Correlation of Heat and Cold Tolerance in Iranian Tall Fescue Ecotypes with Reactive Oxygen Species Scavenging and Osmotic Adjustment

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Abstract. Excessive heat or cold usually reduces the growth and quality of turfgrass. Genetic variations along with efficient biochemical and physiological mechanisms can diversify the tolerance to heat and cold. This study examined the effects of heat and cold stress on several biochemical and physiological parameters in Iranian tall fescue ecotypes (*Festuca arundinacea* L.). The control group of plants was maintained under optimal temperatures, whereas other groups were exposed to heat or cold in a growth chamber. The experiment was designed as a split plot, with stress treatments as the main plots and ecotypes as subplots. Physiologically and biochemically, the results revealed that three ecotypes ('FA1', 'FA3', and 'FA5') of the eight ecotypes examined in this study had better abilities to survive the simulated heat and cold stress. Better tolerance to heat and cold in the 'FA1', 'FA3', and 'FA5' ecotypes were probably due to higher levels of enzymatic and nonenzymatic antioxidant activities, maintenance of lower levels of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂), higher levels of proline and total nonstructural carbohydrates (TNC), along with a more efficient osmotic adjustment. Diamine oxidase (DAO) and polyamine oxidase (PAO) activities increased significantly in 'FA1', 'FA3', and 'FA5' ecotypes. In summary, the strength of tolerance among ecotypes can be ranked as 'FA1' > 'FA3' > 'FA5' > 'FA2' > 'FA6' > 'FA4' > 'FA7' > 'FA8' under heat stress and 'FA5' > 'FA1' > 'FA3' > 'FA2' > 'FA4' > 'FA6' > 'FA7' > 'FA8' under cold stress.

Tall fescue (*F. arundinacea* L.) is one of the main species of the Poaceae family. This cool-season grass has different characteristics including early maturity, drought tolerance, excellent seedling strength, and easy establishment. The tall fescue is commonly used for grassland rehabilitation on light-textured soils in shrub lands and rangelands where precipitation is generally low (Pan et al., 2013).

Heat and cold stress are two major abiotic factors that limit plant growth and reduce the turf quality of cool-season grass by affecting several physiological and biochemical factors (Du et al., 2013; Fan et al., 2014). Heat and cold stress could lead to oxidative damages via the production of reactive oxygen species (ROS), such as superoxide anion (O₂⁻) and H₂O₂ (Meloni et al., 2003). Reactive oxygen species can seriously disrupt normal metabolism in plants via oxidative damages such as lipid peroxidation, protein degradation, and enzyme inactivation

(Etemadi et al., 2015). Different types of turfgrass have various mechanisms for their adaptation to heat and cold stress, including changes in physiological parameters and antioxidant defense systems (Yu et al., 2012). Plants have evolved very efficient antioxidant defense systems to eradicate ROS during oxidative stress. This includes both enzymatic and nonenzymatic components, such as superoxide dismutase (SOD), Glutathione (GSH), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) (Sheikh-Mohamadi et al., 2018).

Turfgrass ecotypes exhibit different levels of tolerance to heat and cold. This has been evaluated by a number of researchers (Du et al., 2009; Jiang and Huang, 2001). Turfgrass ecotypes differ greatly in their ability to resist heat and cold stress. Evaluations of cultivars within a given group of species have been performed previously, although data on Iranian Tall fescue ecotypes are limited. In warm and cold climatic regions, selecting heat- and cold-tolerant species and ecotypes is a hopeful alternative to prevent heat- and cold-induced injuries, and such measures can be a benefit to turfgrass management (Soliman et al., 2012).

The identification of genetic variation and mechanisms that are involved in the tolerance to heat and cold can assist management programs for increasing turfgrass performance under heat and cold stress. Therefore, the present study aimed at evaluating Iranian tall fescue ecotypes along with their biochemical and physiological responses to heat and cold stress. Traits that may be associated with a greater ability to resist heat and cold have been considered.

Materials and Methods

Plant materials and growth conditions.

Eight ecotypes of Iranian tall fescue (*F. arundinacea* L.) were used in this research. Seeds of tall fescue ecotypes were collected in Sept. 2016 from eight sites in Iran (Table 1). The collected seeds were planted in Oct. 2016 in the greenhouse. The seeds were germinated in polyvinyl chloride tubes (10 cm diameter and 40 cm length) filled with sterilized sandy loam soil. The water used for irrigation was characterized by a pH value of 7.2 and an electrical conductivity (EC) of 0.3 mS·cm⁻¹. The water was applied as needed to prevent any visible stress during the establishment phase of grass development. In general, grasses were watered three times weekly to maintain plants under well-watered conditions. The soil moisture was maintained at field capacity and the grass was maintained at a cutting height of 5 cm. The pots were then transferred to growth chambers where treatments were applied under natural light conditions (13-h photoperiod, relative humidity of 50% to 60%, and photosynthetic photon flux density of 500 μmol·m⁻²·s⁻¹ at the canopy level). The temperatures were set at 22 °C day and 18 °C night. Plants were allowed to acclimate to the conditions of the growth chamber for 2 weeks before the start of the experiment.

Treatments and experimental design. Iranian tall fescue ecotypes were subjected to the following three temperature treatments for 24 d. The treatments were 1) optimal temperatures, 2) heat stress, and 3) cold stress. In the first group, plants were maintained at optimal temperature conditions of 22 °C day and 18 °C night; no heat or cold stress was applied. The second group had plants that were subjected to 35/30 °C (day/night) (Abraham et al., 2008; Larkindale and Huang, 2004), and plants of the third group were subjected to 4 °C (day/night) (Fan et al., 2014). The plants were watered once a day to avoid water deficiency. The soil in the pots was kept humid (60% to 80% of water holding capacity) throughout the experiment.

Physiological and biochemical measurements.

Proline content, TNC content, MDA content, H₂O₂ content, DAO (EC 1.4.3.6), PAO (EC 1.5.3.3), SOD (EC 1.15.1.1), APX (EC 1.11.1.11), POD (EC 1.11.1.7), CAT (EC 1.11.1.6) antioxidant enzymes activity, GSH, and turf quality were measured on days 0, 8, 16, and 24, after the beginning of the heat and cold treatments.

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H₂O₂ content and MDA content analysis. Leaf samples (0.5 g) of the ecotypes were ground finely by using liquid nitrogen in ice-chilled mortar and pestle. They were homogenized in 10 mL 10% ice-cold trichloroacetic acid. The homogenate was then centrifuged at 20,000 g_n for 25 min at 4 °C. The supernatant was used to evaluate H₂O₂ content (Zhou et al., 2005) and MDA content (Heath and Parker, 1968).

Proline content, TNC content and GSH content analysis. Free proline content was determined by a spectrophotometer using the ninhydrin method according to Bates et al. (1973) with some modifications. Fresh leaves (0.6 g) were immersed in 6 mL 5% aqueous sulfosalicylic acid and were then centrifuged at 15,000 g_n for 20 min. The supernatant was treated with 1.6 mL of 3% acid ninhydrin dissolved in 1 mL of glacial acetic acid and was boiled for 1 h. Absorbance was recorded at 520 nm with a calibration curve. The

amount of TNC content was quantified according to the method of Fry et al. (1993) with some modifications and was expressed as 0.5 g of each sample (ecotype). Absorbance of the solution was read at 515 nm and was compared with a standard curve to determine the TNC content. GSH content was determined using the method of Griffith (1980) with slight modifications and was expressed as 0.6 g per ecotype samples. Absorbance was calculated at 412 nm and the GSH content was calculated by using the standard curve.

DAO and PAO activities analysis. The activities of DAO and PAO were quantified by a modification of the radiometric method of Aribaud et al. (1994) and Faivre-Rampant et al. (2000). The ecotype samples (3 g) were homogenized in a prechilled mortar using 2 mL of 40 mM potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at 18,000 g_n for 30 min at 4 °C. The supernatant

was used to determine the activities of DAO and PAO. Changes in DAO and PAO activities were determined using the H₂O₂/Ti method (Nag et al., 2000) after treatment with exogenously added polyamines, i.e., putrescine (Put), spermidine (Spd), and spermine (Spm).

Assay of antioxidant enzyme activities. Initially, 0.5 g of each ecotype sample was ground in 4 mL of 40 mM phosphate buffer (pH 7.4) at 6 °C that was then centrifuged at 16,000 g_n for 20 min. The supernatant was gathered for assays of antioxidant enzyme activities. Different antioxidant enzymes were extracted and the activities of APX, SOD, CAT, and POD were assessed by using the methods described by Chen et al. (2009) and Han et al. (2008).

Turf quality. Turf quality was visually rated on a 1-to-9 scale [National Turfgrass Evaluation Program (NTEP)] according to the turfgrass color, texture, density, and

Table 1. Geographical origin of Iranian tall fescue ecotypes.

Code	Species	Region	Longitude (E)	Latitude (N)	Altitude (m)	Avg annual temp (°C)	Annual rainfall (mm/yr)
FA1	<i>Festuca arundinacea</i>	Mobarakeh, Isfahan	52.5132°E	31.8427°N	1,690	24.8	192.6
FA2		Ardakan, Yazd	54.0086°E	32.3082°N	1,035	19.8	69
FA3		Darab, Shiraz	54.3100°E	28.5400°N	1,180	20.4	176.2
FA4		Saveh, Markazi	50.0136°E	35.6980°N	998	16.6	223.5
FA5		Hashtgerd, Alborz	50.6846°E	35.9614°N	1,175	14.6	377
FA6		Arak, Markazi	49.7013°E	34.0954°N	1,750	13.9	311
FA7		Aligudarz, Lorestan	49.6962°E	33.4050°N	2,100	11.6	264.2
FA8		Takestan, Qazvin	49.7013°E	36.0721°N	1,260	18.1	350

Fig. 1. Proline content, total nonstructural carbohydrates content, and malondialdehyde content of tall fescue ecotypes treated with (A, D, and G, respectively) optimal temperature conditions, (B, E, and H, respectively) heat stress, and (C, F, and I, respectively) cold stress. Least significant difference bars indicate significant differences between tall fescue ecotypes within a given day of treatment ($P = 0.05$).

uniformity, where 1 represented a brown, absolutely dried necrotic turf covering. The value 6 represented an acceptable quality of turf, typical of a home lawn, and the value 9 meant optimum color, density, and uniformity (Beard, 1973).

Data analysis. The data were analyzed based on a split-plot design with the temperature treatments as main plots and ecotypes as subplots. Each stress and ecotype treatment had four replications. All pots were placed randomly in the growth chamber. The statistical significance was tested using the analysis of variance procedure in SAS 9.1 (SAS Institute Inc., Cary, NC). Differences between the mean values were determined using the Fisher's protected least significant difference test at the 5% probability level.

Results and Discussion

Heat and cold stress are considered to be major challenges as researchers consider climate change and the management of turfgrass (Du et al., 2013; Fan et al., 2014). Grass species and genotypes vary in their responses to heat and cold conditions. These responses include changes in the morphological, physiological, and biochemical mechanisms (Bocian et al., 2011; Yu et al., 2012). Understanding the mechanisms of grass tolerance to heat and cold stress is important in selecting grass species and genotypes to

facilitate the breeding of varieties that can be more tolerant to heat and cold (Abraham et al., 2008; Bocian et al., 2011).

Plants have different mechanisms that help them cope with environmental stresses such as high and low temperatures, drought, and salinity (Liu et al., 2017). Osmotic adjustment is a mechanism for the maintenance of water potential in plant cells during periods of heat and cold stress (Zaher-Ara et al., 2016). Compatible cytosolutes including proline, TNC, soluble sugars and glycine betaine can reduce the cell's osmotic potential under dehydration (Sheikh-Mohamadi et al., 2017d). Proline is an important compatible osmolyte and a scavenger of ROS in plants (Akbari et al., 2018). During the experiment, proline content remained constant at optimal temperatures in all ecotypes (Fig. 1A). The proline content of 'FA1' and 'FA5' were higher than other ecotypes after 8, 16, and 24 d following the beginning of the heat and cold stress (Fig. 1B–C). Heat and cold stress increased the proline content in 'FA3' on days 16 and 24 (Fig. 1B–C). The increased accumulation of proline content in stressed plants may be an adaptation process and tolerance strategy to avoid abiotic stress in plants (Fan et al., 2014; Liu et al., 2011). Sarmast et al. (2015) found significant variability in proline content among different ecotypes when comparing the proline content among different tall fescue ecotypes under

abiotic stress conditions. Furthermore, it is believed that proline protects plants against stress by different means such as the contribution toward osmotic adjustment, membrane integrity, detoxification of ROS, and the native structures of enzymes and proteins (Bocian et al., 2011; Liu et al., 2017).

The TNC content in plants contains fructans, starch, and water-soluble carbohydrates. Numerous studies have demonstrated that the TNC content increases during heat and cold stress, and TNC accumulation is associated with improvement in the tolerance to heat and cold, for example, in the creeping bentgrass and other plants (Liu and Huang, 2000; Sheikh-Mohamadi et al., 2018). The results of this study also revealed that the control plants of all ecotypes (situated at optimal temperatures) showed a stable TNC content throughout the duration of the study, and no significant differences were observed between the ecotypes (Fig. 1D). On all days of the experiment, the TNC content increased in 'FA1' and 'FA3' under heat stress. The same was observed in 'FA1' and 'FA5' under cold stress (Fig. 1E–F). The TNC content decreased in 'FA6' and 'FA7' under heat stress and it decreased in 'FA4', 'FA6', and 'FA7' under cold stress after 8, 16 and 24 d of the temperature treatments (Fig. 1E–F). Huang and Gao (2000) reported that tolerant cultivars of tall fescue accumulate more TNC.

Fig. 2. H_2O_2 content, diamine oxidase activity, and polyamine oxidase activity of tall fescue ecotypes treated with (A, D, and G, respectively) optimal temperature conditions, (B, E, and H, respectively) heat stress, and (C, F, and I, respectively) cold stress. Least significant difference bars indicate significant differences between tall fescue ecotypes within a given day of treatment ($P = 0.05$).

Malondialdehyde is one of the final products of chemical degradation during the peroxidation of lipids in the cell membrane. Damages to the cell membrane can be caused by free radicals under abiotic stress, and MDA is described as an indicator of the extent to which a plant is damaged (Han et al., 2014). It has commonly been applied to differentiate between heat- and cold-tolerant species and also to distinguish between heat- and cold-susceptible species and ecotypes (Du et al., 2009, 2013). After 8 and 16 d of temperature treatments, there was no change in the MDA content of 'FA1' and 'FA3' under heat and also no changes in 'FA1' and 'FA5' under cold stress (Fig. 1H–I). At the end of the experiment (24 d following the beginning of the temperature treatments), the MDA content increased in 'FA1' and 'FA3' under heat stress. It also increased in 'FA1' and 'FA5' under cold stress (Fig. 1H–I). A lower MDA content has been associated with a better tolerance to environmental stress among plants (Filek et al., 2012).

Stressful conditions encourage plants to overproduce ROS, such as H_2O_2 , which may result in serious damages to vital cellular organelles (Sheikh-Mohammadi et al., 2017a). For instance, H_2O_2 can damage DNA, enzymes, proteins, chlorophyll, membrane lipids, pigments, and nucleic acids (Ben Amor et al., 2006; Xu et al., 2013). The H_2O_2 content in all ecotypes increased

under heat and cold stress, compared with the control (Fig. 2B–C). After 16 and 24 d of the temperature treatments, the H_2O_2 content increased in 'FA1' and 'FA3' under heat stress. Similarly, the content increased in 'FA2' under cold stress (Fig. 2B–C). Heat and cold stress increased the H_2O_2 content in 'FA4', 'FA7', and 'FA8' within 8, 16, and 24 d of the temperature treatments (Fig. 2B–C). After 8 and 24 d of the experiment, the H_2O_2 content increased in 'FA2' and 'FA5' under heat stress. Similar increases were observed in 'FA1', 'FA3', and 'FA5' under cold stress (Fig. 2B–C). Low levels of H_2O_2 have been associated with better tolerance to heat and cold stress (Soliman et al., 2012).

In plants, important roles of DAO and PAO activities occur in a wide range of biochemical and physiological processes that have been reported in germination, defense responses against abiotic stress (e.g., drought, cold, salinity, and high temperature stress) and biotic stress (e.g., bacteria, viruses, fungi, and parasites), flowering and senescence (An et al., 2008; Moschou et al., 2008). It is believed that DAO and PAO play important roles in the production of H_2O_2 through the catabolism of polyamines in plant tissues (Alcázar et al., 2010; Xing et al., 2007). DAO is the main enzyme that catalyzes the oxidative deamination of diamines. DAO catalyzes the oxidative deamination of diamines to putrescine and subsequently into

pyrroline, ammonia, and H_2O_2 (Alcázar et al., 2011). In this study, after 8, 16, and 24 d of temperature treatments, the activity of DAO enzymes increased in 'FA1' and 'FA5' during heat stress (Fig. 2E). The DAO enzyme activity increased in 'FA2' under heat and cold stress on days 8 and 24 of the temperature treatments (Fig. 2E–F). After 16 d of the temperature treatments, the DAO activity increased in 'FA3' under heat stress and in 'FA8' during cold stress but then it decreased (Fig. 2E–F). After 8 and 16 d of the experiment, the DAO enzyme activity increased in 'FA3' under cold stress and then decreased (Fig. 3F). PAO is involved in the catabolism of polyamines and acetyl polyamines. Polyamines are essential factors for growth and survival. PAO is a very important enzyme that regulates the polyamine metabolic pathway (biosynthesis, catabolism, and regulation) in different plant systems (Sánchez-Rodríguez et al., 2016). PAO activities occur frequently in the cell walls of grass, and they constitute the terminal catabolism of polyamines and the production of H_2O_2 (Alcázar et al., 2011). After 8, 16, and 24 d of the temperature treatments, the PAO activity increased in 'FA1' and 'FA3' because of the heat and cold stress (Fig. 2H–I). The PAO enzyme activity increased in 'FA7' under heat stress. The activity also increased in 'FA2' during cold stress after 8 d of the temperature treatments but then showed

Fig. 3. Glutathione content, superoxide dismutase activity, and ascorbate peroxidase activity of tall fescue ecotypes treated with (A, D, and G, respectively) optimal temperature conditions, (B, E, and H, respectively) heat stress, and (C, F, and I, respectively) cold stress. Least significant difference bars indicate significant differences between tall fescue ecotypes within a given day of treatment ($P = 0.05$).

a downward trend (Fig. 2H–I). After 16 d of the temperature treatments, the activity of PAO enzyme increased in ‘FA5’ during cold stress but then decreased (Fig. 3I). Cvikrová et al. (2012), Sánchez-Rodríguez et al. (2016) and Toumi et al. (2010) observed a rapid and continued increase in DAO and PAO activities in cultivars tolerant to abiotic stress. Improved activities of DAO and PAO activities can develop plant tolerance. They have also been reported in several higher plants and during their tolerance to environmental stress (Shoresh et al., 2011; Tang and Newton, 2005). Researchers found that activities of DAO and PAO enzymes produce H_2O_2 in response to environmental and biotic stress. The product may generate mediated signals in the structural defense system by signal molecules or by an induced antioxidant response (Tang and Newton, 2005).

Plants have antioxidant defense systems to protect them against the deleterious effects of ROS, such as H_2O_2 , which are produced at high levels when plants are exposed to abiotic stress conditions (Sheikh-Mohammadi et al., 2017a). The antioxidant defense system consists of several antioxidant enzymes such as SOD, CAT, APX, POD, and some nonenzymatic antioxidants such as GSH, which are capable of eliminating H_2O_2 , neutralizing or scavenging free radicals (Sheikh-Mohammadi et al., 2017b). GSH plays a defensive role in the scavenging of singlet oxygen, hydroxyl

radicals, and peroxides (Guo et al., 2006). Lu et al. (2008) reported that a higher content of GSH under environmental stress is associated with increased stress tolerance in the bermudagrass. On all days of the experiment, the GSH content increased in ‘FA1’ and ‘FA3’ under heat stress and also increased in ‘FA1’ under cold stress (Fig. 3B–C). The GSH content increased in ‘FA5’ during heat stress and also in ‘FA3’ and ‘FA5’ during cold stress, after 8 and 24 d of the temperature treatments (Fig. 3B–C). SOD is a key antioxidant enzyme that catalyzes the conversion of superoxide radicals to H_2O_2 and molecular oxygen (Sheikh-Mohammadi et al., 2017c). SOD enzyme activity in ‘FA1’, ‘FA3’, and ‘FA5’ were higher than in other grasses under heat and cold conditions (Fig. 3E–F). After 8, 16, and 24 d of the temperature treatments, the activity of SOD increased in ‘FA1’ and ‘FA3’ under heat stress and in ‘FA1’ and ‘FA5’ under cold stress (Fig. 3E–F). After 16 d of the temperature treatments, the activity of the SOD enzyme increased in ‘FA2’, ‘FA3’, and ‘FA7’ during cold stress and then the activity decreased (Fig. 3C). In enzymatic antioxidant systems, APX and POD are essential enzymes in the cyclic activities of plants. APX and POD are necessary to transform H_2O_2 to H_2O and O_2 within the GSH–ascorbate cycle in chloroplasts (Sheikh-Mohammadi et al., 2017c). After 8, 16, and 24 d of the temperature

treatments, the activity of APX increased in ‘FA1’ and ‘FA2’ under heat and cold stress (Fig. 3H–I). After 8 and 16 d of the temperature treatments, the APX activity increased in ‘FA3’ under heat stress and in ‘FA3’ and ‘FA5’ under cold stress and then the activity decreased (Fig. 3H–I). Heat stress increased the activity of APX enzymes in ‘FA5’ after 16 and 24 d of the temperature treatments (Fig. 3H). On all days of the experiment, the activity of POD enzyme increased in ‘FA1’, ‘FA3’, and ‘FA5’ under heat stress. It also increased in ‘FA1’ and ‘FA2’ under cold stress (Fig. 4B–C). During the first 16 d of the temperature treatments, the POD activity increased in ‘FA2’ under heat stress and in ‘FA5’ under cold stress, and then the activity decreased (Fig. 4B–C). CAT is able to turn H_2O_2 to H_2O and O_2 which is followed by the removal of this radical oxygen species. CAT controls paroxysmal H_2O_2 without limiting its production (Hosseini et al., 2010). CAT activity increased in ‘FA3’ and ‘FA5’ under heat stress and in ‘FA1’ under cold stress within 8, 16, and 24 d of the treatment (Fig. 4E–F). In ‘FA4’, ‘FA6’, and ‘FA7’, the CAT activity remained constant under both heat and cold stress (Fig. 4E–F). After 8 and 16 d of the temperature treatments, the CAT activity increased in ‘FA1’ and ‘FA2’ under heat stress and in ‘FA2’, ‘FA3’, and ‘FA5’ under cold stress and then the activity decreased (Fig. 4E–F). According to the

Fig. 4. Peroxidase activity, catalase activity, and turf quality of tall fescue ecotypes treated with (A, D, and G, respectively) optimal temperature conditions, (B, E, and H, respectively) heat stress, and (C, F, and I, respectively) cold stress. Least significant difference bars indicate significant differences between tall fescue ecotypes within a given day of treatment ($P = 0.05$).

results of this study, tolerant ecotypes exhibited higher activities of antioxidant enzymes than heat- and cold-sensitive ecotypes. Maintaining a high level of antioxidative enzyme activities may contribute to heat and cold tolerance by improving the protective mechanisms against oxidative stress damages (Du et al., 2013; Fan et al., 2014; He and Huang, 2010; Liu et al., 2017). On the other hand, a decrease was observed in antioxidant enzyme activities including SOD, CAT, APX, and POD in some tall fescue ecotypes during prolonged heat and cold stress (24 d). This was probably because of the toxic effect of the overproduction of O_2 and H_2O_2 or its poisonous ROS derivatives, as observed by the higher H_2O_2 and MDA accumulation in the leaves of the tolerant ecotypes (Fan et al., 2014; Soliman et al., 2012). Higher activities of antioxidant enzymes have been reported in heat-tolerant tall fescue ecotypes (Cui et al., 2006). According to previous research, changes in the activities of antioxidant enzymes under heat and cold stress depend on plant species, ecotypes, the length, and intensity of the stress (Du et al., 2009; Espevig et al., 2012).

In turfgrass research, turf quality is investigated to evaluate the effects of environmental stress in grass species and ecotypes. In our experiment, under heat and cold stress, the turf quality of all ecotypes demonstrated a steady decline, and the rate of decline was different between ecotypes (Fig. 4G). 'FA1' and 'FA3' under heat stress and 'FA1' and 'FA5' under cold stress had significantly higher turf quality than other ecotypes during the experiment and maintained acceptable turf quality (7.0 or higher) within the first 16 d following temperature treatments (Fig. 4H–I). After 8, 16 and 24 d of the temperature treatments, turf quality decreased in 'FA4', 'FA6', and 'FA7' under heat and cold stress (Fig. 4H–I). By the end of the exposure to temperature stress (24 d following temperature treatments), turf quality of all ecotypes decreased to a level below the acceptable turf quality (Fig. 4H–I). Grass species and ecotypes that can maintain high levels of turf quality for a longer period of time under heat and cold stress are indicative of the higher tolerance to heat and cold stress (Du et al., 2009; Ebdon et al., 2002; Yu et al., 2012).

Conclusions

In summary, the results of this study indicate genetic variation in heat and cold tolerance in tall fescue ecotypes. Turf quality of all ecotypes decreased to improper quality rankings at the end of the experiment. In fact, turf quality declined with increasing heat and cold stress. Nevertheless, this decline in the turf visual quality was less severe for ecotypes 'FA1', 'FA3', and 'FA5' that had greater turf quality than other ecotypes under stress conditions. 'FA7' and 'FA8' had the poorest quality among all ecotypes. Based on biochemical and physiological analysis of heat and cold tolerance, the diversity of heat

and cold tolerance among Iranian tall fescue ecotypes was substantial. 'FA1' and 'FA3' were heat tolerant; 'FA5' and 'FA2' were moderately heat tolerant; and 'FA4', 'FA6', 'FA7', 'Regent', and 'FA8' were heat sensitive. Moreover, 'FA1', 'FA5', and 'FA3' were cold tolerant, 'FA2' was moderately cold tolerant, and 'FA6', 'FA7', 'FA4', and 'FA8' were cold-sensitive. The MDA content in 'FA1', 'FA3', and 'FA5' was significantly lower than in other ecotypes under heat and cold stress, indicating that less lipid peroxidation occurred as a result of higher antioxidant enzyme activities. Moreover, a lower H_2O_2 content was observed in 'FA1', 'FA3', and 'FA5'. The data demonstrated that superior heat and cold tolerance in 'FA1', 'FA3', and 'FA5' could be characterized by the higher activities of enzymatic antioxidants and nonenzymatic antioxidants; higher proline and TNC content; lower MDA and H_2O_2 contents; and higher activities of DAO and PAO. The results suggest that the selection and use of ecotypes with higher activities of enzymatic antioxidants (SOD, POD, CAT, and APX), nonenzymatic antioxidants (GHS), DAO, and PAO may improve the quality of tall fescue ecotypes under heat and cold stress. Finally, according to the results, it may be suggested that 'FA1' and 'FA3' are the strongest ecotypes in tolerance to heat and that the 'FA1' and 'FA5' are the strongest ecotypes in tolerance to cold stress. These ecotypes would be advised for future cultivation under conditions that are usually characterized by excessive heat or cold.

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