	26.35±1.57hi	29.32±1.12efg	35.5±1.258bc	26.50±2.95i	29.33±4.29C
	Mean	28.77±2.53C	31.74±2.33B	38.00±2.38A	28.47±2.39C	
POD (Ug^{-1} f.w.)	LDL	61.56±0.93c	63.91±3.98c	87.74±4.43b	105.56±8.42a	79.69±19.27A
	IDL	60.88±0.93c	63.23±3.98c	87.06±4.43b	104.88±8.42a	79.01±19.27A
	SDL	60.43±0.93c	62.78±3.98c	86.61±4.43b	104.43±8.42a	78.56±19.27A
	Mean	60.95±0.97C	63.30±3.63C	87.14±4.04B	104.96±7.63A	
APX (Ug^{-1} f.w.)	LDL	864.64±15.18de	874.64±15.18d	1155.36±15.70a	849.29±25.31ef	935.98±132.16A
	IDL	824.64±15.18gh	834.64±15.18fg	1115.36±15.70b	809.29±25.31hi	895.98±132.16B
	SDL	779.64±15.18jk	789.64±15.18ij	1070.36±15.70c	764.29±25.31k	850.98±132.16C
	Mean	822.97±38.77B	832.97±38.77B	1113.69±38.94A	807.61±42.88C	

*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. Data represent the mean value of four replicates ± SD.

Antioxidant enzyme activities

Plants under water deficit have limited capacity for photosynthesis. However, continued light absorption under drought stress results in excessive energy within and around the photosynthesis apparatus. This excitation energy can be dissipated by reducing molecular oxygen, whereby electrons leaked from chloroplasts and mitochondria interact with oxygen and generate active oxygen species (AOS) these species cause oxidative damage to lipids, nucleic acids, and proteins (Asada 1999). Increases in antioxidant enzyme activity may be induced by presence of AOS (Smirnoff 1993). Some common antioxidant enzymes include catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione reductase, and ascorbate peroxidase (APX). Tolerance to the stresses is associated with maintenance or an increase in antioxidant enzyme activities (Bowler et al. 1992; Lascano et al. 2001; Sairam et al. 2000). 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 2). Sairam et al. (2000) indicated that there were differences in the increase in activities of various antioxidants among tolerant genotypes of wheat, such that one tolerant genotype had very high levels of ascorbic acid and APX, while another tolerant genotype exhibited higher SOD and CAT and intermediate ascorbic acid activities. POD enzyme activities increased with decrease in field capacity levels. The maximum and minimum POD activity was obtained in 25%FC and 100%FC treatments, respectively (Table 2). Other studies have reported increases Zhang et al. (1995), decreases Zhang & Kirkham (1996), and no changes Fangmeier et al. (1994) in POD activity in response to drought stress. CAT and SOD enzymes activities significantly increased with decreasing field capacity from 100% to 50% then, declined in 25%FC treatment (Table 2). SOD catalyzes the dismutation of superoxide into H_2O_2 and O_2 Bowler et al. (1992), and is reported to be one of the most effective antioxidant enzymes in limiting oxidative damage (Asada 1999). In wheat *(Triticum aestivum* L.), SOD activity increased or remained unchanged in the early phase of drought but decreased with further water stress (Zhang et al. 1995). Zhang & Kirkham (1996) also reported that CAT activity was not affected by mild drought. These results indicated that the ability of CAT to quench active oxygen was maintained during initial stress but was limited during a prolonged period of full drying; however, plants were able to maintain some CAT activity even when water was available only in deep soil. Results of present study indicated that regardless of field capacity treatments, APX, 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 2). Interaction between field capacity and photoperiod resulted in the highest and lowest APX, CAT and SOD enzymes activities in 50%FC-LDL and 25%FC-SDL treatments while, maximum and minimum POD enzyme activities obtained in 25%FC-LDL and 100%FC-SDL treatments (Table 2). Similar findings have been previously reported by Burritt & 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 bermudagrss and paspalum. Grace & 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.

CONCLUSION

The results proved that the reduction in intense irrigation led to a decrease in chlorophyll contents, enzymes activities and fresh and dry weight of root and shoot and the extended photoperiod increased performance of turf and the most growth and physiological parameters of tall fescue under drought stress. According to the shortage of water resources and possibility of increasing light duration in some places like parks and stadiums, in turf destructive effect of drought stress can be reduced by increasing photoperiod and reduced the amount of used water.

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