Comparison of osmoprotectants and antioxidant enzymes of different wild Kentucky bluegrass in Shanxi province under high-temperature stress

Fangshan Xia, Zhaosheng Han, Huisen Zhu, Kuanhu Dong and Lixia Du

College of Grassland Science, Shanxi Agricultural University, Taigu, Shanxi, China

Summary

High-temperature stress was one of the biggest threats to cool-season turfgrass, hence breeding new varieties adapted to high temperature was the fundamental way to improve the turf quality. This study was designed to determine the changes in contents of malonaldehyde (MDA), soluble sugar, soluble protein and proline, and activities of superoxide dismutase (SOD) and catalase (CAT) in different accessions of wild Kentucky bluegrass (*Poa pratensis*), which was collected from 13 areas of Shanxi province and subjected to 0, 5, 10, 15, 20 and 25 days of high-temperature stress, to evaluate the adaptability of different accessions of Kentucky bluegrass to high temperature stress. The results showed that the contents of soluble sugar, proline and MDA enhanced in all accessions of Kentucky bluegrass with the increase of high-temperature stress, the soluble protein contents of all accessions of Kentucky bluegrass declined under 0 to 10 days of high-temperature stress, and then increased with the increase of high-temperature stress. Inversely, the SOD and CAT activities of all Kentucky bluegrass augmented under 0 to 10 or 15 days of high-temperature stress, but they all declined with the continuous increase of high-temperature stress. The changes in osmotic and antioxidant protective system were very different in response to high-temperature stress in different accessions of Kentucky bluegrass, and the accessions of 'Qinshui' and 'Hunyuan' had the highest resistance to high-temperature stress, hence they could be used to breed new varieties that are resistant to high-temperature stress.

Keywords

antioxidase, heat stress, lipid peroxidation, osmoregulation substances, *Poa pratensis*

Significance of this study

What is already known on this subject?

- High-temperature stress is the most crucial challenge for cool-season turfgrass, and osmoprotectants and antioxidant enzymes were the traditional and principal protection for plants to resist the damage of adversity.

What are the new findings?

- The influences of high-temperature stress on osmotic and antioxidant protective systems were very different in different accessions of Kentucky bluegrass, and two wild accessions exhibited the excellent resistance to high-temperature stress.

What is the expected impact on horticulture?

- These findings might play a significant role in breeding new varieties that are resistant to high-temperature stress; thereby it might be conducive to establish high quality turf under temperature stress.

and often combine with drought or other stresses (Ergo et al., 2018; Fang et al., 2018). Understanding the impact of high-temperature stress on growth and development in plants could help to reduce the loss of agricultural production.

High-temperature stress induces a series of physiological, biochemical, and molecular changes in plants affecting their growth and development (Harsh et al., 2016). A major contributor to high-temperature stress is widely considered to be the rapid production and over-accumulation of reactive oxygen species (ROS) in plants (Mittler et al., 2011). ROS are detoxified by osmoprotectants and antioxidants in plants under normal physiological conditions (Kotak et al., 2007). Proline, soluble protein and sugar play a role in cellular osmoregulation and also exhibit many protective effects; the increase in their levels exhibits enhanced tolerance to high-temperature stress (Wu et al., 2014; Zou et al., 2016). Superoxide dismutase (SOD) is usually considered as the first line of defense against oxidative stress (Gupta et al., 2013). Catalase (CAT) scavenges H_2O_2 by breaking directly it to H_2O , and an increase in its activity is related to augment in stress tolerance (Kraus et al., 1995). However, the defense system cannot prevent the occurrence of oxidative stress and counterbalance the over-accumulation of ROS under environmental stress (Tutar et al., 2017). Furthermore, current researches manifest that the variation of defense system in plants had great diversity among their different genotypes under high-temperature stress (Wang and Huang,

Introduction

The events of short-term high-temperature stress have occurred frequently across the globe in recent decades and are likely to increase with human-activities climate changes (Gourdji et al., 2013). However, high-temperature stress is one of the most crucial challenges for plants, which generates serious retardation in their growth and development, and even death, hence severely reduces their yields (Rezaei et al., 2015; Tutar et al., 2017; Webber et al., 2017). Therefore, extensive agricultural losses are generated worldwide by the increase in high-temperature stress with global warming,

2004; Almeselmani et al., 2006; Harsh et al., 2016). However, studies to high-temperature stress response on model or economically important plant species showed different thermotolerance and signaling responses were discovered in those with different ecotypes (Tutar et al., 2017). Therefore, studying the impact of high-temperature stress on antioxidant capacity of different genotypes of plants could avail to breeding more adaptable varieties.

Kentucky bluegrass (*Poa pratensis*) is not only an important forage grass for livestock, but also is the most commonly cool-season turfgrass in temperate regions. However, high-temperature stress is the frequently encountered adversity for cool-season turfgrasses during summer, leading to a serious decline in turf quality (Wang and Huang, 2004). Decline in turf quality caused by high-temperature stress is also a momentous concern in establishment and management of Kentucky bluegrass lawn (He, 2010).

Furthermore, Kentucky bluegrass often exists in a wide range of genetic variability to stress tolerance during summer (Bonos and Murphy, 1999). Therefore, understanding their genetic variability for high-temperature stress is necessary for breeding more adaptable varieties in Kentucky bluegrass. So this study was designed to explain the difference of high-temperature resistance by determining some osmoprotectants and antioxidant enzymes of Kentucky bluegrass, thereby providing references for screening out new varieties with high-temperature resistance.

Materials and methods

Seed samples

Seeds of wild Kentucky bluegrass were collected from 13 areas of Shanxi province by the Forage Seed Laboratory of Shanxi Agricultural University in 2014, and were then sealed in plastic bags and stored at -20°C. Detailed collection information was shown in Table 1. 'Midnight' was prepared as the control.

Seedlings cultivation

The experiment was launched in July 2015. Seeds were uniformly sowed in plastic pots (15 cm in height, 12 cm in diam.) filling a sterilized mixture (3:2:2, v/v) of soil, sand and vermiculite. Seed rate was 12 g m⁻². Pots were maintained in a greenhouse for 60 days and were then moved into growth

chambers for 7 days. The greenhouse had an average day/night temperature of 21/14°C, 75% of relative humidity, illumination intensity of 3,000 lux, and a 12-h photoperiod. The chambers were set at 25/20°C (day/night temperature), 75% of relative humidity, illumination intensity of 3,000 lux, and a 12-h photoperiod. Pots were watered 3 times per week by using weighing method.

High-temperature stress treatments

After 7 days in growth chambers, the chambers were set at 38/30°C (day/night temperature), but relative humidity and illumination intensity remained unchanged. Each accession of Kentucky bluegrass was subjected to 6 periods of high-temperature stress (0, 5, 10, 15, 20 and 25 days) and were prepared as the samples for the experiments. Each treatment was set 4 replicates.

Osmotic materials assays

The levels of proline contents in the samples were determined according to the method of Bates et al. (1973). The soluble sugar was estimated by anthrone method (Dubois et al., 1956). Protein contents were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Enzymes extraction and assays

Enzymes were extracted according to Kibinza et al. (2006). 2 g of leaf samples were ground and homogenized using a mortar and pestle in 3.5 mL of potassium phosphate buffer (0.1 mol L⁻¹, pH 7.8) containing 2 mmol L⁻¹ dithiothreitol, 0.1 mmol L⁻¹ ethylenediaminetetraacetic acid, 1.25 mmol L⁻¹ polyethyleneglycol-4000 and 20% polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 g for 15 minutes. The resulting supernatant was stored for analysis of SOD and CAT activities. All steps of the extraction procedure were performed between 1 and 4°C.

SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (Rao and Sresty, 2000). One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction at 560 nm.

CAT activity was measured by the dynamic change in absorbance at 240 nm over one minute due to the decline of extinction of H₂O₂. Supernatant (100 µL) was mixed with

TABLE 1. General information of different accessions of wild Kentucky bluegrass.

Accessions	Collecting location	Geographic location			
		Longitude	Latitude	Elevation (m)	Habitat
Qingshui	Dongda Village, Qingshui County	112°26'16.9"E	35°42'44.3"N	622	Roadside
Huozhou	Qiliyu Village, Huozhou City	112°00'4.9"E	36°36'59.6"N	1,549	Hillside
Xinzhou	Nipo Village, Xinzhou City	112°26'01.1"E	38°37'58.1"N	1,547	Fieldside
Wutai	Xialiyuan Village, Wutai County	113°27'38.6"E	38°56'34.4"N	1,509	Valley
Lingshi	Shigaoshan Village, Lingshi County	111°00'55.7"E	36°00'44.7"N	1,127	Riverside
Lingqiu	Shangchehe Village, Lingqiu County	114°18'46.9"E	39°20'06.4"N	852	Valley
Guangling	Nangeya Village, Guangling County	114°15'27.1"E	39°37'57.1"N	1,298	Valley
Huanyuan	Xuankongsi Village, Hunyuan County	113°42'32.6"E	39°39'41.5"N	1,184	Roadside
Ningwu	Qujiagou Village, Ningwu County	111°56'34.3"E	38°52'51.2"N	2,272	Roadside
Wuzhai	Dianping Village, Wuzhai County	111°54'27.1"E	38°47'24.1"N	1,865	Riverside
Tunliu	Goukou Village, Tunliu County	112°34'44.2"E	36°22'18.1"N	1,000	Riverside
Yingxian	Ximizhuang Village, Yingxian County	113°07'32.6"E	39°36'33.6"N	993	Riverside
Youyu	Mayinghe Village, Youyu County	112°19'46.0"E	40°12'20.1"N	1,267	Roadside

3.4 mL phosphate buffer (25 mmol L⁻¹, pH 7.0, mixed with 0.1 mmol L⁻¹ EDTA) and 200 µL 0.1 mol L⁻¹ H₂O₂. The reaction was started by adding H₂O₂. Catalase activity was measured using the method of Chance and Maehly (1955).

MDA contents assays

Lipid peroxidation was determined as the amount of MDA according to Bailly et al. (1996). 1 g of samples were ground in 5 mL deionised water and homogenised with an equal volume of 0.5% (w/v) 2-thiobarbituric acid in 20% (w/v) trichloroacetic acid. The homogenate was incubated at 100°C for 15 minutes, then cooled immediately and centrifuged at 4,000 rpm for 10 minutes. The supernatant was used for MDA determination. MDA levels were quantified by measuring the absorbance at 532 and 600 nm.

Statistical analyses

Comparisons of mean differences were carried out by the variance analysis using SPSS for Windows version 13.0. Duncan's multiple range test ($P=0.05$) was adopted to appraise the differences in physiological indicators among different treatments.

Results

Changes in soluble sugar contents of different wild Kentucky bluegrass

The soluble sugar contents of all the Kentucky bluegrass increased significantly ($P<0.05$) with the enhancement of high-temperature stress, but they were different in different accessions under same high-temperature stress (Table 2). At 0 days of high-temperature stress, the soluble sugar contents were significantly ($P<0.05$) higher in 'Huozhou', 'Wutai' and 'Tunliu' than in 'Midnight', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 5 days of high-temperature stress, there was no significant ($P>0.05$) difference between 'Hunyuan' and 'Midnight', and the soluble sugar contents in other accessions remained unchanged comparing to 'Midnight'. At 10 days of high-temperature stress, there was no significant ($P>0.05$) difference

among 'Midnight', 'Qinshui', 'Hunyuan' and 'Ningwu', but the soluble sugar contents in other accessions still remained unchanged comparing to 'Midnight'. At 15 days of high-temperature stress, the soluble sugar contents were significantly ($P<0.05$) higher in 'Qinshui', 'Huozhou', 'Wutai', 'Hunyuan', 'Ningwu', 'Wuzhai' and 'Tunliu' than in 'Midnight', and they were still significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 20 days of high-temperature stress, there were no significantly ($P>0.05$) difference between 'Wuzhai' and 'Midnight', and then the soluble sugar contents were remarkable ($P<0.05$) higher in 'Lingqiu' than in 'Midnight', but they still remained unchanged in other accessions comparing to 'Midnight'. At 25 days of high-temperature stress, the soluble sugar contents in 'Huozhou', 'Wutai', 'Lingqiu', 'Hunyuan', 'Ningwu' and 'Tunliu' were significantly ($P<0.05$) higher than in 'Midnight', and no significant ($P>0.05$) difference was observed among 'Midnight', 'Qinshui', and 'Guangling', and they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'.

Changes in soluble protein contents of different wild Kentucky bluegrass

The soluble protein contents of all the Kentucky bluegrass decreased significantly ($P<0.05$) at the initial stage of high-temperature stress, and then they increased significantly ($P<0.05$) with the enhancement of high-temperature stress, but they were different in different accessions under same high-temperature stress (Table 3). At 0 days of high-temperature stress, the soluble protein contents were significantly ($P<0.05$) higher in 'Lingqiu' and 'Hunyuan' than in 'Midnight', there was no significant ($P>0.05$) difference among 'Midnight', 'Qingshui', 'Yingxian' and 'Youyu', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 5 days of high-temperature stress, there was no significant ($P>0.05$) difference among 'Midnight', 'Qingshui', 'Lingshi' and 'Hunyuan', but the soluble protein contents were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 10 days of high-temperature stress, except there was no significant ($P>0.05$) difference among 'Midnight', 'Guangling', and 'Hunyuan', and the soluble protein contents

TABLE 2. Effects of high-temperature stress on soluble sugar contents in leaves of different wild Kentucky bluegrass (mg g⁻¹ FW)

Accessions	Duration of high-temperature stress (days)					
	0	5	10	15	20	25
Midnight	2.37±0.030Fc	2.69±0.025Ed	3.18±0.040Dc	3.58±0.027Cf	4.15±0.038Bd	4.81±0.044Ad
Qinshui	2.11±0.022Fe	2.58±0.020Ee	3.17±0.026Dc	3.85±0.032Ccd	4.28±0.052Bc	4.86±0.036Ad
Huozhou	2.53±0.030Fb	2.78±0.026Ec	3.65±0.024Db	4.08±0.026Cb	4.52±0.035Bb	5.05±0.040Ac
Xinzhou	2.16±0.026Fde	2.45±0.018Ef	2.81±0.032Dd	3.38±0.033Cg	3.91±0.035Be	4.31±0.018Af
Wutai	2.77±0.027Fa	3.33±0.026Ea	3.76±0.012Da	4.21±0.038Ca	4.69±0.041Ba	5.31±0.037Ab
Lingshi	1.48±0.025Fk	1.88±0.023Ei	2.38±0.032De	3.36±0.015Cg	3.61±0.037Bf	4.16±0.015Ag
Lingqiu	1.83±0.029Fg	2.18±0.026Eg	2.83±0.015Dd	3.38±0.018Cg	4.48±0.036Bb	5.04±0.035Ac
Guangling	1.72±0.026Fh	2.26±0.027Eg	2.72±0.018Dd	3.18±0.012Ch	3.92±0.024Be	4.75±0.039Ade
Hunyuan	2.20±0.023Fd	2.76±0.015Ecd	3.20±0.046Dc	3.82±0.032Cde	4.59±0.018Bab	5.80±0.027Aa
Ningwu	2.03±0.029Ff	2.61±0.019Ee	3.12±0.026Dc	3.73±0.044Ce	4.56±0.062Bb	5.42±0.043Ab
Wuzhai	1.57±0.032Fj	2.21±0.037Eg	2.79±0.020Dd	3.72±0.021Ce	4.14±0.031Bd	4.64±0.046Ae
Tunliu	2.72±0.023Fa	3.12±0.015Eb	3.54±0.043Db	3.94±0.043Cc	4.54±0.052Bb	5.07±0.032Ac
Yingxian	1.79±0.029Fgh	2.17±0.018Eg	2.44±0.055De	3.12±0.020Ch	3.83±0.050Be	4.29±0.034Afg
Youyu	1.65±0.015Fi	2.07±0.029Eh	2.38±0.032De	2.99±0.029Ci	3.69±0.024Bf	4.26±0.026Afg

Different capital letters in the same row represent significant differences between treatments at the 0.05 level, different small letters in the same column represent significant differences between accessions at the 0.05 level.

TABLE 3. Effects of high-temperature stress on the soluble protein contents in leaves of different wild Kentucky bluegrass (mg g⁻¹ FW).

Accessions	Duration of high-temperature stress (days)					
	0	5	10	15	20	25
Midnight	6.15±0.010Dc	4.61±0.052Fab	4.83±0.007Eab	8.30±0.050Cc	9.03±0.012Bc	10.26±0.018Ad
Qingshui	6.13±0.017Cc	4.55±0.019Db	3.91±0.036Ed	8.77±0.047Bb	8.90±0.017Bd	11.77±0.051Ab
Huozhou	5.18±0.043Cg	3.94±0.036De	3.10±0.051Ef	5.96±0.036Bf	7.87±0.022Ah	7.75±0.014Ai
Xinzhou	5.25±0.063Dfg	3.73±0.025Ef	2.87±0.030Fh	5.94±0.046Cf	7.24±0.038Bi	8.85±0.025Ag
Wutai	5.75±0.030Dd	3.73±0.033Ef	3.57±0.018Fe	6.82±0.024Ce	8.69±0.032Be	9.20±0.036Af
Lingshi	5.53±0.040De	4.70±0.029Ea	3.37±0.027Ff	6.81±0.022Ce	8.51±0.019Bf	8.80±0.052Ag
Lingqiu	6.95±0.033Ca	3.89±0.034Ee	2.84±0.021Fh	6.77±0.043De	8.44±0.022Bf	9.29±0.027Af
Guangling	6.22±0.046Dbc	4.16±0.022Fd	4.77±0.035Eb	7.10±0.028Cd	8.41±0.036Bf	9.55±0.020Ae
Hunyuan	6.31±0.019Db	4.60±0.022Eab	4.89±0.017Ea	7.10±0.043Cd	9.35±0.030Bb	12.02±0.017Aa
Ningwu	4.49±0.032Ch	3.60±0.020Dg	3.61±0.028De	6.82±0.023Be	7.20±0.020Bi	8.37±0.015Ah
Wuzhai	5.76±0.057Dd	3.93±0.024Ee	4.12±0.020Ec	6.94±0.020Ce	8.72±0.043Be	10.51±0.036Ac
Tunliu	5.36±0.015Df	4.35±0.028Ec	3.82±0.022Fd	6.36±0.055Cf	8.88±0.052Bd	9.21±0.051Af
Yingxian	6.24±0.008Cbc	4.36±0.018Dc	2.91±0.017Eh	10.23±0.049Ba	10.69±0.020Aa	10.56±0.049Ac
Youyu	6.18±0.041Dbc	4.33±0.015Ec	2.91±0.029Fh	7.23±0.027Cd	8.10±0.019Bg	8.88±0.052Ag

Different capital letters in the same row represent significant differences between treatments at the 0.05 level, different small letters in the same column represent significant differences between accessions at the 0.05 level.

were also significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 15 days of high-temperature stress, the soluble protein contents were significantly ($P<0.05$) higher in 'Qingshui' and 'Yingxian' than in 'Midnight', and they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 20 days of high-temperature stress, except the soluble protein contents were significantly ($P<0.05$) higher in 'Hunyuan' and 'Yingxian' than in 'Midnight', and they were also significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 25 days of high-temperature stress, the soluble protein contents were significantly ($P<0.05$) higher in 'Qingshui', 'Hunyuan' and 'Yingxian' than in 'Midnight', and they were also significantly ($P<0.05$) lower in other accessions than in 'Midnight'.

Changes in proline contents of different wild Kentucky bluegrass

The proline contents of all the Kentucky bluegrass increased significantly ($P<0.05$) with the enhancement of high-temperature stress, but they were also different in different accessions under same high-temperature stress (Table 4). At 0 days of high-temperature stress, the proline contents were significantly ($P<0.05$) higher in 'Qingshui', 'Huozhou', 'Lingshi' and 'Hunyuan' than in 'Midnight', and there was no significant ($P>0.05$) difference between 'Midnight' and 'Lingqiu', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 5 days of high-temperature stress, the proline contents were significantly ($P<0.05$) higher in 'Qingshui', 'Guangling', 'Hunyuan' and 'Tunliu' than in 'Midnight', and there was no significant

TABLE 4. Effects of high-temperature stress on the free proline contents in leaves of different wild Kentucky bluegrass (μg g⁻¹ FW).

Accessions	Duration of high-temperature stress (days)					
	0	5	10	15	20	25
Midnight	144.80±2.79Fc	248.98±1.00Ed	352.13±2.79Dh	567.16±3.68Cf	955.43±3.09Be	1,384.20±4.69Ac
Qinshui	155.12±2.42Fb	255.86±0.66Ebc	427.88±2.89Def	617.28±4.58Ce	791.34±0.20Bi	1,357.96±4.50Ad
Huozhou	166.88±2.21Ea	112.44±1.56Fj	347.72±4.20Dh	534.73±2.25Cf	1,123.07±3.46Bb	1,344.78±4.04Ad
Xinzhou	133.65±3.32Fd	241.23±1.15Ee	432.96±3.37Dde	537.85±2.84Cf	898.73±3.94Bg	1,078.11±3.00Ag
Wutai	127.50±1.45Fde	251.25±1.79Ecd	522.30±3.65Db	612.64±2.88Ce	827.14±3.88Bh	1,287.09±3.89Ae
Lingshi	173.57±2.06Fa	222.54±1.07Eg	433.36±3.82Dde	779.49±3.91Ca	1,058.97±4.54Ac	832.92±2.61Bi
Lingqiu	143.16±2.50Fc	212.87±0.97Eh	321.90±2.38Di	718.60±4.95Cc	1,037.30±3.98Bd	1,091.47±2.27Ag
Guangling	84.68±2.27Fg	267.69±1.25Ea	323.38±3.42Di	534.30±3.06Cf	754.63±1.50Bj	979.04±1.81Ah
Hunyuan	172.30±1.88Fa	259.35±0.76Eb	458.61±3.86Dc	636.41±4.89Cd	830.75±2.86Bh	1,082.57±3.80Ag
Ningwu	119.90±1.05Fe	214.87±1.73Eh	444.06±3.11Dcd	746.88±3.89Cb	961.11±4.46Be	1,442.53±4.18Ab
Wuzhai	95.57±0.79Ff	206.40±1.79Ei	414.50±1.75Df	742.23±3.32Cb	1,247.85±4.96Ba	1,555.60±4.72Aa
Tunliu	118.23±2.85Fe	261.29±1.32Eb	386.35±4.76Dg	487.48±0.20Ch	760.42±4.52Bj	969.55±4.43Ah
Yingxian	120.59±1.62Fe	239.81±1.77Eef	350.95±4.32Dh	543.57±1.88Cg	938.15±3.84Bf	1,206.02±1.24Af
Youyu	101.09±3.31Ff	234.84±0.70Ef	561.85±5.96Da	723.94±1.68Cc	1,071.87±4.96Bc	1,349.38±4.04Ad

Different capital letters in the same row represent significant differences between treatments at the 0.05 level, different small letters in the same column represent significant differences between accessions at the 0.05 level.

TABLE 5. Effects of high-temperature stress on SOD activities in leaves of different wild Kentucky bluegrass (U mg⁻¹ protein).

Accessions	Duration of high-temperature stress (days)					
	0	5	10	15	20	25
Midnight	92.48±0.69Df	129.91±0.78Bc	139.67±0.78Ai	143.00±0.933Af	107.51±0.90Ccd	90.18±0.86Dc
Qinshui	109.46±0.96Cd	131.57±0.54Bc	169.03±0.76Ac	130.48±0.58Bh	108.85±1.09Cbc	93.74±0.61Db
Huozhou	85.88±0.55Eg	120.66±0.76Cd	157.86±0.56Bef	166.82±0.63Ab	94.70±0.59Df	42.36±0.79Fj
Xinzhou	108.41±0.68Dd	114.37±0.68Ce	202.64±0.89Aa	130.08±0.86Bh	110.07±0.93Dbc	53.20±0.56Eh
Wutai	63.77±0.63Ei	108.26±0.88Cf	152.91±0.83Ah	115.31±0.78Bi	83.72±0.81Dh	62.70±0.62Eg
Lingshi	73.34±0.66Eh	113.99±0.79Ce	131.76±0.55Bj	148.90±0.66Ad	88.58±0.46Dg	66.54±0.63Ff
Lingqiu	97.73±0.55Ee	131.75±0.83Cc	154.44±0.38Bgh	173.72±0.95Aa	104.55±0.82Dde	83.69±0.80Fd
Guangling	115.07±0.97Dc	130.87±0.19Cc	165.15±0.42Bd	173.72±0.63Aa	127.77±0.89Ca	94.52±0.91Eb
Hunyuan	126.11±0.97Ca	136.26±0.79Bb	163.32±0.77Ad	164.96±0.70Ab	126.06±0.99Ca	110.88±0.89Da
Ningwu	122.19±0.67Cb	149.14±0.75Ba	155.93±0.84Afg	94.18±0.89Dj	86.85±0.94Egh	61.64±0.75Fg
Wuzhai	98.18±0.98De	111.64±0.48Ce	139.55±0.46Bi	144.72±0.80Aef	88.60±1.01Eg	47.18±0.65Fi
Tunliu	64.80±0.75Ei	107.73±0.33Cf	198.59±0.44Ab	160.05±0.81Bc	102.39±0.60De	48.22±0.91Fi
Yingxian	90.86±1.02Ef	118.12±0.85Cd	129.85±0.43Bj	133.61±0.53Ag	112.24±0.85Db	72.58±0.50Fe
Youyu	91.82±0.89Ef	128.74±0.83Cc	159.00±0.48Ae	146.54±0.33Bde	103.94±0.08De	55.96±0.80Fh

Different capital letters in the same row represent significant differences between treatments at the 0.05 level, different small letters in the same column represent significant differences between accessions at the 0.05 level.

($P>0.05$) difference between 'Midnight' and 'Wutai', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 10 days of high-temperature stress, the proline contents were significantly ($P<0.05$) lower in 'Lingqiu', and 'Guangling' than in 'Midnight', and there was no significant ($P>0.05$) difference between 'Midnight' and 'Huozhou', but they were significantly ($P<0.05$) higher in other accessions than in 'Midnight'. At 15 days of high-temperature stress, the proline contents were significantly ($P<0.05$) lower in 'Tunliu' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference among 'Midnight', 'Huozhou', 'Xinzhou' and 'Guangling', but they were significantly ($P<0.05$) higher in other accessions than in 'Midnight'. At 20 days of high-temperature stress, the proline contents were significantly ($P<0.05$) higher in 'Huozhou', 'Lingshi', 'Lingqiu', 'Wuzhai' and 'Youyu' than in 'Midnight', and there was no significant ($P>0.05$) difference between 'Midnight' and 'Ningwu', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 25 days of high-temperature stress, the proline contents only were significantly ($P<0.05$) higher in 'Ningwu' and 'Wuzhai' than in 'Midnight', and they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'.

Changes in SOD activities of different wild Kentucky bluegrass

The SOD activities of all the Kentucky bluegrass increased significantly ($P<0.05$) with the enhancement of high-temperature stress, but they were also different in different accessions under same high-temperature stress (Table 5). At 0 days of high-temperature stress, the SOD activities only were significantly ($P<0.05$) lower in 'Huozhou', 'Wutai', 'Lingshi' and 'Tunliu' than in 'Midnight', and there was no significant ($P>0.05$) difference among 'Midnight', 'Yingxian' and 'Youyu', but they were significantly ($P<0.05$) higher in other accessions than in 'Midnight'. At 5 days of high-temperature stress, the SOD activities only were significantly ($P<0.05$) higher in 'Hunyuan' and 'Ningwu' than in 'Midnight', and there was no significant ($P>0.05$) difference among 'Midnight', 'Qinshui', 'Lingqiu', 'Guangling' and 'Youyu', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 10 days of high-temperature stress, the SOD

activities only were significantly ($P<0.05$) lower in 'Lingshi' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference between 'Midnight' and 'Wuzhai', but they were significantly ($P<0.05$) higher in other accessions than in 'Midnight'. At 15 days of high-temperature stress, the SOD activities were significantly ($P<0.05$) lower in 'Qinshui', 'Xinzhou', 'Wutai', 'Ningwu' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference between 'Midnight' and 'Wuzhai', but they were significantly ($P<0.05$) higher in other accessions than in 'Midnight'. At 20 days of high-temperature stress, the SOD activities were significantly ($P<0.05$) higher in 'Guangling', 'Hunyuan' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference among 'Midnight', 'Qinshui', 'Xinzhou' and 'Lingqiu', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 25 days of high-temperature stress, the SOD activities only were significantly ($P<0.05$) higher in 'Qinshui', 'Guangling' and 'Hunyuan' than in 'Midnight', and they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'.

Changes in CAT activities of different wild Kentucky bluegrass

The CAT activities of all the Kentucky bluegrass increased significantly ($P<0.05$) after 0 to 15 days high-temperature stress, and then they declined significantly ($P<0.05$) with the enhancement of high-temperature stress, but they were also different in different accessions under same high-temperature stress (Table 6). At 0 days of high-temperature stress, there was no significant ($P>0.05$) difference between 'Midnight' and 'Hunyuan', but the CAT activities were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 5 days of high-temperature stress, the CAT activities were significantly ($P<0.05$) higher in 'Qinshui', 'Wutai', 'Lingqiu', 'Hunyuan' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference between 'Midnight' and 'Tunliu', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 10 days of high-temperature stress, the CAT activities only were significantly ($P<0.05$) higher in 'Qinshui', 'Wutai', 'Lingqiu', 'Hunyuan' and 'Yingxian' than in 'Midnight', and they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 15 days of

TABLE 6. Effects of high-temperature stress on CAT activities in leaves of different wild Kentucky bluegrass ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein).

Accessions	Duration of high-temperature stress (days)					
	0	5	10	15	20	25
Midnight	9.82±0.057Ea	11.00±0.047De	13.12±0.022Cf	14.65±0.029Ad	13.87±0.032Bc	7.65±0.045Fa
Qinshui	8.48±0.061Ecd	12.30±0.044Db	14.88±0.009Cb	16.40±0.012Ab	15.74±0.069Ba	7.06±0.063Fc
Huozhou	9.12±0.034Eb	9.16±0.016Dj	10.63±0.023Bm	11.55±0.011Al	9.68±0.021Ck	7.31±0.025Fb
Xinzhou	7.01±0.059Df	10.41±0.026Cg	11.82±0.015Bj	12.74±0.020Aj	11.69±0.018Bh	4.44±0.062Eh
Wutai	8.42±0.060Ed	11.64±0.045Dc	13.70±0.018Bd	14.55±0.029Ae	11.87±0.019Cg	6.00±0.064Fd
Lingshi	7.70±0.058Ee	9.63±0.047Di	11.22±0.020Bl	12.11±0.040Ak	10.85±0.012Cj	5.17±0.058Ff
Lingqiu	7.71±0.055Ee	11.35±0.035Dd	13.24±0.026Be	14.15±0.038Af	12.28±0.019Cf	4.83±0.055Fg
Guangling	8.36±0.029Ed	9.88±0.058Dh	12.25±0.028Ch	14.21±0.018Af	13.53±0.043Bd	7.07±0.046Fc
Hunyuan	9.82±0.057Ea	12.61±0.059Da	15.16±0.042Ca	16.69±0.015Aa	15.42±0.064Bb	7.77±0.061Fa
Ningwu	7.01±0.059Ef	10.42±0.021Dg	11.96±0.009Bi	12.83±0.013Aj	11.20±0.015Ci	4.47±0.050Fh
Wuzhai	6.29±0.024Eg	9.96±0.043Dh	11.69±0.013Bk	13.22±0.009Ah	11.25±0.026Ci	4.92±0.046Fg
Tunliu	7.69±0.046Ee	10.91±0.004De	12.26±0.010Bh	13.05±0.034Ai	11.74±0.032Cgh	5.59±0.074Fe
Yingxian	8.65±0.025Ebc	12.69±0.035Da	14.28±0.009Bc	15.02±0.023Ac	12.94±0.012Ce	6.09±0.030Fd
Youyu	8.44±0.021Ed	10.61±0.058Df	12.54±0.052Cg	13.46±0.029Ag	12.82±0.020Be	5.49±0.041Fe

Different capital letters in the same row represent significant differences between treatments at the 0.05 level, different small letters in the same column represent significant differences between accessions at the 0.05 level.

high-temperature stress, the CAT activities in 'Wutai' and 'Lingqiu' also changed to lower than in 'Midnight', and they still remained unchanged in other accessions comparing to 'Midnight'. At 20 days of high-temperature stress, the CAT activities only were significantly ($P<0.05$) higher in 'Qinshui' and 'Hunyuan' than in 'Midnight', and they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 25 days of high-temperature stress, except there was no significant ($P>0.05$) difference between 'Midnight' and 'Hunyuan', the CAT activities were all significantly ($P<0.05$) lower in other accessions than in 'Midnight'.

Changes in MDA contents of different wild Kentucky bluegrass

The MDA contents of all the Kentucky bluegrass increased significantly ($P<0.05$) with the enhancement of

high-temperature stress, but they were also different in different accessions under same high-temperature stress (Table 7). At 0 days of high-temperature stress, the MDA contents were significantly ($P<0.05$) higher in 'Huozhou', 'Guangling', 'Hunyuan', 'Ningwu' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference among 'Midnight', 'Xinzhou' and 'Wutai', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 5 days of high-temperature stress, the MDA contents were significantly ($P<0.05$) higher in 'Huozhou', 'Xinzhou', 'Wutai' and 'Yingxian' than in 'Midnight', and there was no significant ($P>0.05$) difference among 'Midnight', 'Guangling', 'Hunyuan' and 'Wuzhai', but they were significantly ($P<0.05$) lower in other accessions than in 'Midnight'. At 10 days of high-temperature stress, except there was no significant ($P>0.05$) difference between 'Midnight' and 'Hunyuan', the MDA con-

TABLE 7. Effects of high-temperature stress on MDA contents in leaves of different wild Kentucky bluegrass ($\mu\text{mol g}^{-1} \text{FW}$).

Accessions	Duration of high-temperature stress (days)					
	0	5	10	15	20	25
Midnight	13.12±0.022Cf	4.01±0.032Ed	4.31±0.029Dg	8.01±0.055Cf	10.36±0.022Bf	11.52±0.020Ag
Qinshui	14.88±0.009Cb	3.82±0.035Eef	5.35±0.062De	6.80±0.050Ci	7.55±0.049Bk	9.23±0.040Ak
Huozhou	10.63±0.023Bm	4.78±0.020Ea	6.30±0.072Db	9.49±0.012Cd	11.80±0.032Bb	12.73±0.044Ad
Xinzhou	3.33±0.046Ed	4.40±0.046Db	5.07±0.024Cf	10.08±0.043Bb	10.21±0.067Bg	11.08±0.047Ah
Wutai	3.25±0.027Fde	4.55±0.059Eb	6.47±0.044Db	9.94±0.058Cbc	11.23±0.020Bd	11.98±0.049Af
Lingshi	2.87±0.036Fh	3.78±0.048Eef	5.25±0.023Def	7.40±0.036Ch	9.64±0.043Bh	10.49±0.029Ai
Lingqiu	2.94±0.044Fh	3.73±0.046Ef	6.94±0.045Da	10.21±0.027Ca	11.65±0.044Bc	12.05±0.044Af
Guangling	3.65±0.041Fb	4.07±0.023Ecd	6.30±0.038Db	9.93±0.060Cbc	10.61±0.045Be	11.38±0.019Ag
Hunyuan	3.49±0.013Fc	3.94±0.032Ede	4.42±0.071Dg	7.74±0.086Cg	9.46±0.028Bi	10.21±0.026Aj
Ningwu	3.66±0.024Fb	4.20±0.038Ec	5.62±0.072Dcd	8.78±0.053Ce	9.27±0.035Bj	12.28±0.051Ae
Wuzhai	2.89±0.009Fh	3.94±0.041Ede	5.47±0.070Dde	6.90±0.021Ci	11.72±0.041Bbc	14.48±0.035Aa
Tunliu	3.15±0.022Fef	4.19±0.033Ec	6.95±0.035Da	7.78±0.029Cg	10.14±0.016Bg	13.14±0.046Ac
Yingxian	3.89±0.029Fa	4.54±0.035Eb	6.25±0.044Db	8.86±0.066Ce	12.74±0.021Ba	13.96±0.038Ab
Youyu	2.97±0.024Fgh	3.66±0.036Ef	5.74±0.055Dc	9.78±0.094Cc	10.45±0.028Bf	12.26±0.042Ae

Different capital letters in the same row represent significant differences between treatments at the 0.05 level, different small letters in the same column represent significant differences between accessions at the 0.05 level.

tents were significantly ($P < 0.05$) higher in other accessions than in 'Midnight'. At 15 days of high-temperature stress, the MDA contents were significantly ($P < 0.05$) lower in 'Qinshui', 'Lingshi', 'Hunyuan', 'Wuzhai' and 'Tunliu' than in 'Midnight', and they were significantly ($P < 0.05$) higher in other populations than in 'Midnight'. At 20 days of high-temperature stress, the proline contents were significantly ($P < 0.05$) higher in 'Huozhou', 'Wutai', 'Lingqiu', 'Guangling', 'Wuzhai' and 'Yingxian' than in 'Midnight', and there was no significant ($P > 0.05$) difference between 'Midnight' and 'Youyu', but they were significantly ($P < 0.05$) lower in other accessions than in 'Midnight'. At 25 days of high-temperature stress, the MDA contents only were significantly ($P < 0.05$) lower in 'Qinshui', 'Xinzhou', 'Lingshi' and 'Hunyuan' than in 'Midnight', and there was no significant ($P > 0.05$) difference between 'Midnight' and 'Guangling', they were significantly ($P < 0.05$) higher in other accessions than in 'Midnight'.

Discussion

Disequilibrium of cellular homeostasis is the major cause for oxidative damage to membrane, lipids, proteins and nucleic acids in plants by environmental stresses (Srivalli et al., 2003). Lipid peroxidation, symbolizing with the production of MDA, is an important manifestation of environmental stresses in plants (Gupta et al., 2013). Thus, the MDA contents often augmented prominently in plants under high-temperature stress (Gao et al., 2010). Uniformly, the obvious increase of lipid peroxidation had also been observed in Kentucky bluegrass during high-temperature stress (Wang and Huang, 2004; He et al., 2005). In our study, the MDA contents increased significantly ($P < 0.05$) in all the accessions of Kentucky bluegrass along with the enhancement of high-temperature stress (Table 7), manifesting that lipid peroxidation was enhanced markedly in all the accessions of Kentucky bluegrass under high-temperature stress.

Osmotic adjustment is an important pathway to resist damage from environmental stress in plants (Sangu et al., 2015). Particularly, the accumulation of osmoprotectants, e.g., proline, sugars, proteins, were often regarded as a primary strategy for the protection and survival of plants under environmental stress (Chen et al., 2007). Under high-temperature stress, a remarkable enhancement of the soluble sugar and proline contents was observed in many plants for their osmotic adjustment (Sassi-Aydi et al., 2014; Harsh et al., 2016). In this study, the soluble sugar and proline contents also increased significantly ($P < 0.05$) in all Kentucky bluegrass along with the enhancement of high-temperature stress (Tables 2 and 4), indicating that osmotic adjustment capacity was augmented in Kentucky bluegrass to resist their injury from high-temperature stress. Similar results had been observed in Kentucky bluegrass under high-temperature stress (Du et al., 2011). Nonetheless, the soluble protein contents of all the Kentucky bluegrass decreased significantly ($P < 0.05$) during 5 to 10 days of high-temperature stress in our study (Table 3); this might be due to the inhibition of protein synthesis and the denaturation of some proteins at the initial stage of high-temperature stress (Levitt, 1980). Similarly, a remarkable decline of total soluble protein contents had been reported in leaves of mulberry (*Morus alba*; Chaitanya et al., 2001) and strawberry (*Fragaria × ananassa*; Gulen and Eris, 2004) under high-temperature stress. Conversely, the soluble protein contents of all the Kentucky bluegrass increased significantly ($P < 0.05$) during 15 to 25 days of high-temperature stress; this might be due to the release of membrane proteins by enhancement of lipid peroxidation

and proteins degradation at severe high-temperature stress (Gulen and Eris, 2004). In our study, The MDA contents of all the Kentucky bluegrass increased sharply during 15 to 25 days of high-temperature stress (Table 7), and the levels of SOD and CAT activities also declined significantly ($P < 0.05$) during this period of high-temperature stress (Tables 5 and 6). Thereby, the increase in soluble protein contents of Kentucky bluegrass might not be related to the enhancement of their osmotic adjustment ability, but it might be manifested their severe damage caused by high-temperature stress.

High levels of antioxidant enzymes had been observed in many plants during response to high-temperature stress, and they might have a specific role in the acquisition of tolerance to high-temperature stress (Tutar et al., 2017). The high levels of SOD and CAT activities played an important role in Kentucky bluegrass for response to high-temperature stress (Wang and Huang, 2004; He et al., 2005; He and Huang, 2010). In this study, the SOD activities also increased significantly ($P < 0.05$) in most accessions of Kentucky bluegrass during 0 to 15 days of high-temperature stress (Table 5), and the CAT activities increased significantly ($P < 0.05$) in all the accessions of Kentucky bluegrass during 0 to 15 days of high-temperature stress (Table 6). Simultaneously, The MDA contents increased significantly, but remained at a relatively low level (Table 7). Analogously, Harsh et al. (2016) had also observed that a prominent increase of SOD and CAT activities in most genotypes of moth bean (*Vigna aconitifolia*) accompanying with a remarkable over-accumulation of total sugar and proline under short-term high-temperature stress. These results showed that the high level of SOD and CAT activities delayed the explosion of lipid peroxidation in Kentucky bluegrass under mild and moderate high-temperature stress. However, the SOD and CAT activities declined significantly ($P < 0.05$) in all the accessions of Kentucky bluegrass during 15 to 25 days of high-temperature stress (Tables 5 and 6), and the MDA contents also produced rapidly in all the accessions of Kentucky bluegrass (Table 7). Similarly, an explosion of lipid peroxidation was also observed in many plants under high-temperature stress (Wang and Huang, 2004; Gao et al., 2007). Consequently, these results indicated that the action of SOD and CAT played an important role for Kentucky bluegrass in resisting high-temperature stress.

The defense capability was often diverse in different genotypes of plants to resist high-temperature stress, and their activation levels were also disparate in different genotypes of plants with the enhancement of high-temperature stress (Almeselmani et al., 2006). Under high-temperature stress, the variation of antioxidant capacity and lipid peroxidation was also different in different Kentucky bluegrass, which indicated that their responses to high-temperature stress were different (Wang and Huang, 2004; He, 2010; Du et al., 2011). In this study, the MDA contents of Kentucky bluegrass remained at a relatively low level in 'Qinshui', 'Lingshi' and 'Hunyuan' under high-temperature stress, but remained at a relatively high level in 'Huozhou', 'Wutai' and 'Yingxian', which symbolized that lipid peroxidation of Kentucky bluegrass in 'Qinshui', 'Lingshi' and 'Hunyuan' was more temperate than in 'Huozhou', 'Wutai' and 'Yingxian' under high-temperature stress. Under oxidative stress, O_2^- generated by the electron transport chain, the first to cause lipid peroxidation, often was dismutated to H_2O_2 by SOD in the matrix of plants (Matamoros et al., 2013). In this study, the SOD activities of Kentucky bluegrass still held at a relatively high level in 'Qinshui', 'Guangling' and 'Hunyuan' under high-temperature stress, but they held at a relatively low level in 'Wutai' and

'Lingshi', which manifested that more O_2^- was dismutated to H_2O_2 by SOD in 'Qinshui', 'Guangling' and 'Hunyuan' than in 'Wutai' and 'Lingshi'. However, the CAT activities of Kentucky bluegrass maintained at a relatively high level in 'Qinshui', 'Hunyuan' and 'Yingxian' under high-temperature stress, but they maintained at a relatively low level in 'Huozhou', 'Xinzhou', 'Lingshi', 'Guangling', 'Ningwu', 'Wuzhai', 'Tunliu' and 'Youyu', showing that more H_2O_2 was decomposed into H_2O by CAT in 'Qinshui', 'Hunyuan' and 'Yingxian' than in 'Huozhou', 'Xinzhou', 'Lingshi', 'Guangling', 'Ningwu', 'Wuzhai', 'Tunliu' and 'Youyu'. Similarly, the changes in SOD and CAT activities were also different in 37 genotypes of moth bean under short-term high-temperature stress (Harsh et al., 2016). In this study, the soluble protein contents of Kentucky bluegrass retained at a relatively high level in 'Qinshui', 'Hunyuan' and 'Yingxian' under high-temperature stress, but they retained at a relatively low level in 'Huozhou', 'Xinzhou', 'Wutai', 'Ningwu' and 'Tunliu'; this indicated that more protection might be provided by the enhancement of soluble protein contents in 'Qinshui', 'Hunyuan' and 'Yingxian' than in 'Huozhou', 'Xinzhou', 'Wutai', 'Ningwu' and 'Tunliu' under high-temperature stress. Moreover, the proline contents of Kentucky bluegrass sustained at a relatively high level in 'Qinshui', 'Hunyuan' and 'Wuzhai' under high-temperature stress, but they sustained at a relatively low level in 'Xinzhou', 'Guangling' and 'Yingxian'; this result also showed that there might be a higher capacity for osmotic regulation in 'Qinshui', 'Hunyuan' and 'Wuzhai' than in 'Xinzhou', 'Guangling' and 'Yingxian' under high-temperature stress. Likewise, the soluble sugar contents of Kentucky bluegrass kept at a relatively high level in 'Huozhou', 'Wutai' and 'Tunliu' under high-temperature stress, but they kept at a relatively low level in 'Xinzhou', 'Lingshi', 'Yingxian' and 'Youyu'; this result suggested that the ability of osmotic regulation might be enhanced in 'Huozhou', 'Wutai' and 'Tunliu' than in 'Xinzhou', 'Lingshi', 'Yingxian' and 'Youyu' under high-temperature stress. Consequently, there were different osmotic and antioxidative protection responses to high-temperature stress in different accessions of Kentucky bluegrass. Whereas, the MDA contents of Kentucky bluegrass kept at a relatively low level in 'Qinshui' and 'Hunyuan' under high-temperature stress, both their proline and soluble protein contents and activities of SOD and CAT all maintained at a high level, there results showed that they had a high adaptability to high-temperature stress.

Conclusions

In summary, the contents of MDA, proline and soluble sugar increased in all accessions of Kentucky bluegrass with the enhancement of high-temperature stress, the soluble protein contents decreased in all accessions of Kentucky bluegrass under 0 to 10 days of high-temperature stress, but they increased after 15 days of high-temperature stress. On the contrary, the activities of SOD and CAT increased in all accessions of Kentucky bluegrass under 10 or 15 days of high-temperature stress, and then declined with the continuous enhancement of high-temperature stress. The osmotic and antioxidant responses to high-temperature stress were different in different accessions of Kentucky bluegrass, but the decrease of SOD and CAT activities in 'Qinshui' and 'Hunyuan' were relatively slow, and their MDA contents also increased slowly. Therefore, 'Qinshui' and 'Hunyuan' accessions of Kentucky bluegrass had a high resistance to high-temperature stress, which could be used to develop new varieties which were resistant to high-temperature stress.

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References

- Almeselmani, M., Deshmukh, P.S., Sairam, R.K., Kushwaha, S.R., and Singh, T.P. (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant Sci.* 171, 382–388. <https://doi.org/10.1016/j.plantsci.2006.04.009>.
- Bailly, C., Benamar, A., Corbineau, F., and Côme, D. (1996). Changes in malondialdehyde content and superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiol. Plant.* 97, 104–110. <https://doi.org/10.1111/j.1399-3054.1996.tb00485.x>.
- Bates, L.S., Waldren, R.P., and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207. <https://doi.org/10.1007/BF00018060>.
- Bonos, S., and Murphy, J. (1999). Growth responses and performance of Kentucky bluegrass under summer stress. *Crop Sci.* 39, 770–774. <https://doi.org/10.2135/cropsci1999.0011183X003900030026x>.
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- Chaitanya, K.V., Sundar, D., and Reddy, A.R. (2001). Mulberry leaf metabolism under high temperature stress. *Biol. Plant.* 44, 379–384. <https://doi.org/10.1023/A:1012446811036>.
- Chance, B., and Maehly, A.C. (1955). Assay of catalase and peroxidase. *Method. Enzymol.* 2, 764–775. [https://doi.org/10.1016/S0076-6879\(55\)02300-8](https://doi.org/10.1016/S0076-6879(55)02300-8).
- Chen, Z., Cuin, T.A., Zhou, M., Twomey, A., Naidu, B.P., and Shabala, S. (2007). Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. *J. Exp. Bot.* 58, 4245–4255. <https://doi.org/10.1093/jxb/erm284>.
- Du, H., Wang, Z., Yu, W., Liu, Y., and Huang, B. (2011). Differential metabolic responses of perennial grass *Cynodon transvaalensis* × *Cynodon dactylon* (C4) and *Poa pratensis* (C3) to heat stress. *Physiol. Plant.* 141, 251–264. <https://doi.org/10.1111/j.1399-3054.2010.01432.x>.
- Dubois, M., Gilles, K., Hamilton, J.K., Robers, P.A., and Smith, F. (1956). A colorimetric method for the determination of sugar and related substances. *Anal. Chem.* 28, 350–356. <https://doi.org/10.1021/ac60111a017>.
- Ergo, V.V., Lascano, R., Vega, C.R.C., Parola, R., and Carrera, C.S. (2018). Heat and water stressed field-grown soybean: A multivariate study on the relationship between physiological-biochemical traits and yield. *Environ. Exp. Bot.* 148, 1–11. <https://doi.org/10.1016/j.envexpbot.2017.12.023>.
- Fang, C., Dou, L., Liu, Y., Yu, J., and Tu, J. (2018). Heat stress-responsive transcriptome analysis in heat susceptible and tolerant rice by high-throughput sequencing. *Ecol. Genetics and Genomics* 6, 33–40. <https://doi.org/10.1016/j.egg.2017.12.001>.
- Gao, Y., Guo, Y.K., Lin, S.H., Fang, Y.Y., and Bai, J.G. (2010). Hydrogen peroxide pretreatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat-stressed cucumber leaves. *Sci. Hortic.* 126, 20–26. <https://doi.org/10.1016/j.scienta.2010.06.006>.
- Gourdji, S.M., Sibley, A.M., and Lobell, D.B. (2013). Global crop exposure to critical high temperatures in the reproductive period: Historical trends and future projections. *Environ. Res. Lett.* 8, 24–41. <https://doi.org/10.1088/1748-9326/8/2/024041>.

- Gulen, H., and Eris, A. (2004). Effect of heat stress on peroxidase activity and total protein content in strawberry plants. *Plant Sci.* 166, 739–744. <https://doi.org/10.1016/j.plantsci.2003.11.014>.
- Gupta, N.K., Agarwal, S., Agarwal, V.P., Nathawat, N.S., Gupta, S., and Singh, G. (2013). Effect of short-term heat stress on growth, physiology and antioxidative defence system in wheat seedlings. *Acta Physiol. Plant.* 35, 1837–1842. <https://doi.org/10.1007/s11738-013-1221-1>.
- Harsh, A., Sharma, Y.K., Joshi, U., Rampuria, S., Singh, G., Kumar, S., and Sharma, R. (2016). Effect of short-term heat stress on total sugars, proline and some antioxidant enzymes in moth bean (*Vigna aconitifolia*). *Ann. Agric. Sci.* 61, 57–64. <https://doi.org/10.1016/j.aos.2016.02.001>.
- He, Y., and Huang, B. (2010). Differential responses to heat stress in activities and isozymes of four antioxidant enzymes for two cultivars of Kentucky bluegrass contrasting in heat tolerance. *J. Am. Soc. Hortic. Sci.* 135, 116–124. <https://doi.org/10.21273/JASHS.135.2.116>.
- He, Y., Liu, Y., Cao, W., Huai, M., Xu, B., and Huang, B. (2005). Effects of salicylic acid on heat tolerance associated with antioxidant metabolism in Kentucky bluegrass. *Crop Sci.* 45, 988–995. <https://doi.org/10.2135/cropsci2003.0678>.
- Kibinza, S., Vinel, D., Côme, D., Bailly, C., and Corbineau, F. (2006). Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. *Physiol. Plant.* 128, 496–506. <http://doi.org/10.1111/j.1399-3054.2006.00771.x>.
- Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., and Scharf, K. (2007). Complexity of the heat stress response in plants. *Curr. Opin. Plant Biol.* 10, 310–316. <https://doi.org/10.1016/j.pbi.2007.04.011>.
- Kraus, T.E., McKersie, B.D., and Fletcher, R.A. (1995). Paclobutrazole induced tolerance of wheat leaves to paraquat may involve antioxidant enzyme activity. *J. Plant Physiol.* 145, 570–576. [https://doi.org/10.1016/S0176-1617\(11\)81790-6](https://doi.org/10.1016/S0176-1617(11)81790-6).
- Levitt, J. (1980). Responses of Plants to Environmental Stresses, Vol. I (New York: Academic Press), p. 347–470.
- Matamoros, M.A., Fernández-García, N., Wienkoop, S., Loscos, J., Saiz, A., and Becana, M. (2013). Mitochondria are an early target of oxidative modifications in senescing legume nodules. *New Phytol.* 197, 873–885. <https://doi.org/10.1111/nph.12049>.
- Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G., Tognetti, V.B., Vandepoele, K., Gollery, M., Shulaev, V., and Van Breusegem, F. (2011). ROS signaling: the new wave? *Trends Plant Sci.* 16, 300–309. <https://doi.org/10.1016/j.tplants.2011.03.007>.
- Rao, K.V.M., and Sresty, T.V.S. (2000). Antioxidant parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci.* 157, 113–128. [https://doi.org/10.1016/S0168-9452\(00\)00273-9](https://doi.org/10.1016/S0168-9452(00)00273-9).
- Rezaei, E.E., Webber, H., Gaiser, T., Naab, J., and Ewert, F. (2015). Heat stress in cereals: Mechanisms and modelling. *Eur. J. Agron.* 64, 98–113. <https://doi.org/10.1016/j.eja.2014.10.003>.
- Sangu, E., Tibazarwa, F.I., Nyomora, A., and Symonds, R.C. (2015). Expression of genes for the biosynthesis of compatible solutes during pollen development under heat stress in tomato (*Solanum lycopersicum*). *J. Plant Physiol.* 178, 10–16. <https://doi.org/10.1016/j.jplph.2015.02.002>.
- Sassi-Aydi, S., Aydi, S., and Abdely, C. (2014). Inorganic nitrogen nutrition enhances osmotic stress tolerance in *Phaseolus vulgaris*: Lessons from a drought-sensitive cultivar. *HortScience* 49, 550–555. <https://doi.org/10.21273/HORTSCI.49.5.550>.
- Srivalli, B., Vishanathan, C., and Renu, K.C. (2003). Antioxidant defense in response to abiotic stresses in plants. *J. Plant Biol.* 30, 121–139.
- Tutar, O., Marín-Guirao, L., Ruiz, J.M., and Procaccini, G. (2017). Antioxidant response to heat stress in seagrasses. A gene expression study. *Mar. Environ. Res.* 132, 94–102. <https://doi.org/10.1016/j.marenvres.2017.10.011>.
- Wang, Z., and Huang, B. (2004). Physiological recovery of Kentucky bluegrass from simultaneous drought and heat stress. *Crop Sci.* 44, 1729–1736. <https://doi.org/10.2135/cropsci2004.1729>.
- Webber, H., Martre, P., Asseng, S., Kimball, B., White, J., Ottman, M., Wall, G.W., De Sanctis, G., Doltra, J., Grant, R., Kassie, B., Maiorano, A., Olesen, J.E., Ripoche, D., Rezaei, E.E., Semenov, M.A., Stratonovitch, P., and Ewert, F. (2017). Canopy temperature for simulation of heat stress in irrigated wheat in a semi-arid environment: A multi-model comparison. *Field Crop Res.* 202, 21–35. <https://doi.org/10.1016/j.fcr.2015.10.009>.
- Wu, S.W., Hu, C.X., Tan, Q., Nie, Z.J., and Sun, X.C. (2014). Effects of molybdenum on water utilization, antioxidative defense system and osmotic adjustment ability in winter wheat (*Triticum aestivum*) under drought stress. *Plant Physiol. Biochem.* 83, 365–374. <https://doi.org/10.1016/j.plaphy.2014.08.022>.
- Zou, M., Yuan, L., Zhu, S., Liu, S., Ge, J., and Wang, C. (2016). Response of osmotic adjustment and ascorbate-glutathione cycle to heat stress in a heat-sensitive and a heat-tolerant genotype of wucai (*Brassica campestris* L.). *Sci. Hortic.* 211, 87–94. <https://doi.org/10.1016/j.scienta.2016.08.011>.

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Address of authors:

Fangshan Xia, Zhaosheng Han, Huisen Zhu*, Kuanhu Dong and Lixia Du

College of Grassland Science, Shanxi Agricultural University, Taigu, Shanxi 030801, China

* Corresponding author; E-mail: zhuhuisen@126.com

Tel.: 86+0354-6287563; Fax: 86+0354-6287563