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Biochemical and physiological evaluations of common bermudagrass [*Cynodon dactylon* (L.) Pers.] Iranian accessions under cold stress

M. Akbari, H. Salehi
Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran.

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(*) **Corresponding author:**
hsalehi@shirazu.ac.ir

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: In this study, one foreign cultivar and forty-nine common bermudagrass accessions were collected from 18 provinces of Iran. Turfgrasses were grown at four temperature regimes (24/17, 7.5/0, -7.5/-12 and -15/-15°C day/night cycles) in a factorial experiment based on the completely randomized design with three replications. Physiological traits were evaluated to categorize all accessions as either cold sensitive or tolerant using Hierarchical Clustering with Ward's method in SPSS software. Our results revealed that cold-tolerant common bermudagrass accessions showed higher proline, protein, antioxidant enzymes, color, visual quality and chlorophyll content and cold-sensitive accessions showed more severe cell membrane damage (EL) under cold stress conditions. Fall in temperature from 24°C severely decreased chlorophyll content, visual quality and color in all accessions. The highest antioxidant enzymes activity, chlorophyll content, color and visual quality at -7.5°C were observed in Taft, foreign cultivar, Naein, Malayear, Aligoudarz, Safashahr and Gorgan accessions. The increase in POD, SOD, CAT and APX activity observed in this study led to protection against oxidative damage caused due to high ROS levels. The most cold-tolerant accessions at -15°C were Taft, Naein and Malayear. Great variations in freezing tolerance were observed between Iranian accessions of common bermudagrass. Further molecular studies are needed to clarify better these findings.

1. Introduction

Cold stress is the main serious problem that limits plant growth, agricultural productivity, survival, as well as geography of plant distribution. Common bermudagrass (*Cynodon dactylon* [L.] Pers.), from the grass (Poaceae) family, is a typical creeping grass grows in warmer parts of all continents between about 45 degrees north and 45 degrees south latitude (Harlan and de Wet, 1969; Anderson *et al.*, 2003). This perennial, herbaceous, warm season, C4 grass is commonly known as 'Chaiar' or

'Margh' in Iran. Common bermudagrass's high density, recuperative ability, high tolerance to drought, heat, salinity, wear, flood and most of soils cause the species to be extensively used in tropical and subtropical regions of Iran. This species is a major turfgrass for livestock herbage, golf courses, sport fields, public parks and soil conservation. Despite its good characters, *C. dactylon* has a considerable tendency to be damaged or killed by frost, especially in transition zone (Munshaw *et al*., 2006). An important process in the winter perpetuity of common bermudagrass is acclimatization, which is an adaptation process to overcome the environmental stresses (Levitt, 1980). The most favorable temperature for root and shoot growth of cool-season turfgrass species varied from 10 to 18°C and 18 to 24°C, respectively. Warm season turfgrasses have C4 photosynthetic pathway and are best adapted to warm climatic region of the world and grows well at temperatures between 24 to 29°C and 27 to 35°C for root and shoot growth, respectively (Beard, 1973). During cold stress, plants exhibit different mechanisms to develop their cold hardiness and increase their freezing tolerance (Zhu *et al*., 2004; Knight and Knight, 2012). Some of these processes includes changes in the concentration of amino acids, sugars, proteins, compatible solutes, certain hormones, and changes in the degree of fatty acid saturation level and antioxidant capacity that affect the freezing tolerance (Karpinski *et al*., 2002; Munshaw *et al*., 2006; Zhang and Ervin, 2008).

Genetic resources and wild plant species that genetically related to cultivated variety have gross value in plant breeding programs (Hajjar and Hodgkin, 2007). Today, many investigations have focused on the naturally occurring genetic differences in stress tolerance of many plants such as *Lolium perenne* L., *Brachypodium distachyon* L. and *Festuca arundinacea* Schreb. (Luo *et al*., 2011; Hu *et al*., 2012; Salehi *et al*., 2013). For many years, improvement of warm season turfgrass quality and cold tolerance are the main goals in breeding programs. Natural populations of bermudagrass should have considerable genetic variation for tolerance to environmental stresses. Since bermudagrass is cosmopolite plant, its considerable genetic variation is predictable. There is great diversity among wild populations and cultivars of common bermudagrass for tolerance to freezing (Anderson *et al*., 2003) and other environmental stresses. In spite of that, there is little data about the cold tolerance of common bermudagrass, and many researches are being conducted to improve cold tolerance of this species (Zhang *et al*., 2011; Shi *et al*., 2015). The aims of the present research were to evaluate Iranian common bermudagrass accessions to find accessions with good freezing tolerance and examine physiological changes during cold stress.

2. Materials and Methods

Forty-nine accessions of natural common bermudagrass were collected from 18 provinces of Iran with different climatic conditions ranging from Shiraz city with subtropical condition and Tabriz city with temperate climatic condition (Fig. 1), and one foreign cultivar 'Blackjack' was used as control. All accessions were collected originally from grasslands, roadside, seaside, and around orchards and agriculture fields, and transferred to the School of Agriculture, Shiraz University, under natural greenhouse condition (52°32' E and 29°36' N, elevation

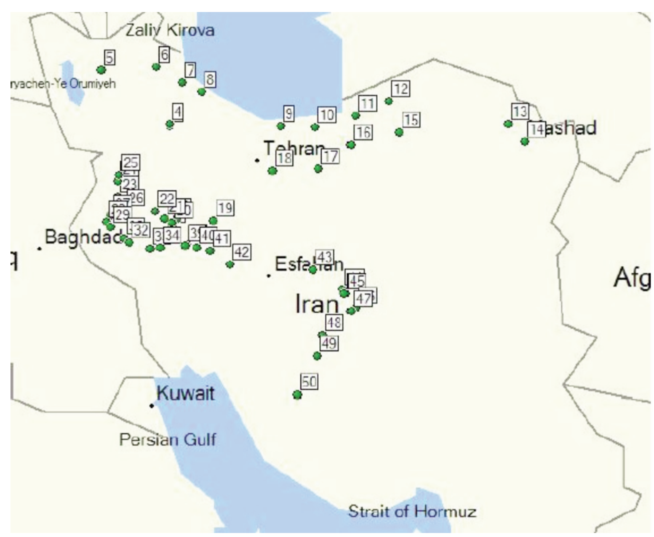

Fig. 1 - Map showing the sampling locations of common bermudagrass accessions from different regions of Iran. 1: Boroujerd, 2: Malayear, 3: Ghidar, 4: Zanjan, 5: Tabriz, 6: Sarein, 7: Talesh, 8: Anzali, 9: Nour, 10: Sari, 11: Gorgan, 12: Minoudasht, 13: Chenaran, 14: Mashhad, 15: Maiami, 16: Damghan, 17: Semnan, 18: Tehran, 19: Arak, 20: Malayear intersection, 21: Nahavand, 22: Firouzan, 23: Kamiaran, 24: Dehgolan, 25: Sanandaj Abidar, 26: Kermanshah Taghbostan, 27: Mahidasht, 28: Islamabad gharb, 29: Homail, 30: Ilam Saymareh bridge, 31: Holailan, 32: Poldokhtar, 33: Mamoulan, 34: Khoram abad, 35: Foreign cultivar, 36: Doroud Nahalestan, 37: Doroud Daneshjo park, 38: Doroud Siahvel, 39: Doroud Babahour, 40: Azna, 41: Aligoudarz, 42: Daran, 43: Naein, 44: Ardakan 1, 45: Ardakan 2, 46: Yazd, 47: Taft, 48: Abarkouh, 49: Safashahr, 50: Shiraz.

1810 m a.s.l.). Each accession was transplanted into 14 cm diameter pots filled with uniform mixture of 1:1:1 (v:v:v) of sand, loamy soil and decomposed manure. Turfgrasses were kept in natural greenhouse condition and were clipped to a height of 5 cm every 2 weeks. Low and freezing temperature treatments were conducted at 24/17, 7.5/0, -7.5/-12 and -15/-15°C day/night cycles and a 10 h light (300 µmol m^{-2} s^{-1}) for 7 days, using a controlled-environment chamber. After each temperature regime, physiological traits including: superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971), catalase (CAT) (Dhindsa *et al*., 1981), ascorbate peroxidase (APX) and peroxidase (POD) (Chance and Maehly, 1995) activities, proline (Bates *et al*., 1973), protein (Bradford, 1976), electrolyte leakage (Saadalla *et al*., 1990) and chlorophyll content (Saini *et al*., 2001) were measured. Turfgrass color and visual quality were rated visually after each treatment (Beard, 1973).

To extract antioxidant enzymes, fresh leaf or stolon samples (0.5 g) were collected and ground to a fine powder in a mortar by adding liquid nitrogen and then homogenized with an ice cold enzyme extraction buffer containing 0.5% polyvinylpyrrolidone (PVP), 3 mM EDTA, and 0.1 M potassium phosphate buffer (pH=7.5). The extracted samples were centrifuged for 10 min at 13500 rpm and 2-4°C and stored on ice until used. The resulting supernatant was used for enzyme analysis. SOD activity was determined according to the procedure used by Beauchamp and Fridovich (1971), CAT activity was determined as described by Dhindsa *et al*. (1981), ascorbate peroxidase and peroxidase activities were determined according to the method described by Chance and Maehly (1995). Proline was determined according to the method described by Bates *et al*. (1973). Using spectrophotometer (UV-120-20, Japan) at 520 nm wavelength, appropriate proline standards were included in calculation of its content in samples. The protein content was quantified using Bradford method with bovine serum albumin (BSA) as standard. Electrical leakage measured, as described by Saadalla *et al*. (1990), using an electrical conductivity meter (Metrohm 644, Swiss) and calculated with the following formula:

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

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

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

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

Turfgrass color and visual quality were measured after each treatment on a 1 to 9 scale where 1 was very poor quality turf, 6 was minimally acceptable turf, and 9 was exceptional turf quality (Beard, 1973). This study was conducted in a factorial experiment based on completely randomize design (CRD) with three replications. Factors were fifty accessions and four different concentrations of low and freezing temperatures (24/17, 7.5/0, and -7.5/-12 day/night cycles). In the case of treatment with -15/-15°C day/night cycles, only the seven most cold tolerant accessions were evaluated. Mean comparisons were performed using the least significant difference (LSD) at P= 0.05 probability level. Physiological traits were evaluated for accession clustering to determine cold sensitive or cold tolerant using Ward's method of Hierarchical cluster analysis in SPSS software.

3. Results

The Ward cluster analysis based on physiological traits before low temperature treatments (at 24/17°C day/night cycles) grouped the 49 accessions and the foreign cultivar into two major groups (Fig. 2). The first group contained 18 accessions with low antioxidant enzymes activity, proline, protein and chlorophyll content including: Abidar Sanandaj, Boroujerd, Holailan, Malayear, Ghidar, Nour, Saymareh bridge, Anzali, Islamabad gharb, Tehran, Tagh bostan, Kermanshah, Homail, Maiami, Minodasht, Mashhad, Poldokhtar, Safashahr and Shiraz. The second group contained other accessions with more antioxidant enzymes activity, proline, protein and chlorophyll content. Two major groups were formed based on physiological characters after cold stress (at 7.5/0°C day/night cycles) (Fig. 3). The first group contained 36 accessions with low proline, protein, chlorophyll content and low antioxidant enzymes activity. Other accessions were in second group and had more proline, protein, chlorophyll content and more antioxidant enzymes activity included fourteen accessions: Arak, Doroud daneshjo park, Azna, Taft, Safashahr, Ardakan 2, Mahidasht, Mamoulan, Naein, Yazd, Chenaran, Semnan, Homail and Daran. The dendrogram from physiological characters after cold stress (at -7.5/-12°C day/night cycles) grouped the 50 accessions in two main clusters (Fig. 4). The dendrogram from physiological characters at -15/-15°C day/night cycles grouped the 7 most cold-tolerant genotypes into two main groups (Fig. 5). The first group contained 3 accessions with lower proline, protein, chlorophyll content and antioxidant enzymes activity than other elite accessions were: Aligoudarz, Gorgan and Safashahr. Other accessions were in second group and had more antioxidant enzymes activity, proline, protein, chlorophyll, color and visual quality, and the least EL included: foreign cultivar, Malayear, Naein and Taft.

POD, CAT, SOD, APX, proline, protein, EL, chlorophyll, color and visual quality were influenced by cold and freeze temperatures. With drop in temperature from 24 to 7.5°C, POD, CAT, SOD, and APX activities, and proline and protein content increased in all accessions; and from 7.5°C to -7.5°C significantly decreased the same parameters. The highest antioxidant enzymes activity, chlorophyll content, color and visual quality at -7.5°C were observed in Taft, foreign

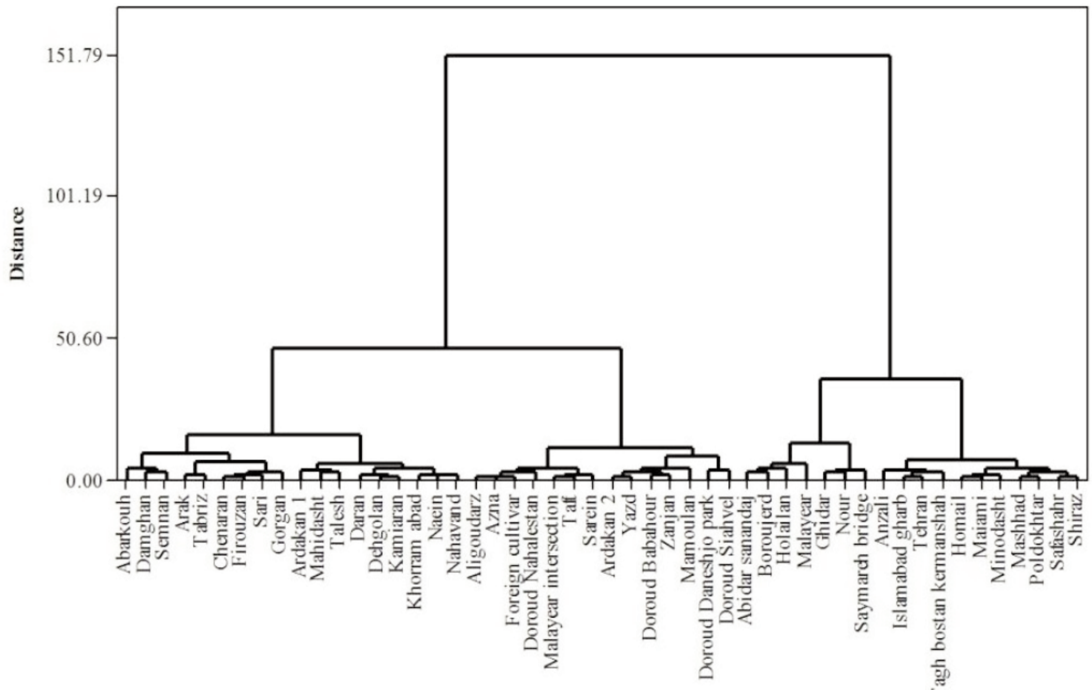

Fig. 2 - Dendrogram of the physiological relationships between 50 accessions of common bermudagrass and control cultivar before cold stress (at 24/17°C day/night cycles).

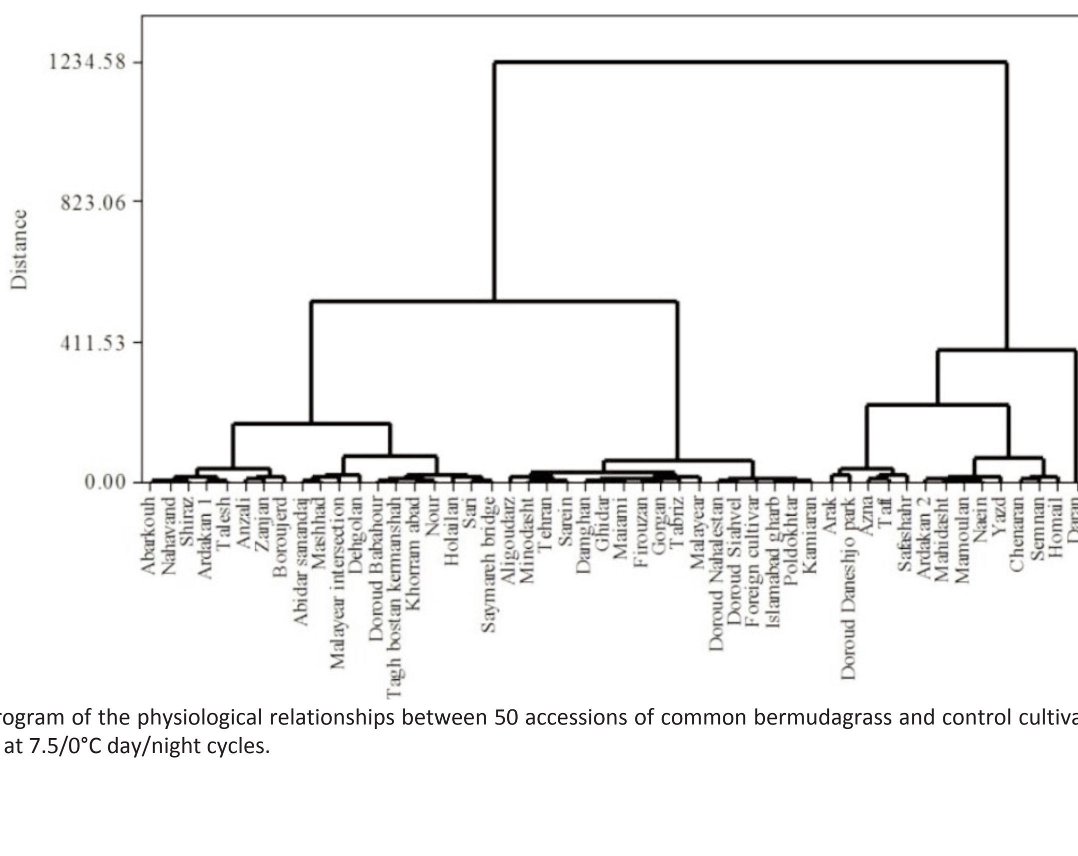

Fig. 3 - Dendrogram of the physiological relationships between 50 accessions of common bermudagrass and control cultivar aftrer cold stress at 7.5/0°C day/night cycles.

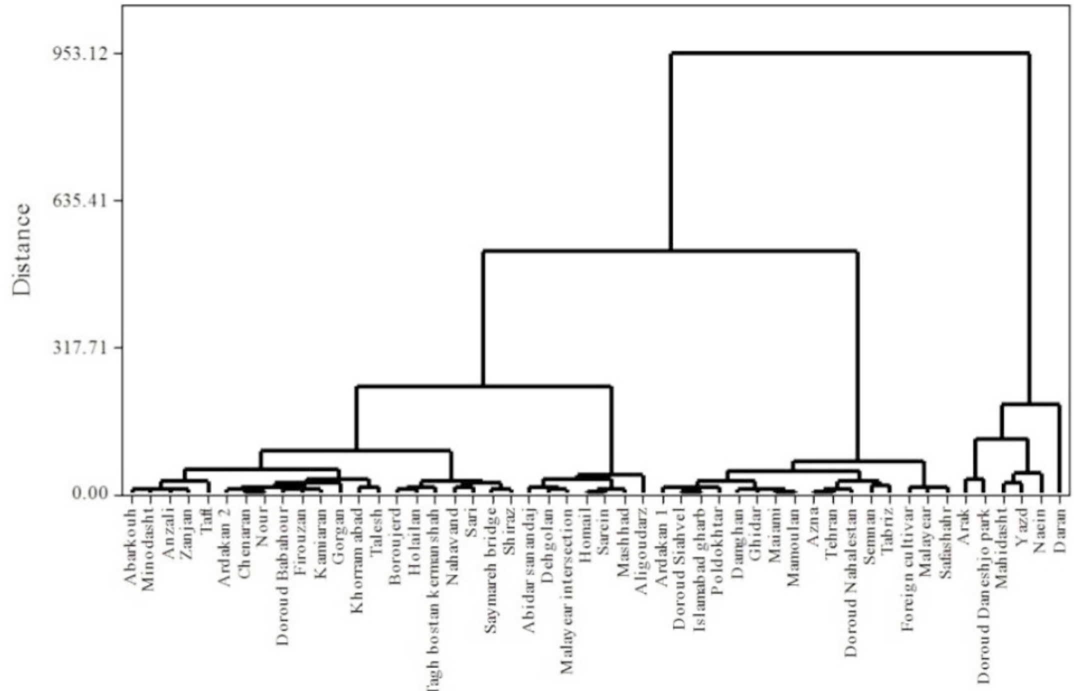

Fig. 4 - Dendrogram of the physiological relationships between 50 accessions of common bermudagrass and control cultivar after cold stress at -7.5/-12°C day/night cycles.

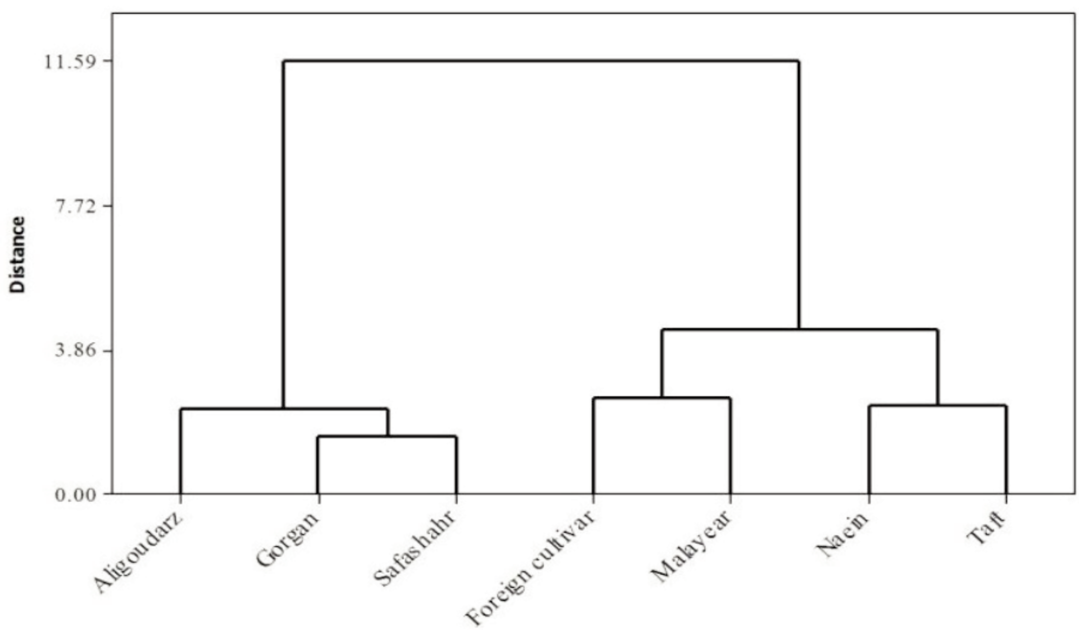

Fig. 5 - Dendrogram of the physiological relationships between 7 cold-tolerant accessions of common bermudagrass after cold stress at -15/-15 °C day/night cycles.

cultivar, Naein, Malayear, Aligoudarz, Safashahr and Gorgan accessions.

Peroxidase

The results of peroxidase assay at 24°C showed that its maximum and minimum activity were belonged to Gorgan and Arak accessions, respectively (Table 1). The highest peroxidase activity at 7.5°C and -7.5°C was observed in Malayear accession. The maximum peroxidase activity at -15°C was observed in Naein accession and the second rank was belonged to common bermudagrass accession collected from Taft.

Catalase

The results of catalase assay at 24°C showed that its maximum activity was belonged to Ghidar and Nour accessions, and its minimum activity was belonged to Abarkouh accession (Table 1). The highest catalase activity at 7.5°C and -7.5°C was observed in Doroud daneshjo park and Taft accessions, respectively (Tables 2 and 3). The maximum catalase activity at -15°C was observed in Taft accession and the second rank was belonged to common bermudagrass accession collected from Malayear (Table 4).

Superoxide dismutase

The results of SOD assay showed that there was difference between SOD activities of different accessions in all temperature regimes. The maximum and minimum superoxide dismutase activity at 24°C was belonged to Doroud nahalestan and Mamoulan

Table 1 - Amount of POD, CAT, SOD, APX, CHL, Proline, EL, protein, color and visual quality of different Iranian accessions and foreign common bermudagrass before cold stress (at 24/17°C day/night cycles)

Location of accession	POD (U mg⁻¹ f.w.)	CAT (U mg⁻¹ f.w.)	SOD (U mg⁻¹ f.w.)	APX U mg⁻¹ f.w.)	CHL	Proline (µmol g⁻¹ f.w.)	EL (%)	Protein (mg g⁻¹ f.w.)	Color	VQ
Doroud Nahalestan	13.26	36.11	120.50	81.25	4.06	13.20	11.90	15.20	7.2	7.1
Abarkouh	13.17	34.12	119.10	79.12	3.68	13.10	13.40	14.30	6.3	6.5
Abidar sanandaj	12.45	35.62	118.60	59.85	3.95	13.20	11.80	14.60	7.1	7.2
Aligoudarz	13.54	36.11	119.50	79.56	4.02	13.40	11.30	14.80	7.0	7.1
Anzali	13.36	34.25	116.40	69.55	3.78	12.80	12.60	14.90	6.2	7.3
Arak	12.11	35.25	119.70	75.25	3.92	13.10	12.70	14.90	7.0	7.1
Ardakan 1	12.26	36.11	116.80	72.12	3.97	12.70	12.30	14.30	7.1	7.2
Ardakan 2	13.21	35.17	117.50	81.21	4.02	13.10	12.50	14.80	7.0	7.0
Azna	13.12	36.24	118.60	80.12	3.96	13.50	11.30	14.90	7.6	8.1
Boroujerd	12.25	35.24	118.50	59.21	4.36	12.10	10.50	14.30	8.5	9.0
Chenaran	12.18	35.14	118.90	78.56	4.35	13.40	11.10	14.30	8.4	8.6
Damghan	12.17	36.24	117.50	77.56	3.81	12.50	13.70	13.80	6.0	6.2
Daran	13.18	35.59	117.30	74.21	4.09	12.90	11.80	13.50	7.0	7.2
Dehgolan	13.11	34.35	117.90	74.32	3.89	13.10	11.60	14.50	7.6	7.2
Doroud Babahour	13.14	36.12	116.50	79.65	3.94	13.40	11.20	15.20	7.8	8.0
Doroud Daneshjo park	13.21	35.59	117.40	84.21	4.41	13.50	10.80	15.30	8.2	8.5
Doroud Siahvel	13.54	36.15	114.50	85.17	4.51	13.30	10.40	15.10	8.5	9.0
Firouzan	12.45	35.14	118.50	77.89	3.89	13.20	11.30	14.30	7.0	7.0
Foreign cultivar	13.55	36.25	119.50	79.81	4.47	13.10	11.20	15.10	8.7	8.5
Ghidar	13.31	36.28	115.80	67.18	3.85	12.70	12.30	13.80	7.7	7.5
Gorgan	13.65	35.17	116.50	77.21	4.38	13.10	10.60	13.90	8.2	8.3
Holailan	12.58	35.18	115.50	58.52	4.12	12.40	12.50	14.50	7.2	7.4
Homail	13.26	35.26	117.80	68.74	3.96	12.90	12.40	14.70	7.6	7.4
Islamabad gharb	13.14	34.16	118.60	69.52	3.76	13.10	13.40	15.30	6.1	6.2
Kamiaran	13.89	34.23	117.60	75.12	4.02	13.40	11.50	14.50	7.2	7.3
Khorram abad	13.23	35.35	115.40	74.25	4.39	12.50	11.90	14.70	8.1	8.4
Mahidasht	13.45	36.21	118.30	72.32	4.32	13.50	10.60	15.10	8.6	9.0
Maiami	12.54	35.25	118.20	68.25	3.98	12.90	11.60	13.90	7.4	7.5
Malayear	13.12	36.12	119.40	63.14	4.18	13.20	11.10	15.20	7.3	8.0
Malayear intersection	13.24	36.25	118.60	81.32	4.32	13.40	11.20	14.50	8.0	8.2
Mamoulan	13.14	35.21	113.70	81.23	3.92	12.30	12.40	14.60	7.4	7.2
Mashhad	13.11	36.15	119.20	69.65	4.32	13.10	10.80	14.40	8.1	8.2
Minodasht	13.14	36.15	117.70	69.54	4.06	13.20	10.90	13.80	7.2	8.4
Naein	13.85	35.16	115.30	75.16	4.06	13.20	11.20	14.20	7.3	7.1
Nahavand	13.55	36.12	116.50	75.14	4.07	13.10	11.70	15.10	7.6	7.4
Nour	12.51	36.28	115.30	65.23	4.41	11.90	13.10	14.80	8.0	7.6
Poldokhtar	12.57	35.18	119.40	69.85	4.37	12.10	11.70	14.80	8.0	8.2
Safashahr	13.35	35.12	118.70	71.21	4.05	13.10	12.20	15.10	7.4	7.5
Sarein	13.15	35.15	119.20	81.25	4.81	13.20	10.90	15.30	9.3	8.4
Sari	13.12	35.21	117.50	78.54	3.96	12.50	11.20	14.60	7.8	8.2
Saymareh bridge	13.21	35.62	117.50	64.21	4.09	12.60	13.10	14.60	7.0	6.7
Semnan	13.32	35.27	116.50	76.5	3.35	12.40	14.70	13.80	5.3	4.2
Shiraz	12.56	35.52	119.10	70.25	3.89	12.80	12.50	14.80	7.1	7.2
Tabriz	12.74	34.18	120.10	75.65	4.38	12.90	11.10	14.60	8.2	7.6
Taft	13.54	36.18	118.60	82.35	4.49	13.20	11.20	14.90	8.3	8.1
Tagh bostan kermanshah	12.46	35.23	117.60	68.65	3.81	12.80	13.10	14.10	6.2	5.7
Talesh	12.18	36.14	117.50	74.32	4.85	13.50	10.80	14.70	9.0	8.1
Tehran	13.21	34.56	118.80	69.95	3.89	12.50	13.80	14.10	6.5	6.2
Yazd	12.96	35.11	116.40	81.24	3.95	12.80	12.60	14.50	7.2	7.5
Zanjan	12.25	35.62	117.20	81.25	3.96	13.40	11.40	15.10	7.5	8.0
LSD (5%)	0.458	0.264	1.860	0.650	0.508	0.320	1.520	0.458	1.452	1.286

Turfgrass visual quality and color based on a scale of 1-9, 1= brown/dead turf, 6= minimal acceptable turf, 9= ideal green, healthy turf.

accessions, respectively. The highest superoxide dismutase activity at 7.5°C and -7.5°C was observed in Doroud daneshjo park accession (Tables 2 and 3). The maximum superoxide dismutase activity at -15°C was observed in Taft accession and the second rank was belonged to common bermudagrass accession collected from Naein (Table 4).

Ascorbate peroxidase

The results of ascorbate peroxidase assay showed that there was difference between ascorbate peroxidase activity of different accessions in all temperature regimes. The maximum and minimum ascorbate peroxidase activity at 24°C was belonged to Doroud siahvel and Holailan accessions, respectively. The

Table 2 - Amount of POD, CAT, SOD, APX, CHL, Proline, EL, protein, color and visual quality of different Iranian accessions and foreign common bermudagrass after cold stress (at 7.5/0°C day/night cycles)

Location of accession	POD (U mg^{-1} f.w.)	CAT (U mg^{-1} f.w.)	SOD (U mg^{-1} f.w.)	APX (U mg^{-1} f.w.)	CHL (mg g^{-1} f.w.)	Proline (µmol g^{-1} f.w.)	EL (%)	Protein (mg g^{-1} f.w.)	Color	VQ
Doroud Nahalestan	20.90	40.86	143.0	125.00	2.12	19.6	59.6	19.1	4.8	4.9
Abarkouh	21.73	42.29	136.0	108.57	2.28	21.9	57.8	21.8	5.7	5.6
Abidar sanandaj	25.64	40.86	132.5	59.29	2.17	20.8	59.3	19.0	5.0	5.1
Aligoudarz	22.18	42.75	138.5	148.57	3.11	26.4	52.5	21.2	7.0	7.1
Anzali	26.39	41.02	147.5	91.43	2.28	21.6	58.2	18.7	5.5	5.6
Arak	23.38	41.17	128.5	249.29	2.31	22.2	56.4	18.4	5.7	5.9
Ardakan 1	22.86	41.12	132.5	105.15	2.27	19.8	57.5	18.5	5.1	5.2
Ardakan 2	22.78	40.96	136.0	185.71	2.31	21.7	56.8	19.2	5.3	5.3
Azna	23.38	42.50	144.0	224.29	2.41	25.3	56.2	20.3	6.0	6.2
Boroujerd	15.19	41.48	140.0	91.43	2.24	21.3	57.4	19.8	5.2	5.3
Chenaran	24.36	43.16	136.0	156.43	2.31	21.9	57.5	18.9	5.8	5.9
Damghan	18.20	41.48	135.5	137.14	2.21	22.3	58.1	19.2	5.1	5.2
Daran	22.78	43.46	139.7	427.14	2.41	27.3	55.1	18.6	6.0	6.2
Dehgolan	23.53	44.69	137.0	42.14	2.34	21.9	58.2	18.6	5.7	5.9
Doroud Babahour	15.94	40.71	132.0	81.43	2.29	21.9	58.2	19.7	5.7	5.6
Doroud Daneshjo park	17.22	44.94	151.0	245.00	2.41	21.6	54.9	20.2	5.9	6.1
Doroud Siahvel	17.82	41.12	137.8	127.86	2.31	21.5	57.5	19.8	5.7	5.8
Firouzan	24.36	43.52	132.5	135.69	2.36	22.4	56.8	19.1	5.7	5.8
Foreign cultivar	22.11	42.24	137.5	122.86	3.28	27.9	51.1	21.5	7.5	7.5
Ghidar	20.08	42.44	138.5	132.71	2.26	21.5	58.5	18.7	5.4	5.5
Gorgan	24.14	41.99	129.5	135.00	2.35	27.3	56.5	18.8	6.4	6.5
Holailan	26.92	42.35	145.5	75.24	2.31	21.9	56.4	18.7	5.8	5.9
Homail	19.40	42.95	142.0	170.00	2.19	19.7	57.2	18.5	5.0	5.2
Islamabad gharb	21.82	41.85	141.4	120.24	2.15	19.5	57.5	18.8	5.0	5.1
Kamiaran	26.62	43.21	139.0	120.00	2.31	21.7	57.4	19.4	5.6	5.5
Khorram abad	24.59	42.55	130.0	85.71	2.35	21.8	57.5	18.7	5.7	5.8
Mahidasht	23.31	44.08	135.5	189.29	2.37	24.9	54.5	18.9	6.1	6.2
Maiami	22.41	41.37	136.0	132.72	2.35	23.2	55.5	18.5	6.0	6.1
Malayear	34.14	41.78	138.0	134.29	3.21	27.2	51.5	21.5	7.4	7.4
Malayear intersection	19.55	41.83	146.0	57.14	2.33	22.4	56.5	19.3	5.8	5.9
Mamoulan	22.78	43.98	139.5	191.43	2.39	24.3	55.5	19.1	6.0	6.1
Mashhad	22.56	42.95	136.0	62.86	2.28	21.5	57.2	19.1	5.7	5.8
Minodasht	20.38	42.09	147.0	140.00	2.25	21.5	57.9	19.1	5.2	5.4
Naein	23.08	42.29	139.0	191.43	3.26	27.5	51.2	18.7	7.5	7.6
Nahavand	24.74	41.22	135.5	110.71	2.26	21.8	57.2	20.8	5.5	5.4
Nour	24.66	42.90	133.5	89.14	2.21	20.7	57.1	18.6	5.0	5.3
Poldokhtar	22.33	40.81	139.2	121.43	2.37	22.5	54.5	18.6	6.0	6.2
Safashahr	21.95	41.53	132.5	212.86	2.38	27.1	53.5	21.3	6.4	6.3
Sarein	23.91	43.31	144.0	140.71	2.31	22.6	56.1	20.5	5.5	5.8
Sari	21.73	43.06	129.5	71.43	2.32	21.8	57.2	18.5	5.8	5.7
Saymareh bridge	25.86	40.96	129.5	75.00	2.34	19.8	57.8	19.4	5.7	5.1
Semnan	23.98	42.18	128.6	161.43	1.86	17.8	59.5	19.5	4.1	4.2
Shiraz	23.08	41.42	137.3	115.29	2.15	19.8	57.8	20.1	5.0	5.2
Tabriz	25.41	41.22	136.5	142.14	2.15	19.8	59.4	18.4	4.9	4.9
Taft	25.26	42.80	142.4	232.14	3.36	27.2	50.5	22.1	7.7	7.7
Tagh bostan kermanshah	21.23	41.46	134.5	81.74	2.14	26.4	59.8	17.1	5.0	4.9
Talesh	21.34	41.36	135.5	102.20	1.15	18.9	62.4	14.9	3.3	3.4
Tehran	19.85	41.27	143.5	140.71	2.24	21.4	57.3	18.7	5.2	5.2
Yazd	25.94	42.65	126.0	184.29	2.11	21.7	58.5	17.4	4.9	4.8
Zanjan	23.76	42.80	145.5	101.43	2.34	26.1	56.1	19.1	6.0	5.9
LSD (5%)	1.014	1.382	7.674	0.458	0.512	0.7279	4.086	1.825	0.988	1.027

Turfgrass visual quality and color based on a scale of 1-9, 1= brown/dead turf, 6= minimal acceptable turf, 9= ideal green, healthy turf.

highest ascorbate peroxidase activity at 7.5°C and -7.5°C was observed in Daran accession (Tables 2 and 3). The maximum ascorbate peroxidase at -15°C was observed in Taft accession and the second rank was belonged to common bermudagrass accession collected from Naein (Table 4).

Total protein

The results presented in Tables 2, 3 and 4 revealed that there was difference between total protein of different accessions in all temperature regimes. The maximum total protein at 24°C was belonged to Doroud daneshjo park, Sarein and

Table 3 - Amount of POD, CAT, SOD, APX, CHL, Proline, EL, protein, color and visual quality of different Iranian accessions and foreign common bermudagrass after cold stress (at -7.5/-12°C day/night cycles)

Location of accession	POD (U mg^{-1} f.w.)	CAT (U mg^{-1} f.w.)	SOD (U mg^{-1} f.w.)	APX (U mg^{-1} f.w.)	CHL (mg g^{-1} f.w.)	Proline (μmol g^{-1} f.w.)	EL (%)	Protein (mg g^{-1} f.w.)	Color	VQ
Doroud Nahalestan	20.90	40.86	143.0	125.00	2.12	19.6	59.6	19.1	4.8	4.9
Abarkouh	21.73	42.29	136.0	108.57	2.28	21.9	57.8	21.8	5.7	5.6
Abidar sanandaj	25.64	40.86	132.5	59.29	2.17	20.8	59.3	19.0	5.0	5.1
Aligoudarz	22.18	42.75	138.5	148.57	3.11	26.4	52.5	21.2	7.0	7.1
Anzali	26.39	41.02	147.5	91.43	2.28	21.6	58.2	18.7	5.5	5.6
Arak	23.38	41.17	128.5	249.29	2.31	22.2	56.4	18.4	5.7	5.9
Ardakan 1	22.86	41.12	132.5	105.15	2.27	19.8	57.5	18.5	5.1	5.2
Ardakan 2	22.78	40.96	136.0	185.71	2.31	21.7	56.8	19.2	5.3	5.3
Azna	23.38	42.50	144.0	224.29	2.41	25.3	56.2	20.3	6.0	6.2
Boroujerd	15.19	41.48	140.0	91.43	2.24	21.3	57.4	19.8	5.2	5.3
Chenaran	24.36	43.16	136.0	156.43	2.31	21.9	57.5	18.9	5.8	5.9
Damghan	18.20	41.48	135.5	137.14	2.21	22.3	58.1	19.2	5.1	5.2
Daran	22.78	43.46	139.7	427.14	2.41	27.3	55.1	18.6	6.0	6.2
Dehgolan	23.53	44.69	137.0	42.14	2.34	21.9	58.2	18.6	5.7	5.9
Doroud Babahour	15.94	40.71	132.0	81.43	2.29	21.9	58.2	19.7	5.7	5.6
Doroud Daneshjo park	17.22	44.94	151.0	245.00	2.41	21.6	54.9	20.2	5.9	6.1
Doroud Siahvel	17.82	41.12	137.8	127.86	2.31	21.5	57.5	19.8	5.7	5.8
Firouzan	24.36	43.52	132.5	135.69	2.36	22.4	56.8	19.1	5.7	5.8
Foreign cultivar	22.11	42.24	137.5	122.86	3.28	27.9	51.1	21.5	7.5	7.5
Ghidar	20.08	42.44	138.5	132.71	2.26	21.5	58.5	18.7	5.4	5.5
Gorgan	24.14	41.99	129.5	135.00	2.35	27.3	56.5	18.8	6.4	6.5
Holailan	26.92	42.35	145.5	75.24	2.31	21.9	56.4	18.7	5.8	5.9
Homail	19.40	42.95	142.0	170.00	2.19	19.7	57.2	18.5	5.0	5.2
Islamabad gharb	21.82	41.85	141.4	120.24	2.15	19.5	57.5	18.8	5.0	5.1
Kamiaran	26.62	43.21	139.0	120.00	2.31	21.7	57.4	19.4	5.6	5.5
Khorram abad	24.59	42.55	130.0	85.71	2.35	21.8	57.5	18.7	5.7	5.8
Mahidasht	23.31	44.08	135.5	189.29	2.37	24.9	54.5	18.9	6.1	6.2
Maiami	22.41	41.37	136.0	132.72	2.35	23.2	55.5	18.5	6.0	6.1
Malayear	34.14	41.78	138.0	134.29	3.21	27.2	51.5	21.5	7.4	7.4
Malayear intersection	19.55	41.83	146.0	57.14	2.33	22.4	56.5	19.3	5.8	5.9
Mamoulan	22.78	43.98	139.5	191.43	2.39	24.3	55.5	19.1	6.0	6.1
Mashhad	22.56	42.95	136.0	62.86	2.28	21.5	57.2	19.1	5.7	5.8
Minodasht	20.38	42.09	147.0	140.00	2.25	21.5	57.9	19.1	5.2	5.4
Naein	23.08	42.29	139.0	191.43	3.26	27.5	51.2	18.7	7.5	7.6
Nahavand	24.74	41.22	135.5	110.71	2.26	21.8	57.2	20.8	5.5	5.4
Nour	24.66	42.90	133.5	89.14	2.21	20.7	57.1	18.6	5.0	5.3
Poldokhtar	22.33	40.81	139.2	121.43	2.37	22.5	54.5	18.6	6.0	6.2
Safashahr	21.95	41.53	132.5	212.86	2.38	27.1	53.5	21.3	6.4	6.3
Sarein	23.91	43.31	144.0	140.71	2.31	22.6	56.1	20.5	5.5	5.8
Sari	21.73	43.06	129.5	71.43	2.32	21.8	57.2	18.5	5.8	5.7
Saymareh bridge	25.86	40.96	129.5	75.00	2.34	19.8	57.8	19.4	5.7	5.1
Semnan	23.98	42.18	128.6	161.43	1.86	17.8	59.5	19.5	4.1	4.2
Shiraz	23.08	41.42	137.3	115.29	2.15	19.8	57.8	20.1	5.0	5.2
Tabriz	25.41	41.22	136.5	142.14	2.15	19.8	59.4	18.4	4.9	4.9
Taft	25.26	42.80	142.4	232.14	3.36	27.2	50.5	22.1	7.7	7.7
Tagh bostan kermanshah	21.23	41.46	134.5	81.74	2.14	26.4	59.8	17.1	5.0	4.9
Talesh	21.34	41.36	135.5	102.20	1.15	18.9	62.4	14.9	3.3	3.4
Tehran	19.85	41.27	143.5	140.71	2.24	21.4	57.3	18.7	5.2	5.2
Yazd	25.94	42.65	126.0	184.29	2.11	21.7	58.5	17.4	4.9	4.8
Zanjan	23.76	42.80	145.5	101.43	2.34	26.1	56.1	19.1	6.0	5.9
LSD (5%)	1.014	1.382	7.674	0.458	0.512	0.7279	4.086	1.825	0.988	1.027

Turfgrass visual quality and color based on a scale of 1-9, 1= brown/dead turf, 6= minimal acceptable turf, 9= ideal green, healthy turf.

Islamabad gharb accessions, and minimum total protein was belonged to Daran accession. The highest total protein at 7.5°C and -7.5°C was observed in Taft accession (Tables 2 and 3). The maximum total protein at -15°C was also observed in Taft accession and the second rank was belonged to common bermudagrass accession collected from Malayear (Table 4).

Proline

Our results revealed that there was difference between proline content of different accessions in all temperature regimes. The maximum proline content at 24°C was belonged to Doroud daneshjo park, Azna, Mahidasht and Talesh accessions, and the minimum proline content was belonged to Nour accession. The highest proline content at 7.5°C and -7.5°C was observed in foreign cultivar and Taft accession, respectively (Tables 2 and 3). The maximum proline content at -15°C was observed in Naein accession and the second rank belonged to common bermudagrass accession collected from Taft (Table 4).

Electrolyte leakage

As shown in Tables 1, 2, 3 and 4, drop in temperature severely increased EL in all accessions. The highest EL at 7.5°C and -7.5°C was observed in Talesh and Abidar accessions, respectively, and the least was seen in Taft accession (Table 2 and 3). The minimum EL at -15°C was observed in Taft accession and the second rank was belonged to common bermudagrass accession collected from Naein (Table 4).

Chlorophyll content, color and visual quality

The highest chlorophyll content, color and visual quality were observed at 24°C and fall in temperature under 24°C severely decreased these characters in all accessions (Tables 1, 2, 3 and 4). The highest chlorophyll content, color and visual quality at -7.5°C were observed in Taft, foreign cultivar, Naein, Malayear, Aligoudarz, Safashahr and Gorgan (Table 3). The maximum chlorophyll content, color and visual quality at -15°C were observed in Taft, Naein and Malayear accessions.

4. Discussion and Conclusions

The 50 *C. dactylon* accessions were clustered into two major groups by Ward's method on the basis of physiological characters at all temperature regimes. Accessions with high POD, CAT, SOD, APX, proline, protein, chlorophyll, color and visual quality, fall in same group. No complete relationships were found between the clustering of the common bermudagrass accessions in dendrograms based on physiological characters and their geographical affiliations. These patterns of physiological variations within common bermudagrass accessions might be due to different genetic background because of various ploidy levels, cross pollination, genetic overlap, germplasm exchange and gene flow. Seven accessions collected from Taft, Naein, Malayear, foreign cultivar, Aligoudarz, Safashahr and Gorgan were the most cold-tolerant genotypes. Because, the highest antioxidant enzymes activity, chlorophyll content, color and visual quality at -7.5°C were observed in these accessions. Iran has a variable climate and we collected these species from different climatic regions of the country that shows its adaptation to wide ranges of climates. The results obtained from our physiological analysis demonstrated that the level of variation was great among Iranian *C. dactylon* accessions. The best color and visual quality in all common bermudagrass accessions were observed before cold stress. Reducing the temperature below 24°C severely decreased chlorophyll content, visual quality and color in all accessions. The results report-

Table 4 - Amount of POD, CAT, SOD,APX, CHL, Proline, EL, protein, color and visual quality of the most cold-tolerant accessions of bermudagrass from Iran after cold stress (at -15/-15°C day/night cycles)

Location of accession	POD (U mg^{-1} f.w.)	CAT (U mg^{-1} f.w.)	SOD (U mg^{-1} f.w.)	APX (U mg^{-1} f.w.)	CHL (mg g^{-1} f.w.)	Proline (µmol g^{-1} f.w.)	EL (%)	Protein (mg g^{-1} f.w.)	Color	VQ
Taft	10.12 a	31.35 a	96.00 a	71.45 c	1.85 a	16.350 a	78.1 d	14.20 a	6.20 a	6.30 a
Naein	10.81 a	26.25 bc	93.86 a	85.39 a	1.74 a	16.41 a	82.3 c	13.70 a	6.10 a	6.00 ab
Malayear	9.55 a	27.28 b	87.08 ab	74.65 c	1.36 e	14.21 ab	87.5 ab	13.90 a	5.36 b	5.30 ab
Foreign cultivar	11.16 a	24.24 cd	76.18 bc	81.26 b	1.24 bc	13.82 ab	85.6 bc	12.80 a	5.10 b	5.10 bc
Aligoudarz	10.13 a	23.36 d	71.22 c	67.25 d	0.94 cd	11.87 b	89.4 ab	11.85 a	4.30 c	4.30 c
Gorgan	8.21 a	23.57 d	74.39 c	52.39 f	0.89 cd	11.68 b	91.2 a	12.10 a	4.20 c	4.50 cd
Safashahr	9.17 a	22.11 d	69.00 c	57.48 e	0.75 d	11.89 b	90.8 a	12.80 a	4.10 c	4.10 d

In each column, means with the same letter are not significantly different according to Least Significant Difference (LSD) test at P= 0.05.
Turfgrass visual quality and color based on a scale of 1-9, 1= brown/dead turf, 6= minimal acceptable turf, 9= ideal green, healthy turf.

ed by Esmaili and Salehi (2012) correspond to the results we obtained. The best temperature for growth and development of tropical grasses is ranging from 27 to 35°C (Beard, 1973). In warm-season turfgrasses, if temperature drops to 10-12.8°C their growth decrease considerably and enters dormancy at close to 0°C (Christians, 2004). McCarty (2001) suggested that a sudden air temperature decline to -5°C or a less rapid fall to below -12°C can cause damage to tropical turfgrasses. Photosynthetic apparatus directly affected by cold stress. In our study, the highest POD, CAT, SOD and APX activity were observed at 7.5°C. As temperature diminished from 7.5 to -15°C, antioxidant enzymes activity decreased. This is in agreement with Manuchehri *et al*. (2014) for *Cynodon dactylon* (California origin). The study made by Zhang *et al*. (2006) on common bermudagrass 'Riviera' and 'Princess-77' showed an increase in the SOD activity during the first seven days of cold acclimation and then after a decline was observed. Cold-acclimatized plants can tolerate freezing stress better than non-acclimated ones due to rapid development of metabolic defenses against freezing stress (Zhang *et al*., 2006). Overall, cold stress produces large amounts of ROS which causes oxidative injury to plants through vast destruction of proteins, carbohydrates, lipids, cellular membranes, DNA and major decline of ATP reserve, and finally cell death (Dionne *et al*., 2001; Gill and Tuteja, 2010). Plants protect their cells from ROS damage by raising the activity of antioxidant enzymes like APX, SOD, CAT and POD (Apel and Hirt, 2004). Results of many studies indicate a positive correlation between freezing tolerance in bermudagrasses and antioxidant enzymes activity (Zhang *et al*., 2006, Manuchehri *et al*., 2014). Over-production of ROS during chilling periods increase oxidative stress and can enhance the activity of antioxidant enzymes and stimulate synthesis of antioxidant metabolites (Karpinski *et al*., 2002). Our results regarding an increase in POD, SOD, CAT and APX activity found in this study is assumed to defense mechanism against oxidative damage caused by cold stress. The central role in the antioxidant defense system is perform by SOD via catalyzing $O_2^{\cdot -}$ into H_2O_2 and O_2, while other antioxidant enzymes are also essential for breakdown of H_2O_2 through various pathways (Mittler, 2002; Apel and Hirt, 2004). CAT can produce O_2 from H_2O_2. APX play a key role in ROS detoxification by conversion of H_2O_2 in to H_2O. The balance between ROS production and elimination is vital mean for the protection of plant cells, and APX plays a major role in maintaining this balance (Asada, 1992; Lin *et al*., 2004). Karpinski *et al*. (2002) showed that tolerant plants have higher antioxidant enzyme activity that has dilatory effect on photooxidative injury during cold stress periods. It is postulated that under cold stress condition, cold tolerant plants increase the activity of their antioxidant enzymes that support active photosynthesis and development of carbohydrate reservation and other compounds such as protein and proline with protective functions. Our findings revealed that higher antioxidant enzymes activity can be attributed to better cold tolerance in common bermudagrass. Decreasing the temperature increased EL, proline and protein content. This negative correlation between temperature and EL can be due to cell membrane damage caused by cold temperatures. The EL method is commonly used to quantify the degree of cell membrane damage induced by cold temperatures and to assay the cold stress tolerance of turfgrasses (Shashikumar and Nus, 1993; Anderson and Taliaferro, 2002). Our results indicated that leaf EL increased during cold acclimatization. Cold acclimatization may induce ROSs production and oxidative stress, and may cause slight damage to cell membrane and later will cause an increase in EL. The production of free radicals under cold stress conditions may initiate the signaling pathways of plant metabolic defense responses, which may reduce cell membrane disruption and EL (Zhang and Ervin, 2008). One of the most important differences between cold tolerant and cold sensitive accessions may be greater development of defense responses to scavenging ROSs and lowering the EL in cold tolerant accessions. Cold acclimation increase proline content in all accessions, but we observed much higher proline content in cold tolerant accessions. This results for proline content in our research, is in accordance with the results presented by Munshaw *et al*. (2004), where they reported a significant increase of stolon proline concentration of the common bermudagrass 'Princess-77' during cold acclimation period. Other supporting results include those of Munshaw *et al*. (2006) and Zhang *et al*. (2006), where they found that higher proline content in bermudagrass cultivars during the winter can be related to greater freezing tolerance. Many studies have pointed to the cryoprotectiveness of proline and their function as osmolytes or compatible solutes (Koster and Leopold, 1988; Santarius, 1992; Karpinski *et al*., 2002). Proline is a cryoprotectant for chloroplast membranes of spinach (*Spinacia oleracea* L.) and plays a crucial role in plant protection against freezing stress (Santarius, 1992). Proline contributes to the acclimatization of plants to cold stress by increasing osmotic potential (reducing the water potential) and decreasing the freezing point of cells. Protein content of all accessions was high during cold acclimation. Higher protein content was observed in cold-tolerant accessions compared to cold-sensitive ones. One of the studies that confirm our results also, is a study by Zhang *et al*. (2006), with their study on *C. dactylon* 'Riviera' as a cold tolerant cultivar with higher protein content, and 'Princess-77' as a cold sensitive cultivar with lower protein content following acclimatization. During cold acclimation plants increase their capacity for synthesis of novel proteins (Cloutier, 1983). This increase in protein content can be attributed to their determinant role in freezing tolerance of common bermudagrass.

Our results showed rapid physiological alterations in common bermudagrass accessions in response to cold stress. Our results also revealed that cold-tolerant common bermudagrass accessions showed higher proline, protein, antioxidant enzymes, color, visual quality and chlorophyll content, and cold-sensitive accessions showed more severe cell membrane damage (EL) under cold stress conditions. We identified drastic natural variations in tolerance between common bermudagrass accessions of Iran in response to freezing stress. Comparative study between bermudagrass accessions based on morphophysiological traits is one of the best method for its cold tolerance improvement. According to our physiological investigations, accessions collected from Taft, Naein and Malayear were the most cold-tolerant genotypes compared to other accessions. Further molecular studies are in progress to clarify better these findings. This is the first report based on physiological characters of Iranian common bermudagrass accessions differences in cold tolerance and provides useful information for breeding programs.

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