Simultaneously evaluation of physiological and biochemical responses in the leaves and roots of Iranian common bermudagrass [Cynodon dactylon L. (Pers.)] accessions under a wide range of temperature fluctuations

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Article

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Abstract

The average temperature of the earth's surface is increasing rapidly, negatively affecting the urban space's green cover. In this study, to identify Iranian common bermudagrass accessions which could endure a wide range of temperature fluctuations, and be identified as cold-heat tolerant accessions, seven cold-tolerant Iranian accessions including Taft, Naein, Malayer, Gardane-Heyran, Aligoudarz, Safashahr, and Gorgan along with Ahvaz accession as a native accession to tropical regions of Iran were subjected to five high-temperature regimes, including 35/30°C (control), 40/35°C (moderate heat stress), 45/40°C (severe heat stress), 50/45°C (extreme heat stress), and 50/50°C (high-extreme heat stress) day/night cycles for 21 days. At the end of this experiment, it was found that Gorgan and Safashahr accessions were able to endure the 50/45°C TR with acceptable turfgrass quality (heat-tolerant accessions). Ahvaz and Aligudarz accessions could withstand extreme heat stress, but their visual quality was weaker than the former group (semi-heat-tolerant). In contrast, Taft, Naein, Malayer and Gardane-Heyran accessions could not tolerate this harsh condition and their shoots were destroyed (sensitive accessions). Evaluation of physiological parameters (Tchl, RWC, EC and RV), osmolytes (proline, TSC and starch) and antioxidants (SOD, APX, CAT and POX) in leaves and roots after moderate heat stress showed that all parameters except RWC and root viability increased in all accessions. With rising temperature in the 45/40°C TR, root viability in sensitive, semi-tolerant and tolerant accessions increased by 170%, 99% and 143% respectively, and its rate in sensitive accessions was almost twice that of tolerant accessions, while there was no significant difference in the amount of stored starch in their roots. In the continuation of the experiment, when the heat stress became extreme, root viability in sensitive accessions was greatly reduced (89%) and its rate was almost 1/4 of that in tolerant accessions. In such a situation, sensitive accessions could only use 4% of their roots' starch reserves, while semi-tolerant and tolerant accessions used 35% and 47%, respectively. These findings provided that heat-tolerant plants with controlling respiratory rate in roots and efficiently breaking down starch storage to carbohydrates could provide the energy required for whole plant metabolic activities. This experiment also highlights the importance of simultaneously investigating evaluated parameters in leaves and roots.

Background

Common bermudagrass [*Cynodon dactylon* L. (Pers.)] is one of the most important typical warm-season grasses extensively [1] which widely used in urban landscaping, sports fields, golf courses, forage, soil stabilization and remediation due to its fast growth rate, trampling and high degrees of tolerance to unfavorable environmental conditions [2–5]. In 2014, the International Panel on Climate Change (IPCC 2014; http://www.ipcc.ch) reported that the number of warm days and nights and the frequency of heat waves have increased in large areas of the world [6,7], and by the end of the 21st century, compared to 1850 to 1900, the earth's surface temperature will have increased by almost 2.6°C in the most optimistic scenario [8]. According to these new global climate change models, plants will soon be exposed to a greater range of heat stress [9]. Heat stress directly results in the denaturation and accumulation of proteins, increasing the fluidity of membrane lipids, and indirectly leads to the inactivation of enzymes in

chloroplast and mitochondria, inhibiting the synthesis and destruction of proteins and the loss of membrane integrity [10,11]. Finally, heat stress limits plant growth and even causes death under extreme conditions [12]. To counteract such detrimental effects, plants use numerous strategies to maintain cellular homeostasis for survival [13,14]. Transcriptomic analysis by RNA-seq in various plant species revealed that the genes involved in carbohydrate, lipid and protein metabolisms, oxidative stress responses, calcium signaling pathway and proline biosynthetic process were up-regulated. In contrast, genes involved in photosynthesis, chlorophyll biosynthesis, cell cycle, and cell wall biosynthesis were down-regulated [12,13,15,16] .In recent decades, studies of the plant adaptation mechanisms to heat stress have shown useful information, but this information is focused on the aboveground organs, and mechanisms involved in root thermotolerance are far less investigated. The root system plays a crucial role in the whole plant's thermotolerance [10,17]. The main roles of roots are to absorb water and nutrients [18] and participate in synthesizing the hormone affecting shoot growth and development. Thus, the main causes of thermal damage to shoots by subjecting roots to high soil temperature are related to the disturbance in the water and nutrients absorption by roots and translocating them to leaves, as well as the disturbance in cytokinin synthesis in roots [19]. Since heat stress severely affects urban space, one of the best strategies for preserving turfgrass quality would be the selection of native heat-tolerant genotypes. With its diverse climate, Iran is one of the centers of genetic diversity in bermudagrass, and valuable plant material with useful genes can be found in it [1,20]. Akbari and Salehi (2018), examining the response of 49 Iranian bermudagrass accessions to cold stress, reported that seven accessions had the highest tolerance to cold stress and can endure the − 7.5/-12°C (day/night) temperature regime well [20]. Therefore, examining Iranian indigenous populations' response to heat stress makes it possible to select accessions that can tolerate a wide range of temperature fluctuations. To address this hypothesis, this comparative research focused on plant physiological and biochemical responses in leaves and roots of eight Iranian common bermudagrass accessions after four long-term high-temperature regimes. Also, due to the importance of root function in whole plant resistance to heat stress, the desired parameters were also measured in leaves and roots simultaneously.

Materials And Methods

Plant Materials and Growth Conditions. In continuation of Akbari and Salehi's research (2018) [20], to select accessions that can withstand a wide range of temperature fluctuations, seven accessions among the 49 Iranian accessions available in the School of Agriculture, Shiraz University, Shiraz, Iran, that had the highest tolerance to cold stress along with Ahvaz accession as a native accession to tropical regions were used in this study (Table S1 and Fig. 1). Every specimen was sampled from each location according to Romani et al. [21]. The permission to collect bermudagrass plants from wild natural resources was obtained from the Iranian Organization of Forests and Rangeland (Government Organization). After collection, the accessions were conserved in Eram Botanical Garden of Shiraz University, Shiraz, Iran (Longitude: 52°31'E, Latitude: 29˚38'N and Altitude: 1798 (m)). Based on phenotypic traits described by Harlan and de Wet [22] and Harlan et. Al. [23] all collected samples were morphologically evaluated to be

common bermudagrass (all plants were identified by corresponding author, an expert in turfgrass science). The voucher specimens were deposited at the Eram Botanical Garden. Stolons (12–20 cm in length with two nodes) of each bermudagrass accession were collected from stock plants and were planted in plastic pots (with a diameter of 12 cm and a depth of 20 cm) filled with a mixture of 1:1:1 (v:v:v) of sand, loam, and decomposed manure. Plants obtained from stolons were kept in a greenhouse (at latitude 31° 20' N and longitude 48° 41' E with an elevation of 22 m) with a maximum temperature of 30°C and a minimum of 20°C, average relative humidity of 50%, and active natural light radiation. To avoid a confounding effect of drought stress, plants were watered daily and hand-clipped every two weeks at a height of 5 cm. After 60 days, all stolons generated roots and grew well. Pots were transferred to the growth chamber (RUMED Light Thermostats, Rubarth Apparate GmbH, Germany). Five temperature regimes were used as 35/30°C (control), 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles for 21 days. During the experiment, the growth chamber was maintained for a light/dark cycle of 14/10 hours, with a photosynthetic photon flux density (PPFD) of 400 µmol m − 2 s − 1, and 60% relative humidity.

Sampling. After each temperature regime, leaf samples were harvested, got frozen with liquid nitrogen, and immediately stored at -80°C until further processing. Root samples had to be washed, so to avoid any new temperature stress, the samples were washed with isothermal double-distilled water at the desired temperature regime and after absorbing excess water, immediately got frozen in liquid nitrogen.

Turf Quality. Turf quality was rated on a scale of 1–9 by visual observations of the plant canopy color, density, and uniformity. 9 represented a healthy and dense green canopy, 1 represented dead plants, and 6 was of minimum acceptable quality [24].

Total Chlorophyll. One g of leaves was thoroughly homogenized in 5ml of 80% acetone and centrifuged (Model K280R, Benchtop Centrifuges Centurion Scientific Ltd., UK) at 4000×g for 5 min. Total chlorophyll was assayed in a spectrophotometer (Jenway, Model 7315, Bibby Scientific Ltd, UK) and calculated as $C_t = 20.2 \times A_{645} + 8.02 \times A_{663}$ [25].

Relative water content (RWC) and Electrolyte leakage (EL). RWC was measured by using 0.2 g of fresh leaves. Fresh leaf blades were soaked overnight in double distilled water and weighed to determine the swelling weight. Swollen leaf blades were oven dried at 80°C for 3 days. RWC was calculated as [(fresh weight - dry weight)/ (threshold weight - dry weight)] × 100 [14]. To estimate cell membrane damage, EL was measured using a conductivity meter (ST3100C, OHAUS Corporation, USA). 0.2 g of fresh leaf and root tips were placed in a plastic tube containing 30 ml of deionized water and shaken overnight at room temperature. Initial conductivity was measured, and tissues were killed by autoclaving at 120°C for 20 min. Killed tissues were incubated in the shaker overnight at room temperature and final conductivity was measured. The EL was calculated as (initial conductivity/final conductivity) × 100 [14].

Root Viability. Root viability was measured using the method of Katuwal et al., 2019 [26]. Fresh root pieces (0.2 g) were incubated in a 5 ml sodium phosphate buffer containing 0.6% 2,3,5-triphenyl tetrazolium chloride (TTC) and 0.05 Triton X-100 at 30°C overnight. Root samples were rinsed with distilled water.

Then formazan was extracted in 5 ml of 95% (v/v) ethanol for 10 minutes at 85°C. The absorbance of the extract was read at the wavelength of 490 nm. Samples were dried, and root viability was determined as absorbance at 490 nm/root dry weight.

Antioxidant Enzyme Activities. One g of leaves and roots were homogenized in 100 mg polyvinylpyrrolidone (PVP) and 3 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM sodium metabisulfite. Supernatants were used to assay the activity of antioxidant enzymes. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the photochemical method as described by Sunker [27]. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitrobluetetrazolium (NBT) reduction as measured at 560 nm. Ascorbate peroxide (APX, EC 1.11.1.11) activity was determined by measuring the decrease in absorbance at 290nm, using the extinction coefficient of 2.8 mM cm^{-1} [28]. Catalase (CAT, EC 1.11.1.6) activity was measured by recording the decrease in absorbance at 240 nm. The activity was by using the extinction coefficient of 39.4 mM cm^{-1} [29]. Peroxidase (POX, EC 1.11.1.7) activity was assayed using the method of Hemeda and Klein [30]. The activity was measured by the increase in absorbance at 470 nm and using the extinction coefficient 26.6 mM cm^{-1}. Total soluble protein was assayed according to Bradford [31].

Proline. Proline content was measured using an acid ninhydrin reagent [32]. 0.1 g of leaf and root samples were homogenized in 5 ml of 80% ethanol. 2ml of glacial acetic acid and 2 ml of acid-ninhydrin were added to the supernatants and incubated at 100°C in a water bath for one hour. The reactions were terminated in an ice bath. Then 4 ml toluene was added to the reaction mixtures. After vortexing, the phases separated, and the absorbance of the upper phase was read at 520 nm. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows: µmol Proline/g of fresh weight = [(µg Proline/ml×ml toluene)/115.5 µg/µmol]/[(g sample)/5].

Total soluble carbohydrates and Starch. After drying the frozen leaf and root samples to remove water, 0.1 g of samples were ground and washed with 100% acetone to remove interfering pigments. The powders were dissolved in 5 ml 80% ethanol and incubated at 80°C in a water bath for 30 minutes. Supernatants were mixed in anthrone reagent and kept at 100°C for 10 min. The absorbance was read at 625nm using a spectrophotometer (Jenway, Model 7315, Bibby Scientific Ltd, UK.). Total soluble carbohydrates were calculated by the standard curve method. To determine the starch content, 3 ml of water was added to the centrifuged pellet to dissolve it at 100°C for 10 minutes, and then 2 ml of 1.1% (v/v) HCL was added to degrade starch and soluble sugars. After centrifugation at 4800 × g for 20 minutes, the same procedures were performed to determine the sugar content of the supernatant, representing the starch content [33].

Statistical Analysis. Treatments were arranged in a split-plot design, where temperature regimes were allocated to main plots and accessions to subplots. Each accession was randomly assigned to three pots within each temperature regime, representing 3 replicates. Data were analyzed using two-way variance analysis (ANOVA) to determine the effects of temperature, accession and their interaction at a confidence level of 95% ($p \leq 0.05$). ANOVA, correlation were assessed by using the SPSS program (IBM SPSS V 260

Chicago, IL, USA), and Tukey's multiple range test for mean comparisons. Minitab software was used for principal component analysis based on a correlation matrix. Heat map analysis and hierarchical clustering for measured parameters and accessions were conducted by one matrix CIMminer (https://discover.nci.nih.gov/cimminer/oneMatrix.do).

Results

All temperature regimes showed significant ($p \leq 0.05$) effects on all measured parameters in all accessions. The ANOVA results are illustrated in Table S2. During this experiment, Gorgan, Safashahr and Ahvaz accessions were able to endure extreme high-temperature regimes, including 50/45°C and 50/50°C TRs, and their shoots and roots survived in those harsh conditions. Although Aligudarz could withstand 50/45°C TR, its visual quality was weaker than the former group. Shoots of Taft, Naein, Malayer and Gardane-Heyran could not tolerate extreme heat stress (50/45°C), their shoots were destroyed, whereas their roots survived.

Physiological responses to high-temperature regimes. physiological traits including Tchl, leaf-EL and root-El increased in all accessions at the 40/35°C TR compared to the 35/30°C TR (control) (Fig. 2). Among accessions, Gorgan recorded the best visual quality (Fig. 2a), the highest amount of Tchl (Fig. 2b), and the lowest amount of root-EL (Fig. 3b). In contrast, Malayer recorded the lowest amount of Tchl. Generally, Gorgan, Safashahr and Aligudarz showed higher Tchl content and visual quality than Taft, Naein, Malayer, Gardane-Heyran and Ahvaz. Conversely, leaf-EL and root-EL were higher in Taft, Naein, Malayer and Gardane-Heyran (Fig. 3). With increasing temperature, during 45/40°C TR in all accessions, Tchl and RWC decreased, while EL increased in both leaves and roots. Gorgan had the highest amount of Tchl and RWC and the lowest amount of root-EL. The 50/45°C and 50/50°C TRs, significantly increased leaf-EL and root-EL compared to the control. Ahvaz depicted the minimum value of Tchl and the maximum value of RWC during 50/45°C TR. During the 50/50°C TR, Gorgan and Safashahr illustrated higher Tchl and RWC, and lower leaf-EL than Ahvaz.

High-temperature regimes affected root viability. During this experiment, root viability varied significantly ($p \leq 0.05$) in all the accessions (Fig. 2d). It illustrated a significant increase in all accessions from the 35/30°C TR up to the 45/40°C TR. In other words, in the temperature range of 35 to 45°C (for the day) and 30 to 40°C (for the night), there was a strong positive correlation between temperature and root viability. In this temperature range, root viability in sensitive accessions, including Taft, Naein, Malayer and Gardane-Heyran was higher than that in the other accessions. With the increasing temperature at the 50/45°C and 50/50°C TRs, root viability decreased in all bermudagrass accessions, and its reduction rate was lower in Gorgan, Safashahr, Ahvaz and Aligudarz accessions than that in other accessions.

Effects of high-temperature regimes on antioxidant enzyme activities. During all temperature regimes, activity of SOD, APX, CAT and POX illustrated significant ($P \leq 0.05$) changes in leaves and roots of all accessions (Fig. 4). Changes in the activities of SOD and APX had a similar trend in this experiment (Fig. 4a and b). During 40/35°C TR SOD and APX activities increased in leaves and roots of all

accessions. With increasing temperature and intensifying heat stress, the activities of these two enzymes gradually decreased. There was no correlation between accession tolerance and the activities of these enzymes in 35/30°C and 40/35°C TRs. But, in the 45/40°C TR where the differences between accessions became apparent, SOD activity in both leaves and roots and APX activity in leaves of Gorgan, Safashahr, Ahvaz and Aligudarz accessions were more than those in Taft, Naein, Malayer and Gardane-Heyran accessions. Also, during the 50/45°C TR, leaf-APX activity in Safashahr and Gorgan was higher than that in Ahvaz and Aligudarz. CAT and POX enzymes showed a different pattern of change compared to SOD and APX (Fig. 5). CAT activity in both tissues increased with rising temperature until the end of the 45/40°C TR (Fig. 5a and b). POX activity revealed different trends in leaves and roots during this experiment (Fig. 5c and d). In leaves, POX activity increased with rising temperature in all accessions (Fig. 5c). While in roots, a clear difference was observed among accessions (Fig. 5d). Root-POX activity increased up to the end of 45/40°C TR. in Taft, Naein, Malayer and Gardane-Heyran accessions. However, with rising temperatures during 50/45°C and 50/50°C TRs, root-POX activity decreased in these accessions. In Aligudarz, Gorgan and Safashahr, root-POX activity increased with increasing temperature and intensification of heat stress, while in Ahvaz root-POX activity was enhanced until the end of 50/45°C TR and then decreased.

Dry weight and total soluble protein. During the experiment, DW varied significantly ($p \leq 0.05$) in all TRs and all accessions. DW of leaves and roots increased in all Iranian accessions after 40/35°C TR compared to the control (Fig. 6). After 40/35°C TR leaf-DW in Naein, Malayer and Gardane-Heyran accessions was lower than that in Taft, Aligudarz, Gorgan, Safashahr and Ahvaz (Fig. 6a). After 40/35°C TR, Gorgan showed the highest amount of leaf-DW and root-DW. At the same time, Naein and Ahvaz recorded the lowest amount of leaf-DW and root-DW, respectively. During high-temperature regimes, including 45/40°C, 50/45°C and 50/50°C, root-DW decreased in all accessions(Fig. 6b). While in high-temperature regimes, leaf-DW showed no significant changes in Gorgan accession (Fig. 6a). TSP also significantly ($p \leq 0.05$) enhanced in leaves and roots of all Iranian accessions at the 40/35°C TR compared to the control (Fig. 7a and b). There was no significant difference among accessions in the leaf-TSP at 40/35°C TR (Fig. 7a). In contrast, the root-TSP in Taft, Naein and Malayer accessions was higher than that in the other accessions (Fig. 7b). During high-temperature regimes, including 45/40°C and 50/45°C, the leaf-TSP and root-TSP decreased in Taft, Naein, Malayer, Gardane-Heyran and Aligudarz accessions. At the same time, Ahvaz, Gorgan and Safashahr did not show a significant difference. Malayer recorded the lowest quantity of root-TSP at the 45/40°C and 50/45°C TRs. At the end of the 50/50°C TR compared to the 50/45°C TR, leaf-TSP in Ahvaz and Gorgan and root-TSP in Ahvaz, Gorgan and Safashahr revealed dramatic reduction. However, leaf-TSP in Safashahr revealed no significant change.

Osmolytes analysis in response to High-temperature regimes. Proline significantly ($p \leq 0.05$) was increased in leaves and roots of all accessions and at 40/35°C TR compared to the control (Fig. 7c and d). At 40/35°C TR leaf-proline in Gorgan, Gardane-Heyran and Safashahr were higher than that of the other accessions (Fig. 7c). During high-temperature regimes, including 45/40°C, 50/45°C and 50/50°C, leaf-proline is reduced in all accessions. In contrast, root-proline showed no significant changes, and the

differences among accessions were insignificant (Fig. 7d). Total Soluble Carbohydrates increased with rising temperature in both leaves and roots of all accessions and exhibited a strong relationship with increasing temperature in Gorgan, Safashahr and Ahvaz accessions esp (Fig. 8a and b). No significant difference ($p \leq 0.05$) was observed among accessions in leaf-TSC during 35/30°C and 40/35°C TRs (Fig. 8a). In contrast, in other high-temperature regimes, the differences among accessions were significant and Safashahr, Gorgan and Ahvaz revealed the maximum amount of leaf-TSC. Root-TSC illustrated significant differences among all accessions during all TRs, and it was higher in Ahvaz, Gorgan and Safashahr than that in the other accessions (Fig. 8b). The effects of high-temperature regimes on starch content were different among accessions and tissues (Fig. 8c and d). Starch enhanced in leaves and roots of all accessions after 40/35°C TR compared to the control, and Gardane-Heyran and Naein recorded the highest and the lowest contents of leaf starch, respectively (Fig. 8c). With further increase in temperature, differences among accessions appeared, and heat-tolerant and heat-sensitive accessions behaved differently. In Taft, Naein, Malayer, Gardane-Heyran and Aligudarz leaf starch content gradually decreased, while in Safashahr, Gorgan and Ahvaz accessions, leaf starch increased at the 50/45°C TR compared to 45/40°C TR. A 5°C increase in night temperature caused leaf starch to significantly reduce even in Gorgan and Safashahr accessions. Root starch illustrated a dramatic reduction in Taft, Naein, Malayer, and Gardane-Heyran at 45/40°C TR compared to 40/35°C TR (Fig. 8d). At the same time, no significant change was observed in root-starch content of these accessions at 50/45°C TR compared to 45/40°C TR. In Gorgan, Safashahr, Ahvaz and Aligudarz, root starch content depicted a contrary trend with a significant decrease at 50/45°C and 50/50°C TRs. Gorgan revealed the minimum root starch content at 50/45°C TR.

Heat map and principal component (PC) analyses. The hierarchical clustering analysis of heat map summarized the response of all measured parameters during the experiment. (Fig. 9). It indicated that the physiological traits, osmolytes and antioxidant enzymes could cluster the eight accessions into two main clusters and four sub-clusters (left of Fig. 9). The first main cluster (A) with low EL, root viability and root starch storage, and high visual quality, Tchl, RWC, antioxidant enzyme activities, TSC and leaf starch storage contained Ahvaz, Gorgan and Safashahr. These accessions could endure high-temperature regimes (50/45°C and 50/50°C TRs). Although Ahvaz could withstand high-extreme heat stress, it was placed in sub-cluster due to lower visual quality, Tchl and antioxidant enzymes activities than those in Gorgan and Safashar (sub-cluster) which could endure extreme heat stress with minimally acceptable turf quality. The second main cluster (B) with high EL and root starch storage, and low visual quality, Tchl, RWC, antioxidant activities, TSC and leaf starch storage contained sub-clusters and . The sub-cluster contained only Aligoudarz which could tolerate the critical temperature regime (50/45°C Day/night cycles) but its shoot was destroyed at the 50/50°C TR. The sub-cluster contained Malayer, Naein, Taft, Gardane-Heyran and Aligoudarz which could not endure the critical temperature regime, and their shoots were completely destroyed. The hierarchical clustering analysis of the heat map also clustered all the measured parameters into two distinct groups (up of Fig. 9). Group a included EL, root viability and root-starch, which showed the rate damage of cell membranes, rate of root respiration and plant ability to supply essential energy to carry out plant metabolic activities, respectively. While Group b included Tchl,

RWC and TSC, leaf-starch, WD as well as proline and antioxidant enzymes activities which show the photosynthetic activity, the ability to retain water in the plant and supply the energy needed by the shoot, as well as the plant's defense power, respectively.

To understand the role of measured parameters in the response of accessions to heat stress, a principal component analysis (PCA) was conducted (Fig. 10). Three PCs were extracted. The first PC explained 47.45% of the variance in the data, and the second PC and third PC explained 22.37% and 12.69% of the variance, respectively. In the biplot of PC analysis, Tchl and RWC as well as WD were positioned in the same direction as antioxidant enzyme activities, TSC, leaf-starch and proline, indicating a positive correlation between the parameters. In addition, these parameters were located close to Gorgan and Safashahr accessions but opposite to Malayer, Taft, Naein and Gardane-Hyran, which further confirmed under heat stress, plant growth was reduced in sensitive accessions. In contrast, EL, root viability and root-starch were close to sensitive accessions and opposite to tolerant accessions.

Discussion

The World Meteorological Organization (WMO) defined heat stress as periods of five consecutive days with air temperatures more than 5°C above the maximum growth temperature [34]. The optimal temperature for the growth of C4 plants has been determined in the range of 27–35°C [35]. On the other hand, the effect of global warming on the rising day and night temperatures is not the same, and at night, it is greater than during days [36]. According to these facts, in this research, the difference between day and night temperature in the investigated regimes was considered 5°C. Thus, these temperature regimes, including 35/30°C, 40/35°C, 45/40°C, 50/45°C and 50/50°C Day/night cycles, were defined as control, moderate heat stress, severe heat stress, extreme heat stress and high-extreme heat stress, respectively. The results showed moderate heat stress increased the amount of chlorophyll (Fig. 2b) and had no negative effect on RWC (Fig. 2c), leading to an increase in the visual quality (Fig. 2a) and the growth (Fig. 6) of all accessions. Hu et. al. (2015) has previously reported such a result on salinity. They showed that moderate salinity induced root growth and reduced their mortality [37]. These data reveal that the useful stress memory stimulated by moderate abiotic stress develops plant defense systems to trigger more effective scavenging mechanisms against subsequent stress [38]. Generally, during moderate heat stress, plants try to stabilize their tissues' water content when abundant water is available in the soil [6]. Despite huge quantities of water in the soil, with intensifying heat stress, a significant decrease in RWC was observed, especially in sensitive accessions (Fig. 2c). Severe heat stress has a negative effect on the root conductance, so by reducing the water uptake by roots and increasing the water loss through the leaves disturbs water balance in whole-plant, leading to a decrease in RWC [39]. Exposure to heat stress and reduced water availability results in a decrease in chlorophyll content [6]. This may be due to either decreased biosynthesis of chlorophyll, or enhanced degradation, or a combined effect of both. In addition, damage of the thylakoid membranes due to heat stress can cause more chlorophyll reduction [40]. Heat-induced reduction of chlorophyll and deactivation of rubisco and rubisco binding proteins reduce photosynthesis and consequently reduce plant growth [41]. In this experiment, a strong negative correlation

was observed between EL (of leaves and roots) and Tchl, RWC, TSP (of leaves and roots) and DW (of leaves and roots) in sensitive accessions (Taft, Naein, Malayer and Gardane-Heyran) and heat semi-tolerant (Aligudarz and Ahvaz) (Tables S3, S4 and S5).

Numerous studies have shown that one of the abilities of plants to cope with heat stress and maintain homeostasis is to change the amount of osmolytes such as carbohydrates, proteins and amino acids during heat stress [42]. Proline is well known for its role as an osmolyte for osmotic adjustment, a signal molecule, and a source of energy [13]. It is also involved in stabilizing cell membranes and proteins, scavenging free radicals, balancing redox potential, and maintaining $NADP^+$/NADPH ratios [43,44]. RNA-seq transcriptome profile in *Agrostis stolonifera* showed that the transcript level P5CS, a key enzyme in the proline biosynthetic pathway, is up-regulated by moderate heat stress [13]. In this study, the amount of proline increased during moderate heat stress compared to the control (Fig. 7c and d). However, as heat stress severity intensified, the proline content in the leaves and roots decreased, because P5CS transcript levels or enzyme activity would be decreased under severe or prolonged heat stress [45–47]. The significant increase in proline content during long-term moderate heat stress (21 days) in this study showed the importance of proline in adapting C*ynodon dactylon* to prolonged moderate heat stress.

Among the antioxidant enzymes, SODs act as the first line of defense against oxidative stress and transform superoxide radicals into hydrogen peroxide. In the next stage, H_2O_2 is detoxified into H_2O by APXs, CATs and POXs [48]. In this experiment, results showed that antioxidant enzyme activities increased in leaves and roots of bermudagrass during moderate heat stress, and accessions were not different (Figs. 4 and 5). But during severe heat stress, where the differences among accessions became apparent, SOD activity in both leaves and roots, APX activity in leaves and POX activity in roots of Gorgan, Safashahr, Ahvaz and Aligudarz accessions were higher than those of Taft, Naein, Malayer and Gardane-Heyran accessions. Studies on heat-acclimated and non-acclimated cool-season turfgrass species proposed that they had lower ROS production because they could increase the activities of different antioxidant enzymes [49]. It has also been reported melatonin (N-acetyl-5-methoxytryptamine) can improve heat tolerance in bermudagrass and kiwifruit plants by promoting antioxidant enzyme activities [50,51]. These results indicate that the antioxidant enzymes play important roles in scavenging the ROS and protecting plants against oxidative stress [52]. Correspondingly, in this study, accessions that exhibited higher activity of SODs, APXs and POXs, had a higher potential for thermotolerance. This claim is confirmed by the lower EL in tolerant and semi-tolerant accessions (Fig. 3). EL is an indicator of cell membrane stability and can be used to select heat-tolerant plants [53,54]. In this experiment Taft, Naein, Malayer, and Gardane-Heyran accessions (in both leaves and roots) showed higher EL than Gorgan, Safashahr, Ahvaz and Aligudarz accessions after 45/40°C temperature regime. This suggests that prolonged severe heat stress affects membrane thermostability of the first group more adversely than others. Therefore, the lower EL of Gorgan and Safashahr under severe heat stress indicates that they can maintain cell membrane stability and be used as a heat-tolerant accession [55].

Measuring root viability is a method of assessing the viability of root tissue based on the positive relationship between the quantity of viable root tissue and the reduction rate of TTC [56]. In this method, the colorless solution of tetrazolium is reduced to a red substance called formazan in the presence of the respiratory enzyme dehydrogenase [57]. Therefore, there is a strong positive correlation between the rate of tetrazolium reduction and the rate of respiration [58]. In this experiment, root viability increased with rising temperature up to 50/45°C TR in all accessions (Fig. 2d). Root viability was higher in heat-sensitive accessions than in heat-tolerant and semi-heat-tolerant accessions. Other researchers reported that during heat stress, the increase in root viability and respiration rate was higher in *Agrostis stolonifera* (a heat-sensitive species) than in *A. scabra* (an adapted species to high-temperature soil) because plants with enhanced thermotolerance could control respiratory rates at a lower rate instead of increasing with temperature and use more efficient respiratory pathways [59]. With a further increase in temperature, root viability decreased in all accessions. The reduction rate was higher in sensitive and semi-heat-tolerant accessions than in heat-tolerant ones. Respiration typically increases with temperature, reaching a maximum of 40–50°C [60]. However, above 50°C or during prolonged periods of 50°C, respiration significantly decreases due to damage of respiration mechanisms. In this experiment, during the 40/35°C TR (moderate heat stress), starch in both leaves and roots increased (Fig. 8c and d) along with increasing the growth (Fig. 6), but with increasing extreme and high-extreme heat stress, starch content increased in leaves and roots. It has shown that during prolonged severe heat stress, starch accumulates in leaves due to reduced strength of sinks or increasing resistance to the transfer of photoassimilates in the phloem, inhibiting the photoassimilates translocation from shoots to roots [19,61]. During the experiment period, with increasing the severity of heat stress, TSC in leaves and roots increased, and the correlation between TSC and temperature was positive (Figs. 8a and b). Heat stress increases respiration while decreasing photosynthesis. Thus, the balance between carbon supply and demand for carbon is disturbed. Under these conditions, starch is broken down into carbohydrates and provides the energy required for plant metabolic activities [62]. The results indicated that despite the higher respiration in sensitive accessions, TSC content in their roots was much lower than in semi-heat tolerant and heat-tolerant accessions after extreme heat stress (50/45°C). While the amount of starch in roots of sensitive accessions was higher than in semi-heat tolerant and heat-tolerant accessions (Fig. 8). Carbohydrate availability in roots is one of the main factors in maintaining root activity in heat stress conditions [63]. Interrupting carbohydrate metabolisms cause root dysfunction and inhibit their activity and plant growth at high soil temperatures [64]. Therefore, to survive at high soil temperatures, roots must be able to use carbon more efficiently when carbon fixation is reduced and carbon supply is limited [19]. In this research, heat-tolerant accessions, including Gorgan and Safashahr, were able to maintain carbohydrate metabolism under severe heat stress and use carbon supply more efficiently to produce acquired energy to maintain plant metabolic activities.

The average temperature of the earth's surface is increasing swiftly [65], negatively affecting the urban space's green cover. In this situation using local genetic variation based on retrieving native populations has been proposed as a useful option to deal with global warming and preserve turfgrass quality. In this

research, to identify tolerant bermudagrass accessions that can endure a wide range of temperature fluctuations, we simultaneously evaluated the physiological, biochemical and osmolyte responses in leaves and roots of eight Iranian natural bermudagrass. The hierarchical clustering analysis of heat map grouped eight accessions into two main clusters (Fig. 9). The first main cluster with lower El and root starch storage and higher visual quality, Tchl, RWC, antioxidant enzymes activity, TSC and leaf starch storage contained Ahvaz, Gorgan and Safashahr which could withstand extreme and high-extreme heat stresses. Gorgan and Safashahr could endure extreme heat stress with minimally acceptable turf quality, and their shoots were destroyed after 2 weeks of high-extreme heat stress, thus they were identified as heat-tolerant accessions. The second main cluster with high EL and root starch storage, and low visual quality, Tchl, RWC, antioxidant activities, TSC and leaf starch storage contained Malayer, Naein, Taft, Gardane-Heyran and Aligoudarz. Malayer, Naein, Taft, and Gardane-Heyran which their shoots were destroyed during extreme heat stress, were identified as heat-sensitive accessions. Aligudarz tolerated extreme heat stress, but could not endure the high-extreme heat stress. Although Ahvaz could withstand high-extreme heat stress, it was identified in the heat semi-tolerant accession like Aligoudarz due to its very low visual quality at extreme heat stress.

Conclusion

Between heat-tolerant accessions, Gorgan was recognized as the best accession for hot regions such as Ahvaz city due to its higher chlorophyll and RWC contents and, consequently, better turfgrass visual quality at high-extreme heat stress. According to Akbari and Salehi (2018), Taft, Naein, Malayer, Aligudarz, Safashahr and Gorgan accession can endure the − 7.5/-12°C (day/night)temperature regime well [20], and this experiment also showed that Safashahr and Gorgan accessions could endure the 50/45°C TR appropriately. It seems that Gorgan and Safashahr can be introduced as cold- and heat-tolerant accessions that could endure a wide range of temperature fluctuations by almost 65°C. Ahvaz accession is widely grown in the southern regions of Iran with tropical climates. But based on this study, it is unsuitable for this region, and Gorgan can be replaced.

Abbreviations

APX: Ascorbate peroxidase; ATP: Adenosine-triphosphate; CAT : Catalase; DW: Drought weight; EDTA: Ethylenediaminetetraacetic acid; EL: Electrolyte leakage; FW: Fresh weight; H2O2: Hydrogen peroxide; NADPH: Nicotinamide adenine dinucleotide phosphate; P5CS: delta-1-pyrroline-5-carboxylate synthase; POX: Peroxidase; ROS: Reactive oxygen species; RWC: Relative water content; RV: Root viability SOD:Superoxide dismutase; SW: Saturated weight; Tchl: Total chlorophyll; TR: Temperature regime; TSC: Total soluble carbohydrates; TSP: Total Soluble Protein; TTC: 2,3,5-triphenyl tetrazolium chloride.

Declarations

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Authors' contributions

ZA performed the experiments, analyzed data and wrote the manuscript; HS assisted with writing and revised the manuscript. MC and ME read the manuscript. All authors have read and agreed on the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Research involving plants

The authors confirm that study complied with local and national regulations for using plants.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

References

1. Huang, S., Wang, C. & Liang, J. Genetic resources and genetic transformation in bermudagrass–a review. *Biotechnology & Biotechnological Equipment* **32**, 1-9 (2018).
2. Zhang, B., Liu, J., Wang, X. & Wei, Z. Full-length RNA sequencing reveals unique transcriptome composition in bermudagrass. *Plant physiology and biochemistry* **132**, 95-103 (2018).
3. Chen, S., Xu, X., Ma, Z., Liu, J. & Zhang, B. Organ-Specific Transcriptome Analysis Identifies Candidate Genes Involved in the Stem Specialization of Bermudagrass (Cynodon dactylon L.). *Frontiers in Genetics* **12** (2021).
4. Xu, X. *et al.* Genetic manipulation of bermudagrass photosynthetic biosynthesis using Agrobacterium-mediated transformation. *Physiologia Plantarum*, e13710 (2022).
5. Fang, Z. *et al.* Integrated transcriptome and proteome analyses provide insight into abiotic stress crosstalks in bermudagrass. *Environmental and Experimental Botany* **199**, 104864 (2022).
6. Fahad, S. *et al.* Crop production under drought and heat stress: plant responses and management options. *Frontiers in plant science*, 1147 (2017).
7. Jagadish, S. K., Way, D. A. & Sharkey, T. D. Plant heat stress: Concepts directing future research. *Plant, cell & environment* **44**, 1992-2005 (2021).

8. Ortiz, A. C., De Smet, I., Sozzani, R. & Locke, A. M. Field-grown soybean shows genotypic variation in physiological and seed composition responses to heat stress during seed development. *Environmental and Experimental Botany* **195**, 104768 (2022).
9. Okereke, C. N. *et al.* Impact of heat stress of varying severity on papaya (Carica papaya) leaves: major changes in stress volatile signatures, but surprisingly small enhancements of total emissions. *Environmental and Experimental Botany* **195**, 104777 (2022).
10. Balfagón, D., Rambla, J. L., Granell, A., Arbona, V. & Gómez-Cadenas, A. Grafting improves tolerance to combined drought and heat stresses by modifying metabolism in citrus scion. *Environmental and Experimental Botany* **195**, 104793 (2022).
11. Zhao, Q. *et al.* Proteomics and phosphoproteomics of heat stress-responsive mechanisms in spinach. *Frontiers in plant science* **9**, 800 (2018).
12. Yu, B. *et al.* Heat Stress Resistance Mechanisms of Two Cucumber Varieties from Different Regions. *International Journal of Molecular Sciences* **23**, 1817 (2022).
13. Xu, Y. & Huang, B. Comparative transcriptomic analysis reveals common molecular factors responsive to heat and drought stress in Agrostis stolonifera. *Scientific reports* **8**, 1-12 (2018).
14. Zhou, Y. *et al.* Small HSPs play an important role in crosstalk between HSF-HSP and ROS pathways in heat stress response through transcriptomic analysis in lilies (Lilium longiflorum). *BMC plant biology* **22**, 1-16 (2022).
15. Min, L. *et al.* Sugar and auxin signaling pathways respond to high-temperature stress during anther development as revealed by transcript profiling analysis in cotton. *Plant Physiology* **164**, 1293-1308 (2014).
16. Lukoszek, R., Feist, P. & Ignatova, Z. Insights into the adaptive response of Arabidopsis thaliana to prolonged thermal stress by ribosomal profiling and RNA-Seq. *BMC plant biology* **16**, 1-13 (2016).
17. Heckathorn, S. A., Giri, A., Mishra, S. & Bista, D. Heat stress and roots. *Climate change and plant abiotic stress tolerance*, 109-136 (2013).
18. Yang, X. *et al.* Primary root response to combined drought and heat stress is regulated via salicylic acid metabolism in maize. *BMC Plant Biology* **22**, 1-18 (2022).
19. Huang, B., Rachmilevitch, S. & Xu, J. Root carbon and protein metabolism associated with heat tolerance. *Journal of experimental botany* **63**, 3455-3465 (2012).
20. Akbari, M., Salehi, H. & Niazi, A. Evaluation of diversity based on morphological variabilities and ISSR molecular markers in Iranian Cynodon dactylon (L.) Pers. accessions to select and introduce cold-tolerant genotypes. *Molecular biotechnology* **60**, 259-270 (2018).
21. Romani, M., Piano, E. & Pecetti, L. Collection and preliminary evaluation of native turfgrass accessions in Italy. *Genetic Resources and Crop Evolution* **49**, 341-349 (2002). https://doi.org:10.1023/A:1020655815121
22. Harlan, J. R. & De Wet, J. Sources of variation in Cynodon dactylon (L). Pers. 1. *Crop Science* **9**, 774-778 (1969).

23. Harlan, J., De Wet, J. & Rawal, K. Geographic distribution of the species of Cynodon LC Rich (Gramineae). *East African Agricultural and Forestry Journal* **36**, 220-226 (1970).
24. Krans, J. V. & Morris, K. Determining a profile of protocols and standards used in the visual field assessment of turfgrasses: A survey of national turfgrass evaluation program-sponsored university scientists. (2007).
25. Arnon, D. I. J. P. p. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. **24**, 1 (1949).
26. Katuwal, K. B., Schwartz, B. & Jespersen, D. Desiccation avoidance and drought tolerance strategies in bermudagrasses. *Environmental and Experimental Botany* **171**, 103947 (2020).
27. Sunkar, R. Plant stress tolerance. *Methods Mol Biol* **639**, 401 (2010).
28. Nakano, Y., Asada, K. J. P. & physiology, c. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. **28**, 131-140 (1987).
29. Aebi, H. in *Methods in enzymology* Vol. 105 121-126 (Elsevier, 1984).
30. Hemeda, H. & Klein, B. J. J. o. F. S. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. **55**, 184-185 (1990).
31. Bradford, M. M. J. A. b. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **72**, 248-254 (1976).
32. Bates, L. S., Waldren, R. P., Teare, I. J. P. & soil. Rapid determination of free proline for water-stress studies. **39**, 205-207 (1973).
33. Zhang, B., Fan, J. & Liu, J. Comparative proteomic analysis provides new insights into the specialization of shoots and stolons in bermudagrass (Cynodon dactylon L.). *BMC genomics* **20**, 1-15 (2019).
34. Wang, D., Heckathorn, S. A., Mainali, K. & Tripathee, R. Timing effects of heat-stress on plant ecophysiological characteristics and growth. *Frontiers in Plant Science* **7**, 1629 (2016).
35. Du, H., Wang, Z., Yu, W., Liu, Y. & Huang, B. J. P. P. Differential metabolic responses of perennial grass Cynodon transvaalensis× Cynodon dactylon (C4) and Poa Pratensis (C3) to heat stress. **141**, 251-264 (2011).
36. Fan, Y. *et al.* Pre-anthesis night warming improves post-anthesis physiological activity and plant productivity to post-anthesis heat stress in winter wheat (Triticum aestivum L.). *Environmental and Experimental Botany* **197**, 104819 (2022).
37. Hu, L. *et al.* RNA-seq for gene identification and transcript profiling in relation to root growth of bermudagrass (Cynodon dactylon) under salinity stress. *BMC genomics* **16**, 1-12 (2015).
38. Ru, C., Hu, X., Chen, D., Wang, W. & Song, T. Heat and drought priming induce tolerance to subsequent heat and drought stress by regulating leaf photosynthesis, root morphology, and antioxidant defense in maize seedlings. *Environmental and Experimental Botany*, 105010 (2022).

39. Morales, D. *et al.* High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biologia Plantarum* **47**, 203-208 (2003).
40. Alghabari, F., Shah, Z. H., Elfeel, A. A. & Alyami, J. H. Biochemical and Physiological Responses of Thermostable Wheat Genotypes for Agronomic Yield under Heat Stress during Reproductive Stages. *Agronomy* **11**, 2080 (2021).
41. Darko, E. *et al.* Differing metabolic responses to salt stress in wheat-barley addition lines containing different 7H chromosomal fragments. *PLOS one* **12**, e0174170 (2017).
42. Shah, Z. H. *et al.* Redox and ionic homeostasis regulations against oxidative, salinity and drought stress in wheat (a systems biology approach). *Frontiers in genetics* **8**, 141 (2017).
43. Ashraf, M. & Foolad, M. R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany* **59**, 206-216 (2007).
44. Qin, Q. *et al.* A maize heat shock factor ZmHsf11 negatively regulates heat stress tolerance in transgenic plants. *BMC plant biology* **22**, 1-14 (2022).
45. Zheng, L. *et al.* Isolation and characterization of a Δ1-pyrroline-5-carboxylate synthetase (NtP5CS) from Nitraria tangutorum Bobr. and functional comparison with its Arabidopsis homologue. *Molecular biology reports* **41**, 563-572 (2014).
46. Rivero, R. M. *et al.* The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. *Plant, cell & environment* **37**, 1059-1073 (2014).
47. Song, S., Lei, Y. & Tian, X. Proline metabolism and cross-tolerance to salinity and heat stress in germinating wheat seeds. *Russian Journal of Plant Physiology* **52**, 793-800 (2005).
48. Wang, X., Huang, W., Yang, Z., Liu, J. & Huang, B. Transcriptional regulation of heat shock proteins and ascorbate peroxidase by CtHsfA2b from African bermudagrass conferring heat tolerance in Arabidopsis. *Scientific reports* **6**, 1-12 (2016).
49. Xu, S., Li, J., Zhang, X., Wei, H. & Cui, L. Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. *Environmental and Experimental Botany* **56**, 274-285 (2006).
50. Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V. & Gómez-Cadenas, A. J. P. p. Plant adaptations to the combination of drought and high temperatures. **162**, 2-12 (2018).
51. Liang, D. *et al.* Melatonin improves heat tolerance in kiwifruit seedlings through promoting antioxidant enzymatic activity and glutathione S-transferase transcription. *Molecules* **23**, 584 (2018).
52. Zhou, R. *et al.* Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. *Environmental and Experimental Botany* **118**, 1-11 (2015).
53. Jiang, Y. & Huang, B. Physiological responses to heat stress alone or in combination with drought: A comparison between tall fescue and perennial ryegrass. *HortScience* **36**, 682-686 (2001).

54. Sailaja, B. *et al.* Comparative study of susceptible and tolerant genotype reveals efficient recovery and root system contributes to heat stress tolerance in rice. *Plant molecular biology reporter* **32**, 1228-1240 (2014).
55. Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R. & Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International journal of molecular sciences* **14**, 9643-9684 (2013).
56. Joslin, J. & Henderson, G. The determination of percentages of living tissue in woody fine root samples using triphenyltetrazolium chloride. *Forest Science* **30**, 965-970 (1984).
57. Jiang, Y. & Huang, B. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop science* **41**, 436-442 (2001).
58. Comas, L. H., Eissenstat, D. M. & Lakso, A. N. Assessing root death and root system dynamics in a study of grape canopy pruning. *The New Phytologist* **147**, 171-178 (2000).
59. Rachmilevitch, S., Xu, Y., Gonzalez-Meler, M. A., Huang, B. & Lambers, H. Cytochrome and alternative pathway activity in roots of thermal and non-thermal Agrostis species in response to high soil temperature. *Physiologia Plantarum* **129**, 163-174 (2007).
60. Hassan, M. U. *et al.* Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—A review. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* **155**, 211-234 (2021).
61. Julius, B. T., Leach, K. A., Tran, T. M., Mertz, R. A. & Braun, D. M. Sugar transporters in plants: new insights and discoveries. *Plant and Cell Physiology* **58**, 1442-1460 (2017).
62. Zhou, R. *et al.* Physiological response to heat stress during seedling and anthesis stage in tomato genotypes differing in heat tolerance. *Journal of Agronomy and Crop Science* **203**, 68-80 (2017).
63. Lambers, H., CHAPIN, F. & Pons, T. Plant physiological ecology springer-Verlag. *New York, USA* (1998).
64. Du, Y. C. & Tachibana, S. Photosynthesis, photosynthate translocation and metabolism in cucumber roots held at supraoptimal temperature. *Journal of the Japanese Society for Horticultural Science* **63**, 401-408 (1994).
65. Ezin, V., Ahanchede, W. W., Ayenan, M. A. T. & Ahanchede, A. Physiological and agronomical evaluation of elite rice varieties for adaptation to heat stress. *BMC Plant Biology* **22**, 1-14 (2022).

Figures

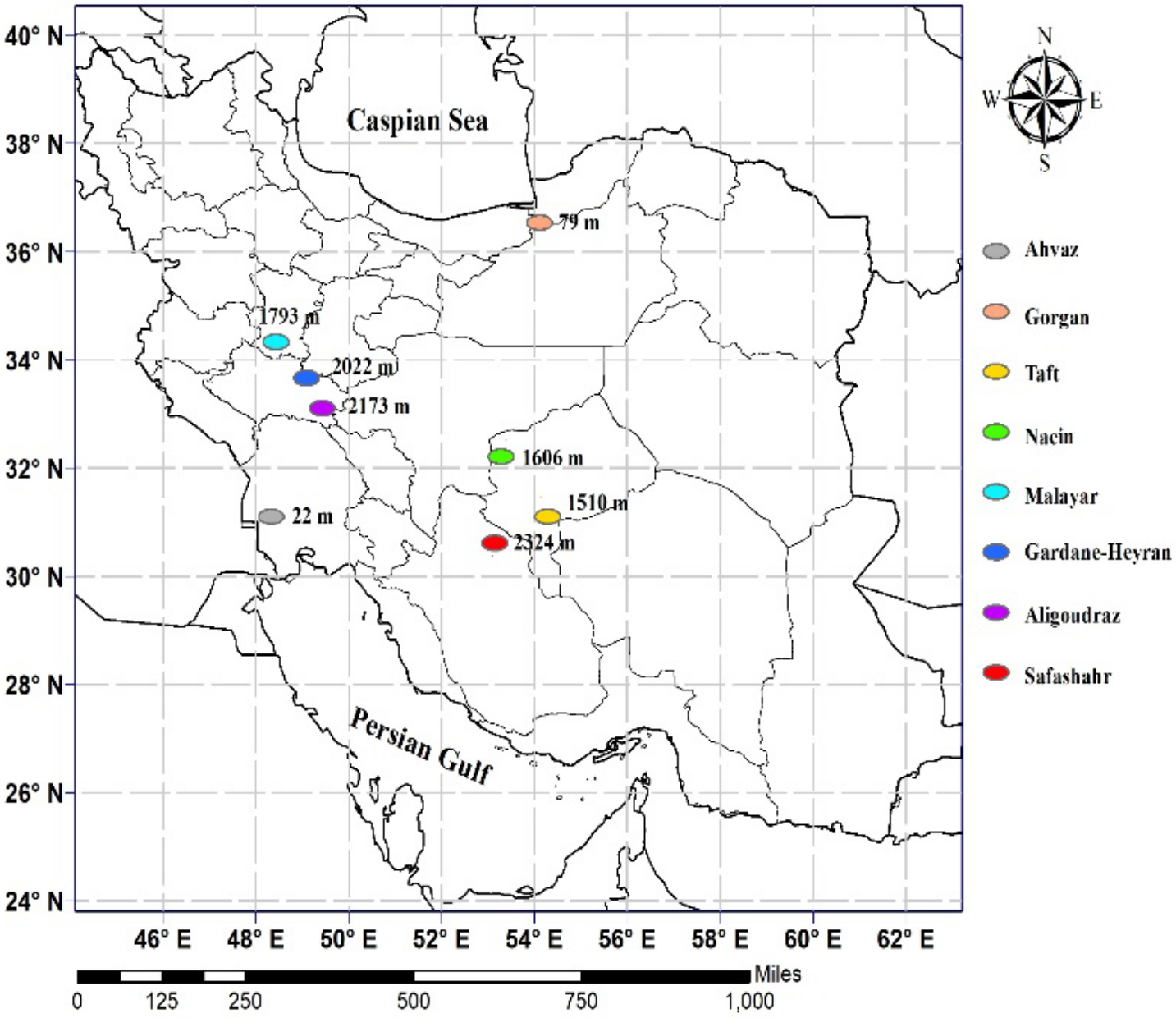

Figure 1

The collection sites of Cynodon dactylon (L.) Pers. accessions used in the present (this figure is created using ArcGIS 10.8; URL: https://support.esri.com/en/products/desktop/arcgis/desktop/arcmap/10-8).

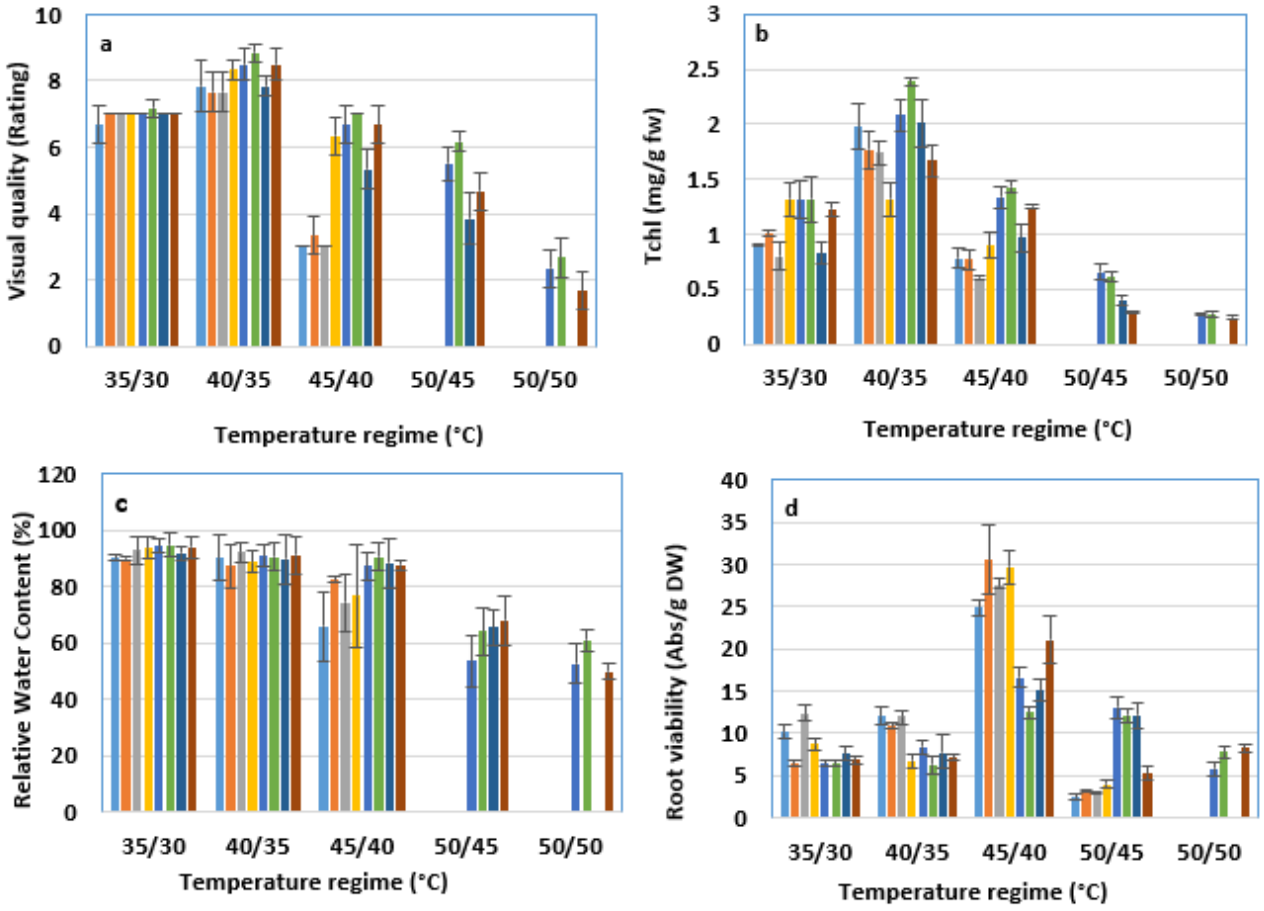

Figure 2. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on physiological traits in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) including Taft ■, Naein ■, Malayer ■, Gardane-Heyran ■, Safashahr ■, Gorgan ■, Aligudarz ■ (as cold tolerant accessions) and Ahvaz ■ (as a native accession to tropical regions): (a) visual quality, (b) Tchl; (c) RWC; (d) root viability. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 2

See image above for figure legend

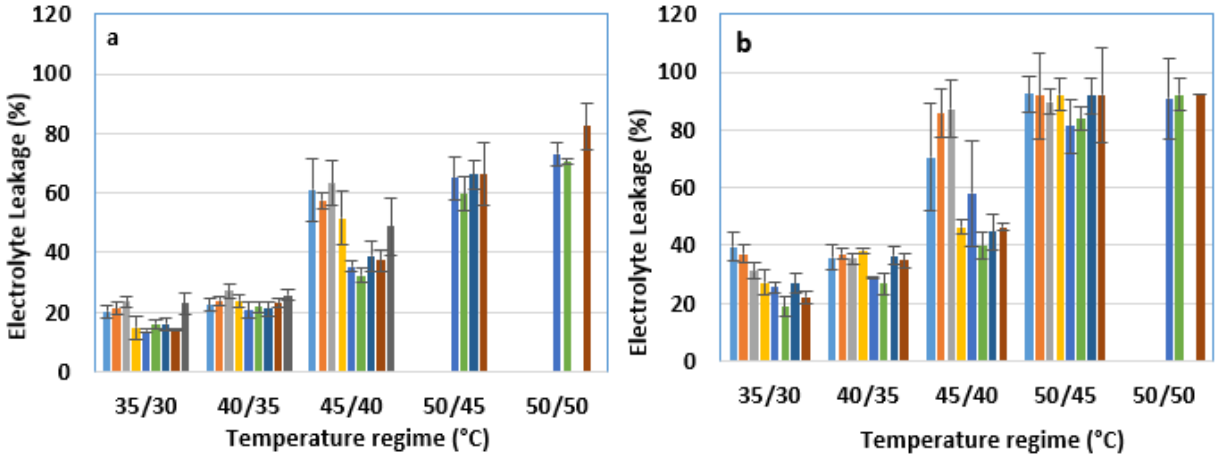

Figure 3. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on electrolyte leakage in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) including Taft, Naein, Malayer, Gardane-Heyran, Safashahr, Gorgan, Aligudarz (as cold tolerant accessions) and Ahvaz (as a native accession to tropical regions): (a) Leaf-EL; (b) Root-EL. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 3

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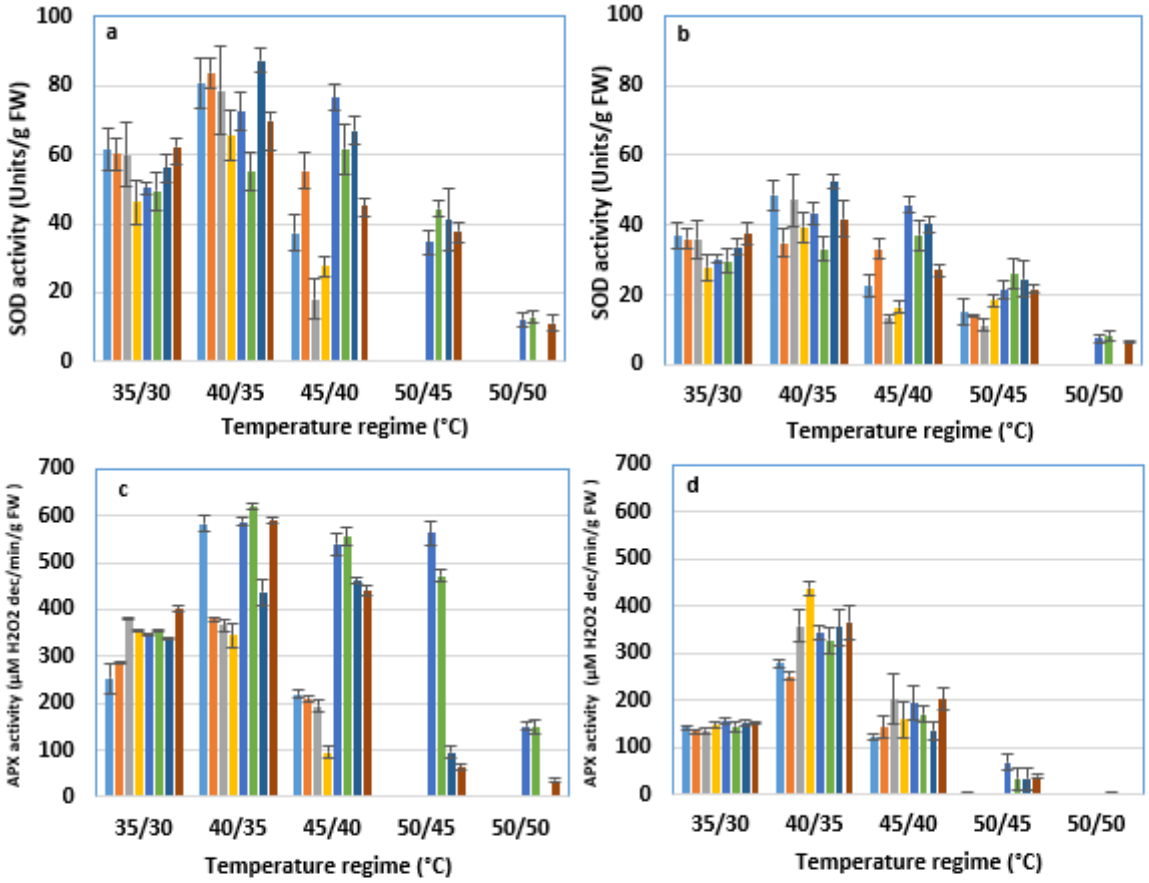

Figure 4. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on superoxide dismutase and ascorbate peroxidase enzyme activities in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) including Taft, Naein, Malayer, Gardane-Heyran, Safashahr, Gorgan, Aligudarz (as cold tolerant accessions) and Ahvaz (as a native accession to tropical regions): (a) Leaf-SOD; (b) Root-SOD; (c) Leaf-APX; (d) Root-APX. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 4

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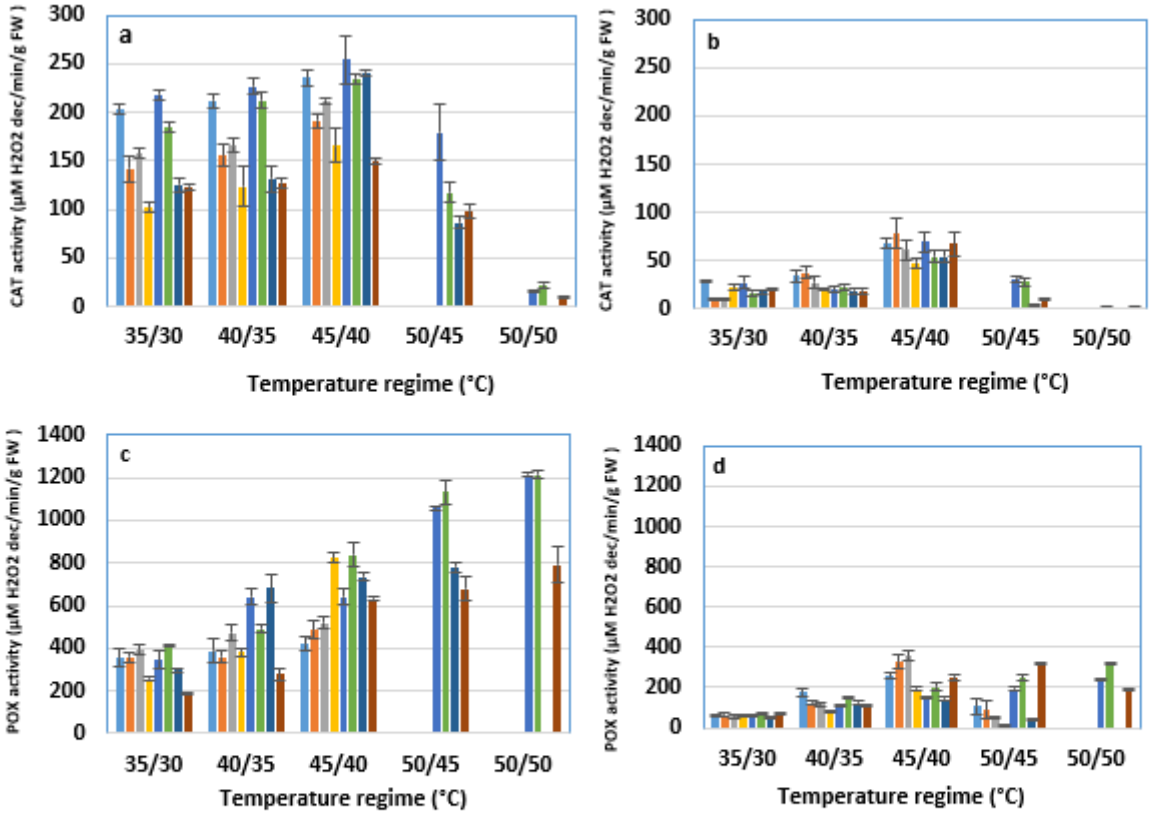

Figure 5. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on catalase and peroxidase enzyme activities in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) including Taft, Naein, Malayer, Gardane-Heyran, Safashahr, Gorgan, Aligudarz (as cold tolerant accessions) and Ahvaz (as a native accession to tropical regions): (a) Leaf-CAT, (b) Root-CAT; (c) Leaf-POX; (d) Root-POX. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 5

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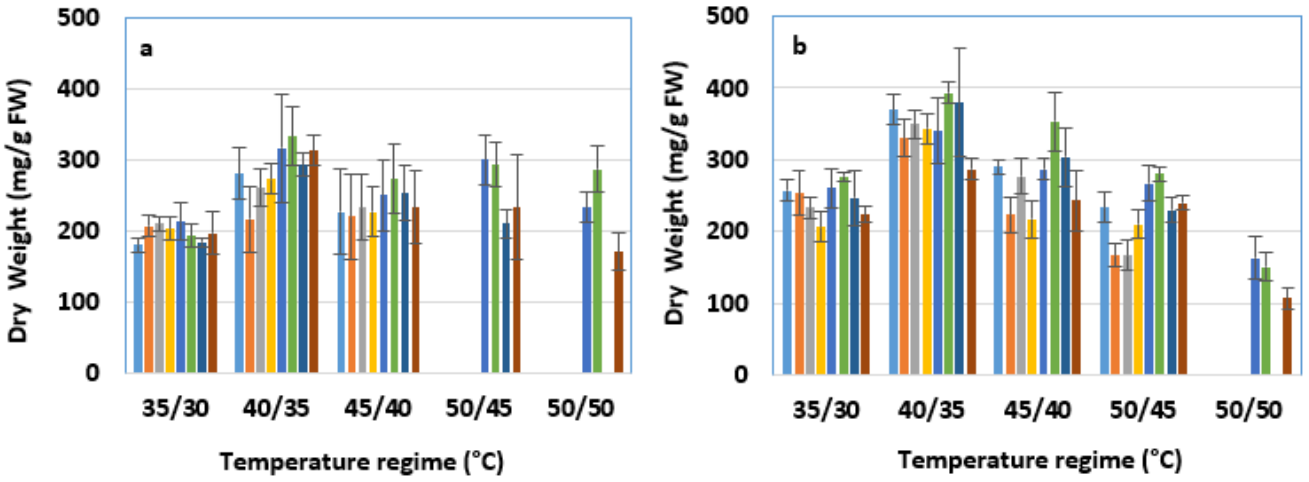

Figure 6. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on drought weight in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) including Taft, Naein, Malayer, Gardane-Heyran, Safashahr, Gorgan, Aligudarz (as cold tolerant accessions) and Ahvaz (as a native accession to tropical regions): (a) Leaf-DW, (b) Root-DW. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 6

See image above for figure legend

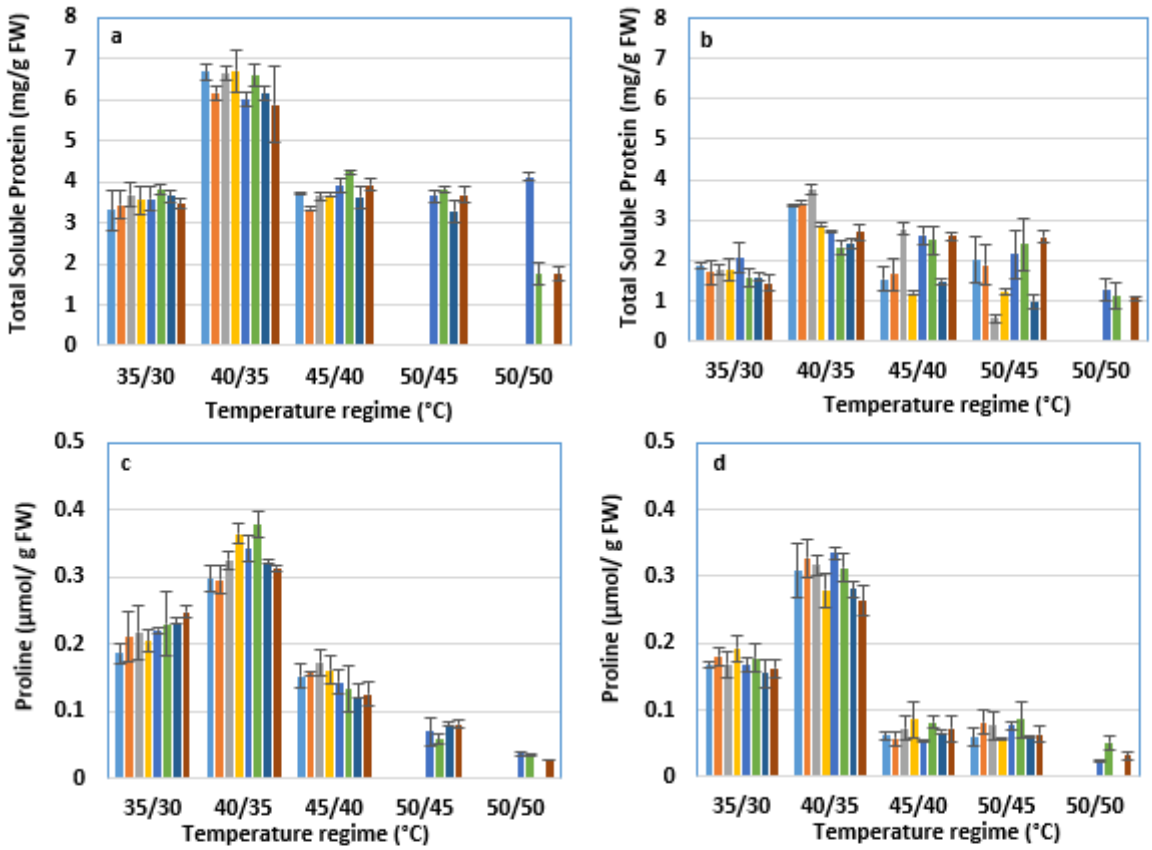

Figure 7. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on total soluble protein and proline contents in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) collected from eight Iranian provinces with different climatic conditions including Taft, Naein, Malayer, Gardane-Hyran, Safashahr, Gorgan, Aligudarz (as cold tolerant accessions) and Ahvaz (as a native accession to tropical regions): (a) Leaf-TSP; (b) Root-TSP; (c) Leaf-Proline; (d) Root-Proline. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 7

See image above for figure legend

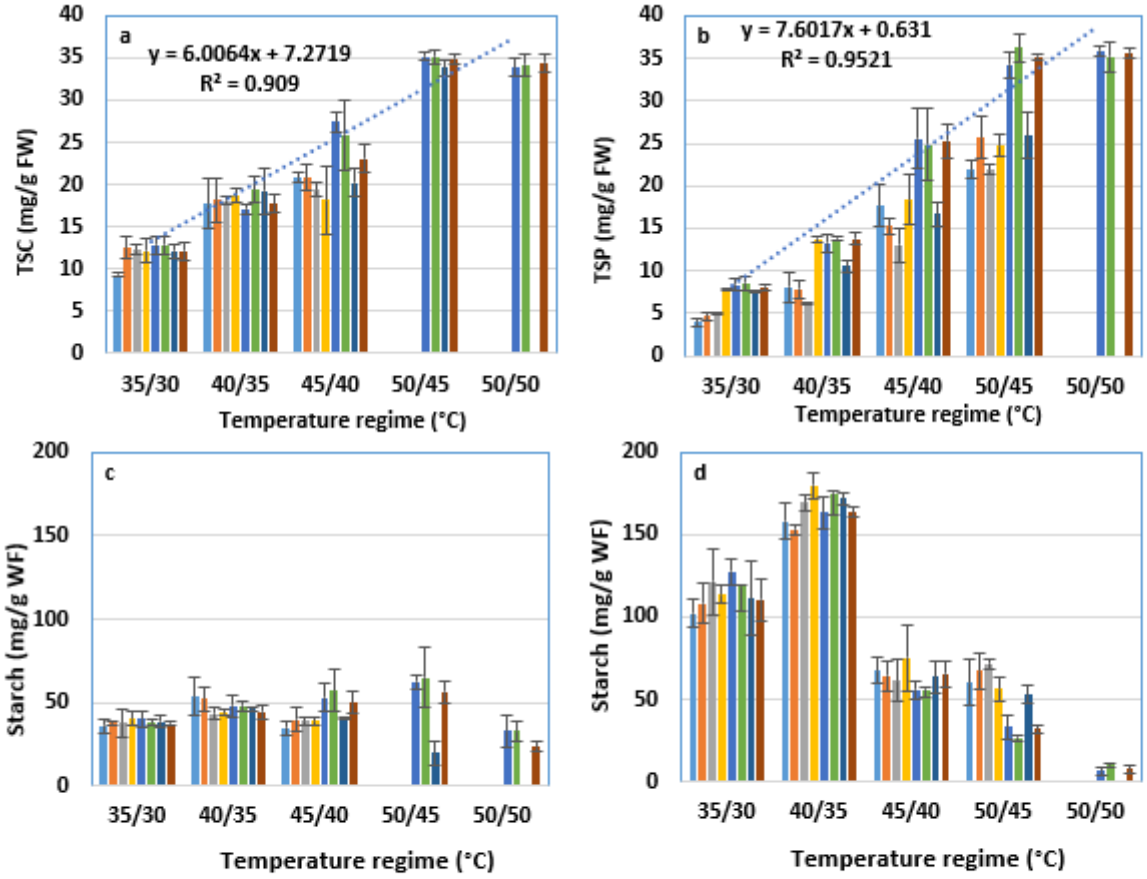

Figure 8. Effects of five temperature regimes including 35/30°C, 40/35°C, 45/40°C, 50/45°C, and 50/50° day/night cycles on total soluble carbohydrates and starch contents in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) including Taft, Naein, Malayer, Gardane-Heyran, Safashahr, Gorgan, Aligudarz (as cold tolerant accessions) and Ahvaz (as a native accession to tropical regions): (a) Leaf-TSC, (b) Root-TSC; (c) Leaf-Starch; (d) Root-Starch. Values indicated are mean ± SD of tri-replicate at $p \leq 0.05$ after 21 days of temperature treatment imposition.

Figure 8

See image above for figure legend

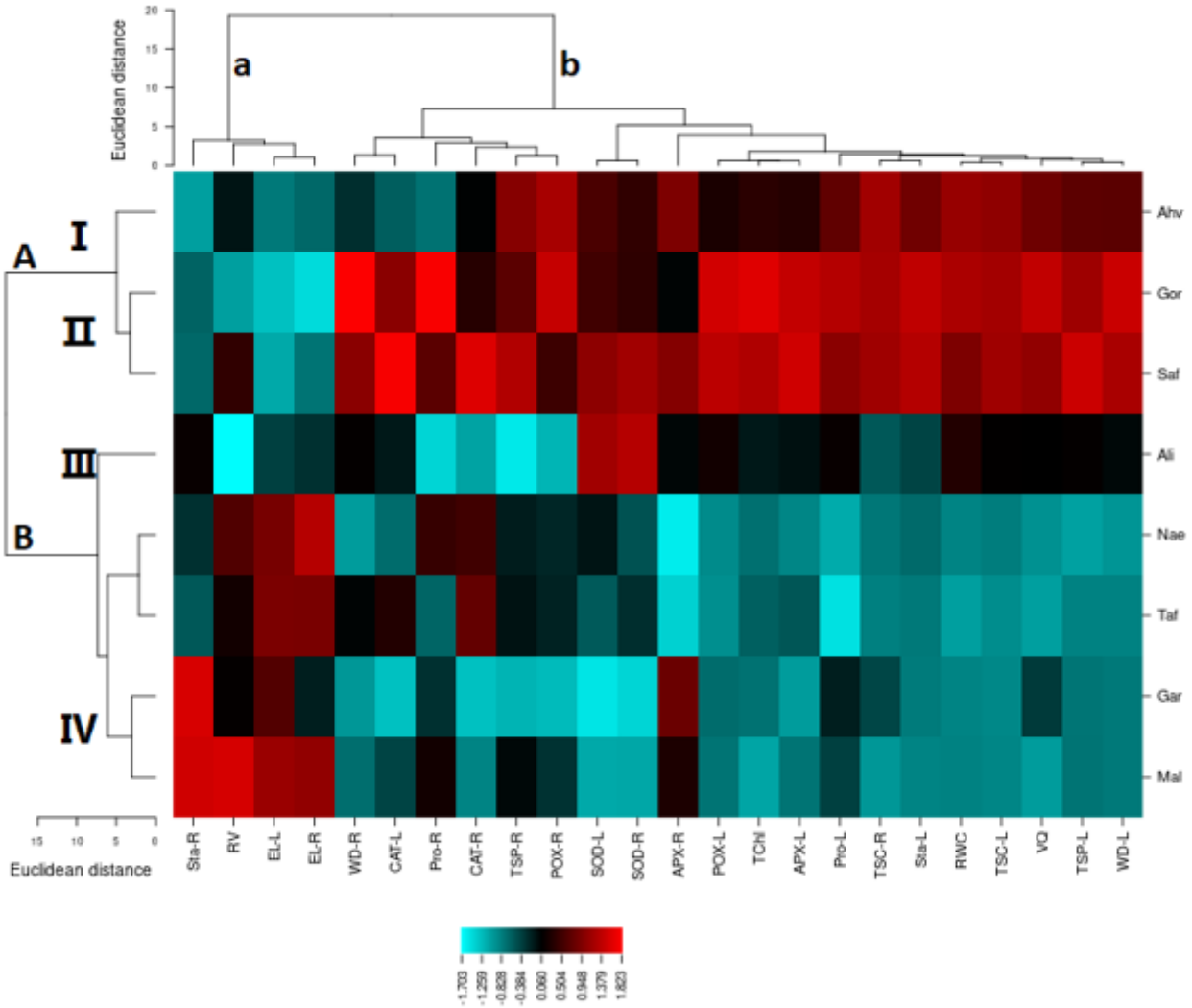

Figure 9

the heat map hierarchical clustering analysis for 14 physiological, biochemical and osmolytes parameters in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) under high-temperature regimes conditions.

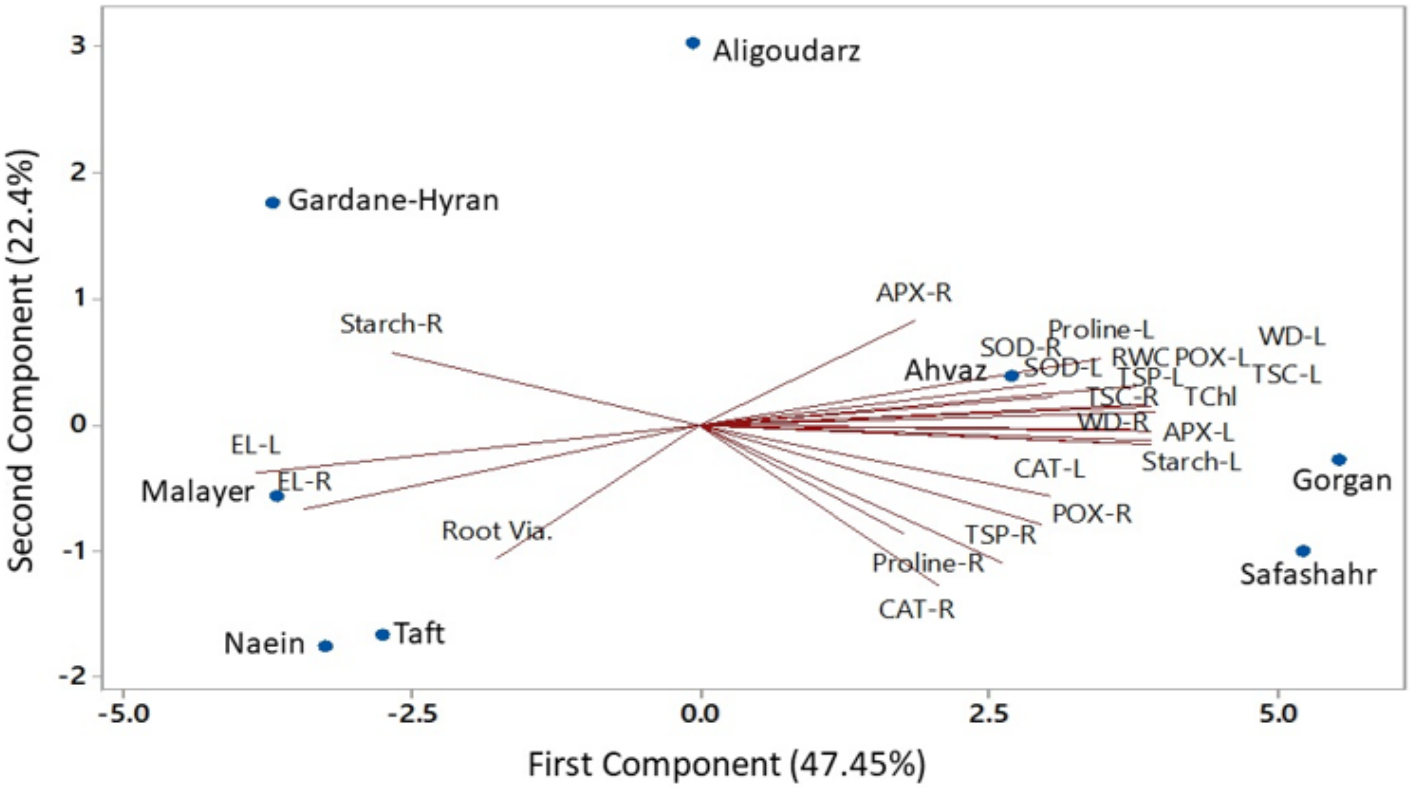

Figure 10

Principal components analysis biplot of 14 physiological, biochemical and osmolytes parameters in leaves and roots of eight Iranian accessions of common bermudagrass (*Cynodon dactylon* L. Pers.) under high-temperature regimes conditions. Red lines indicate measured parameters with various lengths based on the impact of each parameter on the separation of accessions.

Supplementary Files

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