Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 10, No. 3, 2024
https://jcns.ahvaz.iau.ir/ OPEN ACCESS

The Effect of Water Deficit Conditions on the Physiological Responses of Seven Amenity Grass Species

Sayed Alireza Seyedmohammadi[1], Ali Ashraf Jafari*[1,2], Zahra Khodarahmpour[3], Shahab Sadat[1]
1- Department of Genetics and Plant Breeding, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.
2- Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
3- Department of Agronomy and Plant Breeding, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran.

RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *28 Jun. 2023*
Received in revised form: *27 Jul. 2024*
Accepted Date: *29 Aug. 2024*
Available online: *7 Sep. 2024*

To Cite This Article:
Sayed Alireza Seyedmohammadi, Ali Ashraf Jafari, Zahra Khodarahmpour, Shahab Sadat. The Effect of Water Deficit Conditions on the Physiological Responses of Seven Amenity Grass Species. ***J. Crop. Nutr. Sci.,*** 10(3): 45-56, 2024.

ABSTRACT

BACKGROUND: Drought stress is the main challenge in grassland cultivation and management. Introduction of drought-resistant species in hot climates such as Khuzestan (Iran) is a priority.

OBJECTIVES: This study was aimed to assess responses of Seven amenity grass species for morphological and physiological traits in two pot and laboratory conditions. Seven amenity grass species as *Cynodon dactylon*, *Dichondra repens*, *Festuca arundinaceae* (cv. Finelawn, Starlette and Talladega), *Festuca rubra* (cv.Napoli and Simone), *Lolium perenne* (cv. Capri, Danilo and Delaware), *Poa pratensis* (cv. Mardona) and two cv. Sport seed provided from Italy and Netherlands were subjected to four levels of drought stress. In pot seeds were sown in pots and exposed to outdoors.

METHODS: A factorial experiment was conducted using four levels of water stresses namely well-watered (100% of field capacity) as control, (80%FC, 60%FC and 40% FC) in pot condition and well-watered (0MPa) as control, (-0.3 MPa and -0.6 MPa and -0.9 MPa) in laboratory condition as the first factor and turfgrass species as the second factor on a Completely Randomized Design (CRD) with 3 replications in 2018, Ahvaz, Iran. Data collected for 14 physiological traits of the seedlings.

RESULT: The result of ANOVA showed significant effects of species and water stress levels for all traits ($p<0.01$). Interaction effects of species and water stress were significant for all traits except contine of catalase in glasshouse condition indicating that the grass species had different responses to water stress. Results of means comparison between water stress levels showed in pots condition the values of RWC, recovery after drought and regrowth after cutting were decreased by drought stress and the lower values were observed in 40%FC. The higher values of RWC, carbohydrates, Proline and Catalase, SOD and APX content were obtained in *C. dactylon*, indicating that this species was more tolerated to drought stress than other species and recommended for cultivation in green space of Iran. Turf quality and chlorophyll content was decreased due to drought stress. Proline content was increased in three species with prolonged withholding irrigation.

CONCLUSION: The highest prolin was observed in *C. dactylon* in 80% FC. There was significant difference in activities of superoxide dismutase and catalase between stressed plants and control plants. The highest activities of enzymes were showed in *C. dactylon.*

KEYWORDS: *Carotenoid, Chlorophyll, Enzymes, Grass, Pot.*

***Corresponding Author:** Ali Ashraf Jafari ✉ aajafari@rifr-ac.ir

1. BACKGROUND

One of the main pillars of green space are cover plants and grass is one of the most important cover plants of the world. Grass has the greatest role in purifying and reducing air pollution. Plant growth and development are under influence of genetics characters and environmental conditions. Some factor like temperature, moisture, radiation, feed and gases can influence plant growth and development and cause reduction and increase in their function. One of the important environmental stresses is drought stress (Farzamisepehr *et al*., 2021). In arid and semiarid areas, irrigation water supply for turfgrass is a major problem (Riaz *et al.*, 2010). Grass has the greatest role in purifying and reducing air pollution for urban environments (Kafi and Kaviani, 2002, Kuok and Tang, 2023 and Mathew, 2021). In recent years, increasing deleterious effects on agricultural productivity has been observed, especially in arid and semiarid regions where rainfall is low and evapotranspiration is high (Jha *et al*., 2019). Plants have developed varied mechanisms responsible for both avoidance and tolerance to stress in cells (Acosta *et al*., 2017). Increasing drought stress in thymes causing the reduce traits such as plant height, number of lateral shoots, dry and fresh weight of biomass, and in contrast, leading to increase the root volume, root dry weight and root length (Amini Dehaghi and Babaee, 2010 and Azimi *et al*., 2018). Proline as an amino acid is compatible solutes for cell osmotic adjustment and protection of cell components during dehydration (Zhang *et al*., 2009). Drought stress increases proline content in *Brassica napus* (Mirzaee, 2013), Sainfoin (Veisipoor *et al*., 2013), grape cuttings (Meng *et al*., 2014) and soybean (Ghorbanali and Niakan, 2007). Drought stress also increases soluble carbohydrate and protein content (Kabiri *et al*., 2018; Mirzaee, 2013; Meng *et al*., 2014; Ghorbanali and Niakan, 2007). In contrast, photosynthesis is limited by drought stress due to stomatal closure (Flexas *et al*., 2004; Chaves *et al*., 2009; Mafakheri *et al*., 2010), consequently drought stress decreases the chlorophyll content in plant species (Alaei *et al*., 2013). Many of important turfgrass species are currently harvested in their natural habitats in Iran. These can lead to the destruction of a large part of the germplasm of valuable species. Therefore, domestication and cultivation of important turfgrass species in dryland farming conditions are high priority here in Iran.

2. OBJECTIVES

This study was aimed to assess responses of Seven amenity grass species for morphological and physiological traits in two pot and laboratory conditions.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

Seven amenity grass species as *Cynodon dactylon*, *Dichondra repens*, *Festuca arundinaceae* (cv. Finelawn, Starlette and Talladega), *Festuca rubra* (cv.Napoli and Simone), *Lolium perenne* (cv. Capri, Danilo and Delaware), *Poa pratensis* (cv. Mardona) and two cv. Sport seed provided from Italy and Netherlands were subjected to four levels of drought stress. This experiment was conducted in pot condition. In pots condition plastic pots in size of 20 cm were used. Pots had some holes for drainage. A bit of gravel was placed at the bottom of the pots to facilitate the depletion of water. The pots were filled with soils. Seeds were disinfected by Mancoseb fungicide with the 1:2000 ratios to prevent the fungal contamination. In each pots 20 seeds sown in 2018 in ahvaz, Iran. Pots were irrigated regularly until the seeds go to germination and seedlings were appeared. Then, the pots were kept outdoor under shelter during winter. In March 2018, the pots were arranged using a factorial experiment based on a completely randomized design (CRD) with three replications. The first factor was drought stress levels as control 100% Field Capacity (FC), 80% FC , 60% FC and 40% FC in pot condition and four levels of water stresses namely well-watered (0MPa) as control, (-0.3 MPa and -0.6 MPa and -0.9 MPa) in laboratory condition. The second factor was turfgrass species. To determine the amount of water requirements for pots, at the beginning of the experiment, the soil FC was determined by pots weighing method. To this end, water was gradually added to the dry soil in a pot after saturation and the withdrawal of excess water, the pots were weighed again.

3.2. Measured Traits

The pots were weighed every two days interval on the specified weight for each treatment, the amount of needed water was added to each pot Irrigation treatments were continued for three months. Then, data collected for physiological traits in May 2018. The leaf pigments, chlorophyll a and b and carotenoid content were quantified according to the protocol of Lichtenthaler and Wellburn (1983). Proline content was determined using the method of Bates *et al.* (1973) and soluble sugars, measured using methods of Irigoyen *et al*. (1992). The leaf relative water content (RWC) determined according to the method of Ritchie *et al.* (1990) and meanwhile, the proxidase and catalase enzyme activities were measured using Elstner *et al.*, (1995) method.

3.3. *Statistical Analysis*

At the end of the experiments, the analysis of variance was carried out and the mean comparisons were made using Duncan's method for all traits. SAS (Ver.9) and Excel (Ver.2016) software were used for statistical analyses.

4. RESULT

4.1. *Physiological traits*

The results of the analysis of variance showed significant effects of species, water stress for often of physiological traits ($p<0.01$). In pot condition interaction effects were significant for all traits exept APX ($p<0.01$).(Table 1). The result of mean comparisons showed, *Cynodon dactylon* and *F. arundinaceae* (Finelawn) with average values of 56.10 % and 55.80 % had higher RWC, respectively, indicating their ability to retain more water in their leaves under drought stress (Table 2). The results of mean comparison between treatments showed that the values of RWC were 55%, 52%, 52 and 47% for FC 100%, FC 80%, FC 60% and FC 40%, respectively and all of species had dissimilar trends for RWC (Table 2). Similar to the results of this study, reported by Farkhondeh *et al*. (2012) that drought stress conditions decresed RWC due to reduced leaf water potential. *Poapratensis* in the 80% FC treatments gave the highest Relative Water Content value in greenhohse condition (Fig 2). For recovery after drought, the highest values 4.01 were observed in *Cynodon dactylon* (Table 2). In comparisons between drought stress levels, the values of 3.86, 3.28, 2.24 and 1.55 were observed in FC 100%, FC 80%, FC 60% and FC 40%, respectively, indicating that recovery after drought values decreased by increasing drought stress (Table 2). The result showed that in all species, a decrease in recovery after drought occurred after an increase in drought (Fig 1). *Cynodon dactylon* in the control treatments (100% FC) gave the highest recovery after drought value in greenhohse condition (Fig 1). For regrowth of cutting, the highest values 4.33 were observed in *Cynodon dactylon* (Table 2). In comparisons between drought stress levels, the values of 4.17, 3.73, 3.49 and 3.42 were observed in FC 100%, FC 80%, FC 60% and FC 40%, respectively, indicating that regrowth of cutting values decreased by increasing drought stress (Table 2). The result showed that in all species, a decrease in regrowth of cutting occurred after an increase in drought (Fig 1). For proline content, the highest and lowest values of 6.29 and 5.52 mgg^{-1}F/W were observed in *Cynodon dactylon* and *F.arundinaceae* (Finelawn) species, respectively (Table 2). In comparisons between drought stress levels, the highest value of 4.73 mgg^{-1}F/W was obtained in FC 40% that was significantly higher than the two other stress levels FC 60% and FC 80%. In Lolium perenne, proline concentrations in four species were all increased under drought stress (Gholamian *et al*., 2019). Indicating the amount of proline content increased with increasing drought stress, but this increase was not linear. Due to the lack of proline content variation among plant species under water stress, all 14 varieties and seven species exhibited a similar trend in carbohydrate levels. The highest values were often observed in the 40% field capacity (FC 40%) treatment (Fig.2). For carbohydrates content, the highest and lowest values of 43.9 and 33.76 mgg^{-1}F/W were obtained in *F.arundinacea* (Talladega) and *Lolium perenne*, respectively (Table 2). In comparisons between drought stress levels, the highest value of 39.84 and 39.29 mgg^{-1}F/W was obtained in FC 40% and FC 100% that was significantly higher than the two other stress levels FC 60% and FC 80%. Indicating the amount of carbohydrates increased with increasing drought stress, Due to the lack of carbohydrates content, species by water stress interaction, all 14 variety and seven species had a similar trend of carbohydrate and the lowest values in often of them was observed in FC 60% (Fig 2). For catalase enzyme activity the highest and lowest values of 29.99 and 29.77 mgg^{-1}F/W were obtained in *Cynodon dactylon* and *F. arundinaceae* (Finelawn), respectively (Table 2). In comparing between four levels of drought stress, the highest value of 29.61 mgg^{-1}F/W was observed in FC 40% that was significantly highest in the four levels of drought stress, in the other words, the amount of this enzyme increased with increasing drought stress, but this increase was not linear (Table 2). For superoxide dismutase enzyme activity (SOD), the highest and lowest values of 1.39 and 0.80 were obtained in *Cynodon dactylon* and *Poa pratensis* (Mardona), respectively (Table 2). In comparing between four levels of drought stress, the highest value was observed in FC 40% and FC 100% that was significantly higher than the two other stress levels FC 60% and FC

80%. The amount of this enzyme decreased with increasing drought stress, but this decrease was not linear (Table 2 and Fig. 2). For Ascorbat Peroxidase enzyme activity (APX), although the highest and lowest values of 7.23 and 5.18 were obtained in *Cynodon dactylon* and Sport sed NL respectively, but the difference between varieties was not significant (Table 2). In comparing between four levels of drought stress, the highest value was observed in FC 40% that was significantly higher than the three other stress levels. The amount of this enzyme decreased with increasing drought stress, but this decrease was not linear (Table 2 and Fig. 2).

4.2. *Leaf pigments*

The results of the analysis of variance showed significant effects of species, for all of leaves pigments. The effects of water stress and species by water stress interaction were significant for all of leaves pigments (Chlorophyll a, chlorophyll b and carotenoid), (Table 2). The results of mean comparing showed that the higher values were obtained in Sport seed NL. (Table 2). In mean comparison between drought stress treatments, the highest and lowest values of chlorophyll a (0.80 and 0.68 mgg^{-1}F/W), chlorophyll b (with 0.47 and 0.43 mgg^{-1}F/W) and carotenoid (with 0.35 and 0.33) were obtained in normal (FC 100%) and severe (FC 40%) stress, respectively (Table 2). Due to the lack of species by water stress interaction, all 14 variety had a similar trend for leaves pigment changes and the highest and lowest values always were observed in FC 100% and FC 40%, respectively (Fig. 1 and Fig. 2). In Lolium perenne, in 25% FC showed the lowest chlorophyll content (12.6 $mg.g^{-1}$ FW) and chlorophyll content was higher at 100% FC in Poa pratensis (44.24 mgg^{-1} FW) compare to other species (Gholamian *et al*., 2019).

Table 1. ANOVA of physiological traits in seven turfgrass species under four levels of drought Stress in pot conditions

S.O.V.	df	Green color	Leaf burn	Recovery After drought	Regrowth of cutting	chlorophyll a	Chlorophyll b	Carotenoid	RWC	Proline	Carbohydrates	Catalase	SOD	APX
Drought (D)	3	0.86 **	14.12 **	30.13 **	3.25 **	0.10 **	0.02 **	0.01 **	266.66 **	1.50 *	119.94 **	77.82 **	0.42 **	26.91 **
Genotype (G)	13	2.18 **	1.55 **	3.05 **	2.42 **	0.08 **	0.03 **	0.01 **	108.03 **	8.46 **	69.94 **	20.60 **	0.22 **	2.56 ns
G x D	39	0.36 **	1.04 **	1.08 **	0.69 **	0.06 **	0.01 **	0.01 **	139.10 **	5.19 **	18.97 **	2.46 ns	0.10 **	1.29 **
Error	168	0.06	0.08	0.11	0.31	0.01	0.00	0.00	22.58	0.43	8.12	4.75	0.01	5.15
%CV		7.98	9.94	12.40	15.11	10.51	7.84	5.80	9.24	14.77	7.53	8.02	10.84	34.50

ns, * and ** are respectively, non-significant and significant at 5 and 1% probability levels.

Table 2. Means of physiological traits in four levels of drought Stress Treatments

Treatments	Green Color Score	Leaf Burn Score	Recovery of drought Score	Regrowth of cutting Score	Chlorophyll a mg g-1F/W	Chlorophyll b mg g-1F/W	Carotenoid mg g-1F/W	RWC	Proline mg g^{-1}F/W	Carbohydrates mg g^{-1}F/W	Catalase U/mingfw	SOD U/mingfw	APX U/mingfw
Drought Stress													
%100 FC	**3.24** a	**3.70** a	**3.86** a	**4.17** a	**0.80** a	**0.47** a	**0.35** a	**54.78** a	**4.40** a	**39.29** a	26.62 b	1.20 a	6.41 b
%80 FC	3.06 b	3.11 b	3.28 b	3.73 b	0.70 b	0.46 a	0.34 ab	51.94 b	4.39 ab	36.83 b	26.42 b	1.01 b	6.13 b
%60 FC	2.89 c	2.63 c	2.24 c	3.49 b	0.67 b	0.43	0.33 b	51.74 b	4.17 b	35.45 b	25.93 b	0.98 b	5.78 b
%40 FC	2.86 c	2.03 d	1.55 d	3.42 b	0.68 b	0.43 b	0.33 b	47.31 c	4.73 a	39.84 a	**29.61** a	**1.21** a	**8.00** a
Species/ cultivar													
Cynodon dactylon	2.68 ef	**3.39** ab	**4.01** a	**4.33** a	0.56 h	**0.49** b	0.32 de	**56.10** a	**6.29** a	40.04 bc	**29.99** a	**1.39** a	**7.23** a
Dichondra repens	3.29 c	1.86 g	2.05 e	2.98 e	0.81 abc	0.46 bc	0.35 c	48.42 def	2.54 i	37.27 cde	26.50 de	0.91 de	6.19 ab
F. arundinaceae (Finelawn)	3.71 ab	**3.46** a	**3.76** a	4.09 abc	0.81 abc	0.48 b	0.35 c	**55.80** a	**5.52** b	**42.51** ab	**29.77** ab	1.20 bc	**7.36** a
F. arundinacea (Starlette)	2.79 de	3.29 abc	3.09 b	**4.20** ab	0.74 cde	0.42 def	0.31 ef	54.87 ab	5.22 b	36.42 def	27.03 cd	1.17 bc	6.69 ab
F.arundinacea (Talladega)	3.03 d	3.11 cde	3.30 b	4.14 ab	0.76 bcd	0.47 bc	0.35 c	54.87 ab	4.60 cde	**43.90** a	29.41 abc	**1.28** ab	6.95 ab
F. rubra (Napoli)	2.94 de	3.04 cd	2.58 d	3.84 abc	0.74 cde	0.45 bcd	0.34 c	54.57 abc	4.81 cd	38.14 cd	27.18 bcd	1.17 bc	6.93 ab
Festuca rubra (Simone)	2.80 de	2.91 de	2.98 bc	4.08 abc	0.73 de	0.46 bc	0.39 b	53.65 bc	4.13 ef	36.71 def	27.00 cd	1.21 bc	6.94 ab
L. perenne (Capri)	2.75 e	2.91 de	2.64 cd	3.96 abc	0.69 efd	0.43 cde	0.31 ef	53.16 bc	4.84 cd	38.41 cd	26.70 de	1.13 c	6.54 ab
L. perenne (Danilo)	2.34 g	3.05 cd	2.44 d	3.60 bcd	0.65 fg	0.40 fg	0.30 efg	49.27 de	3.76 fg	37.58 cde	26.37 de	1.14 c	6.44 ab
L. perenne (Delaware)	2.28 g	2.18 f	2.29 de	3.60 bcd	0.54 h	0.35 h	0.29 g	50.19 b-f	2.88 hi	40.16 bc	27.48 bcd	1.14 c	6.72 ab
Lolium perenne	2.49 fg	2.79 de	2.56 d	3.96 abc	0.60 gh	0.37 gh	0.30 efg	49.57 b-f	4.74 cde	33.76 f	25.94 de	0.98 d	6.78 ab
Poa pratensis (Mardona)	3.50 bc	2.68 e	1.99 e	2.35 f	0.67 efg	0.42 def	0.31 ef	45.35 f	3.32 gh	35.17 def	24.25 e	0.80 e	5.94 b
Sport seed Italy	**3.75** ab	2.64 e	2.31 de	3.48 cde	**0.84** ab	0.48 b	**0.39** b	48.12 ef	4.96 bcd	35.22 def	25.78 de	0.94 d	6.24 ab
Sport seed NL.	**3.81** a	2.81 de	2.28 de	3.21 de	**0.87** a	**0.60** a	**0.41** a	46.27 f	4.30 def	34.65 ef	26.61 de	0.91 de	5.18 b

Means followed by the same letter in in the columns have no significant differences at 5% probability by duncan test.

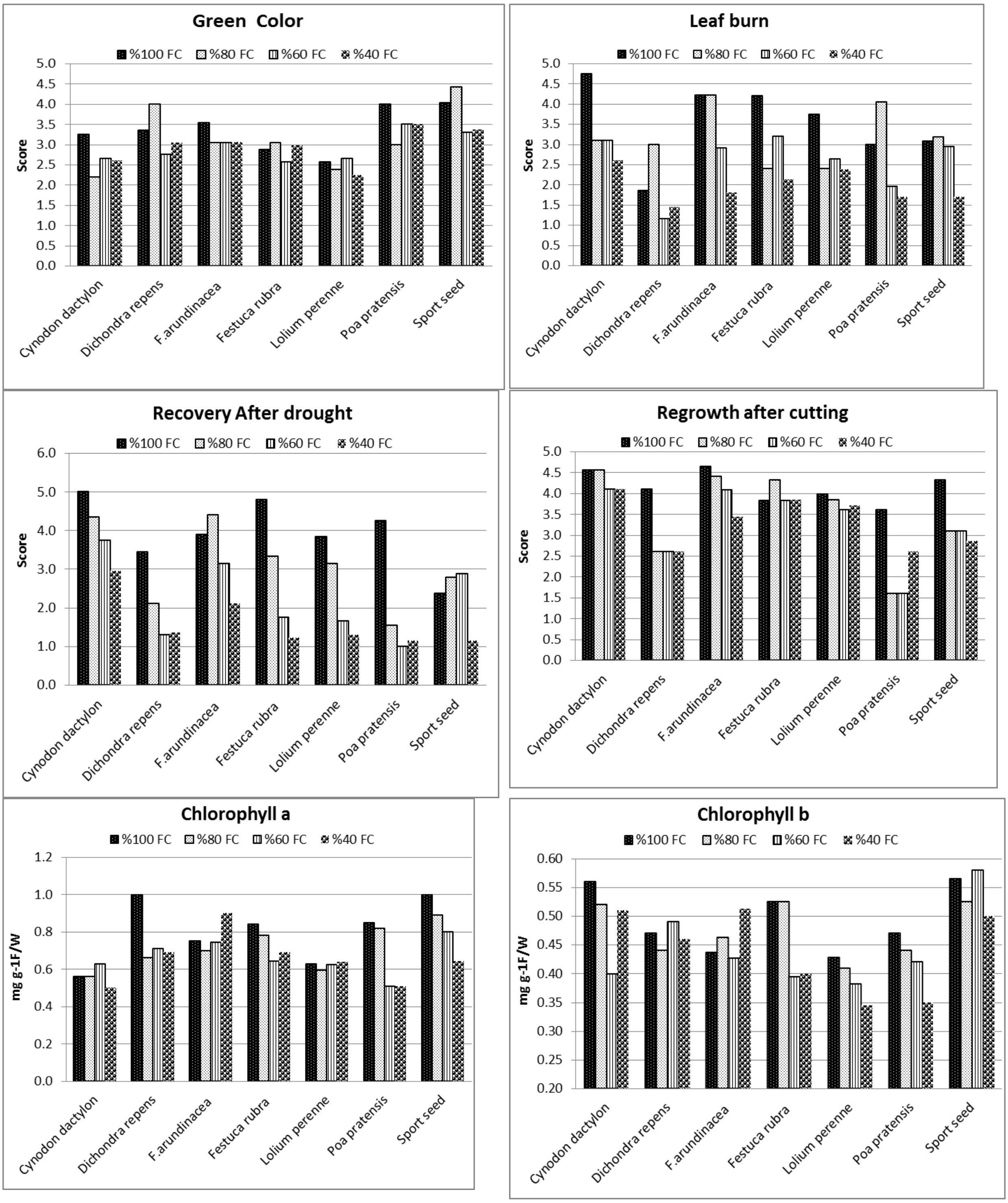

Fig. 1. Means of physiological traits in three turfgrass species under four levels of drought stress in pot conditions.

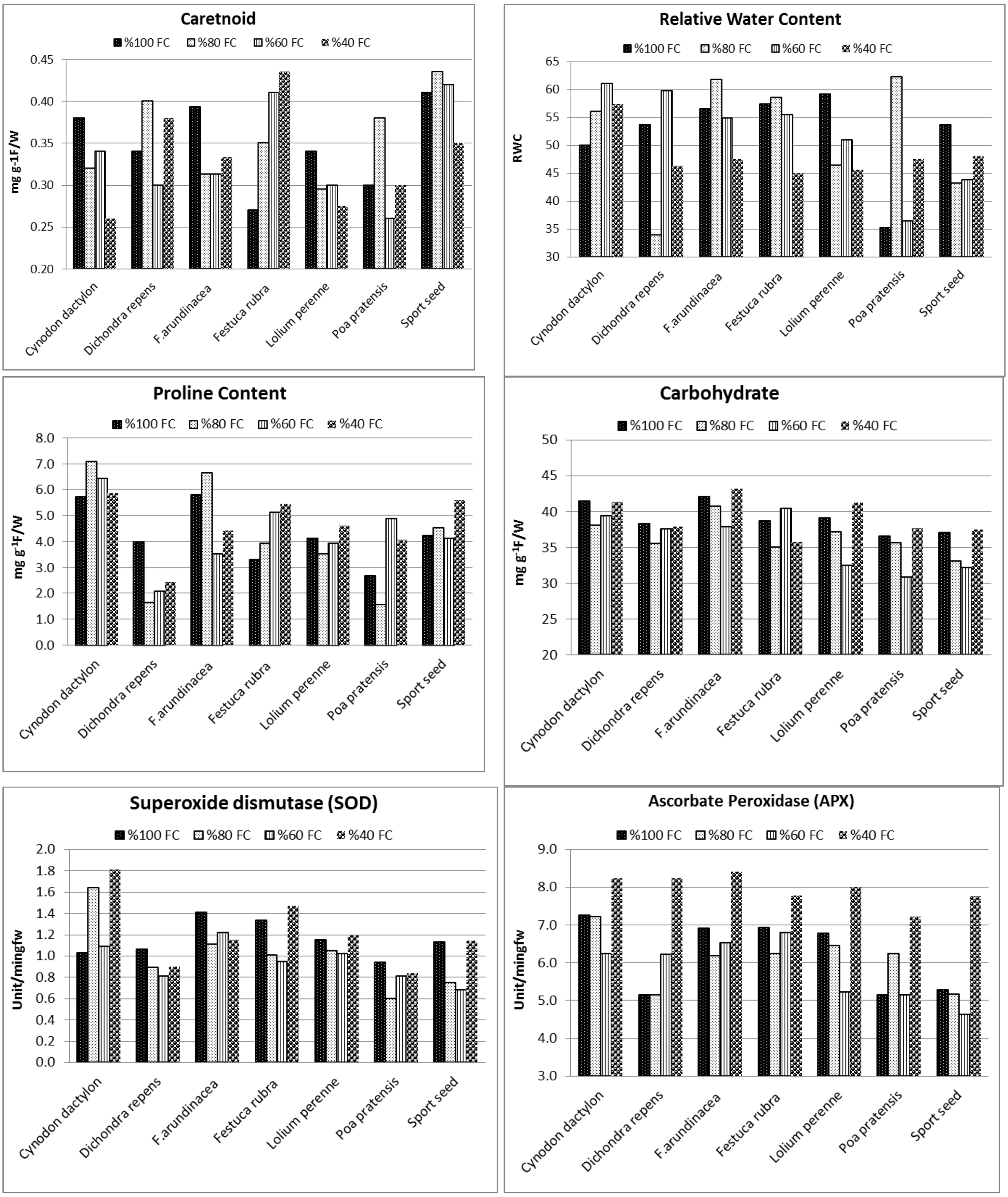

Fig. 2. Means of physiological traits in three turfgrass species under four levels of drought stress in pot conditions.

5. DISCUSSION

For physiological traits the trends of osmotic solutes (proline) and antioxidant enzymes (SOD, APX and catalase) were similar and different levels of drought stress nonlinearly, they caused a change in the amount of traits. But, the RWC and pigments by increasing drought stress values decreased. For RWC, *Cynodon dactylon* and *F. arundinaceae* (Finelawn) with average values of 56.10 and 55.80 had higher RWC than other species, respectively, indicating their ability to retain more water in leaves under drought stress (Table 2). The RWC values were decreased by drought, but, the trend of decreases were not similar in oll of species and the slope of decrease was different in species (Fig 2). Plant recovery is possible by increasing concentrations of solutes in dehydrated cells. In Our study, although, the RWC had decreased down to 60% in severe stress (40% FC), but in all of the species this effect was reversed in the severe stresses. For carbohydrates content, the highest and lowest values of 43.9 and 33.76 mgg-1F/W were obtained in *F.arundinacea* (Talladega) and *Lolium perenne*, respectively (Table 2). Due to the lack of species by water stress interaction, often seven species had a relatively similar trend of carbohydrate and the highest values was observed in FC 40% (Fig 2). Drought stress increases soluble carbohydrate activity in *Dracocephalum moldavica* (Kabiri *et al*., 2018), canola (Mirzaee, 2013), and grape (Meng *et al.*, 2014). The highest and lowest values of 6.29 and 5.52 mgg-1F/W proline content were observed in *Cynodon dactylon* and *F.arundinaceae* (Finelawn) species. The response of species to drought stress were not similar, so that the highest amount of proline was obtained in *Cynodon dactylon* in FC 80% (Fig 2). In mild winters, it has a good recovery rate, it is a popular grass for golf and sports fields (Hanna *et.al*., 2013). Proline accumulation is a common physiological response in many plants in response to drought stress. Similar to our results, drought stress increases proline content in Sainfoin (Veisipoor *et al.*, 2013), grape cuttings (Meng *et al*., 2014) soybean (Ghorbanali and Niakan, 2007). For catalase enzyme activity, the amount of this enzyme increased with increasing drought stress, but this increase was not linear. For Ascorbat Peroxidase (APX), there was no significant species by water stress interaction effect, therefore, the trend of its activity in all seven species were similar and enzyme activity increased by increasing drought stress (Fig 2). For the chlorophyll a, chlorophyll b and carotenoid there was significant difference between the 14 variaty. However, For chlorophyll a, chlorophyll b and carotenoid the higher values were obtained in Sport seed NL. The trends of pigment decreases in all of species were similar and the highest many reports that photosynthesis is limited by drought stress due to stomatal closure (Flexas *et al.*, 2004; Chaves *et al*., 2009; Mafakheri, *et al*., 2010), consequently drought stress decreases the chlorophyll content in plant species.

6. CONCLUSION

The higher values of RWC, carbohydrate, proline and catalase, SOD and APX content observed in Cynodon dactylon show that this species is more tolerant to drought stress than other species and it is recommended for cultivation in green spaces of Iran. In terms of the set of physiological traits, this species has shown the highest resistance to drought in the conditions of drought stress, and it is recommended to cultivate this grass in the conditions of hot and dry weather and limited water resources.

ACKNOWLEDGMENT

The authors thank all colleagues and other participants, who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This study was done by scientific support of Department of Department of Genetics and Plant Breeding, Islamic Azad University, Ahvaz Branch.

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