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RESEARCH ARTICLE

Polyamines and antioxidant defense system are associated with cold tolerance in centipedegrass

Jingjing CHEN[1,3*], Junyang FANG[1*], Zhenfei GUO (✉)[2], Shaoyun LU (✉)[1]

1 College of Life Sciences, Guangdong Engineering Research Center for Grassland Science/State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, South China Agricultural University, Guangzhou 510642, China
2 College of Grassland Science, Nanjing Agricultural University, Nanjing 210095, China
3 Key Laboratory of Tropical Fruit Biology of Ministry of Agriculture/South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Science, Zhanjiang 524091, China

Abstract Physiological responses to low temperature were analyzed in a chilling-tolerant centipedegrass (*Eremochloa ophiuroides*) accession, Shao Guan (SG), in comparison to the commercial cultivar, Common. Lower levels of ion leakage and higher chlorophyll (Chl) concentration were observed in SG than in Common during chilling stress. The maximum photochemical efficiency, the actual photosystem II (PSII) efficiency, photochemical quenching efficiency, and net photosynthetic rate were decreased during chilling stress in both genotypes, with higher levels of these parameters shown by SG than Common. In addition, higher activities of superoxide dismutase (SOD), catalase (CAT), ascorbate-peroxidase (APX) and glutathione reductase (GR), and higher concentrations of ascorbic acid (AsA) and glutathione (GSH) were observed in SG than in Common. Moreover, higher concentrations of putrescine (Put), spermidine (Spd), and spermine (Spm) were observed in SG than in Common. Correlation analysis indicated that SOD, CAT, APX and GR activities, and AsA and GSH concentrations showed high correlation to Put, while APX, GR, and AsA concentrations were correlated to Spd. Exogenous Put or Spd increased antioxidant enzyme activities and chilling tolerance. The results suggested that polyamine-regulated antioxidants are important for chilling tolerance in centipedegrass and protect plants against chilling induced oxidative damage.

Keywords antioxidants, centipedegrass, chilling, photosynthesis, polyamines

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Correspondences: zfguo@njau.edu.cn, turflab@scau.edu.cn
*These authors contribute equally to the work

1 Introduction

Tropical plant species are usually sensitive to low temperature. The enzyme activities in the Calvin–Benson cycle are more sensitive to chilling than the photochemical reactions, which results in a reduced utilization of absorbed light energy for CO_2 assimilation[1]. This imbalance between production and utilization of photo-generated reductants leads to an increased photosynthetic electron flux to O_2 to produce reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide (H_2O_2) and hydroxyl radicals. Accumulated ROS may function in signaling to regulate expression of many genes involving in adaptation responses and ROS may also result in oxidative damage to photosynthetic apparatus when they are not be effectively scavenged[2,3]. A protection mechanism consisting of both enzymatic and non-enzymatic antioxidant systems exists in plant cells for scavenging the accumulated ROS. Superoxide radicals are detoxified by superoxide dismutase (SOD), while H_2O_2 is scavenged by catalase (CAT) in peroxisomes and the ascorbate-glutathione cycle including ascorbate-peroxidase (APX), glutathione reductase (GR), ascorbate (AsA) and glutathione (GSH) exerts its antioxidative roles in chloroplast[2,3]. Numerous investigations have documented that the antioxidative system has an important role in chilling tolerance in various crops[4–7]. Higher levels of antioxidant enzyme activities are observed in chilling-tolerant cultivars than in chilling-sensitive cultivars under chilling stress[6,8].

Putrescine (Put), spermidine (Spd) and spermine (Spm) are major polyamines (PAs) in plant cells and are involved in adaptation responses to environmental stresses[9,10]. Put, Spd and Spm accumulate in cucumber (*Cucumis sativus*) during chilling stress, and pretreatment with Put or Spd increases chilling tolerance associated with higher antioxidant enzyme activities[11,12]. Cold tolerance is enhanced

by upregulating polyamine synthesis through overexpression of genes for arginine decarboxylase (*ADC*)[13,14], S-adenosylmethionine decarboxylase (*SAMDC*)[15] or spermidine synthase (*SPDS*)[16]. Downregulation of *OsSAMDC2* results in decreased tolerance to chilling, drought and salinity in transgenic rice with reduced Spd and Spm concentrations and antioxidant enzyme activities[17]. However, the effects of PAs on chilling tolerance and antioxidants in centipedegrass (*Eremochloa ophiuroides*) have not been reported.

Centipedegrass is a warm-season turfgrass species with medium texture, aggressive and uniform growth, thick sod formation, and excellent adaptation to low pH and poor soil. It is a low maintenance grass and requires infrequent mowing due to its slow-growing habit. Thus, it is increasingly used in residential lawns, recreational turf and soil conservation in subtropical to tropical regions[18,19]. However, centipedegrass has low genetic diversity[18,20], which hinders breeding efforts to improve it and consequently a limited number of cultivars have been released. Centipedegrass lacks cold tolerance, so low temperature is a major environmental factor limiting its use. Cold tolerance is increased by hardening centipedegrass plants before exposing to freezing[21] and sucrose accumulates in stolons of during cold acclimation[22]. In a recent study, genetic mapping and QTL analysis for seed yield, vegetative characters and cold tolerance in centipedegrass based on a population of 87 F_1 plants from crossing between two ecotypes were investigated[23]. Gamma irradiation has been used to broaden the genetic and morphological variations in centipedegrass[24,25] and our laboratory selected a chilling-tolerant mutant which maintained higher concentrations of antioxidants and polyamines during chilling stress compared with the wild type[8]. Despite the genetic studies on cold tolerance in centipedegrass, the physiological responses and adaptation mechanisms to low temperature are not fully understood.

China is thought to be the center of origin centipedegrass[18]. Our field evaluation found that a local centipedegrass accession, Shao Guan (SG), had better performance, with a shorter dormancy period in winter than the commercial cultivar, Common (unpublished data). The objective of this study was to investigate the physiological responses to chilling in SG in comparison with Common, and the effects of PAs on antioxidants and chilling tolerance for a better understanding of chilling tolerance mechanisms in centipedegrass.

2 Materials and methods

2.1 Plant growth and treatments

SG was collected from Shaoguan City, Guangdong Province, China, and vegetatively propagated in the field of South China Agricultural University (23.2419918° N, 113.6400595° E). The soil contained 0.36 $g \cdot kg^{-1}$ available N, 2.2 $mg \cdot kg^{-1}$ available P, 61.2 $mg \cdot kg^{-1}$ available K, and 0.81% organic matter (pH 5.7). Field evaluation showed that SG had better performance with a shorter dormancy period in winter than cv. Common (unpublished data). We used Common as a control in this study, as it is a widely used cultivar[18]. Similar-size plugs of SG and Common were transplanted from the field to 10-cm-diameter plastic pots containing a mixture of peat and perlite (3 : 1 v/v). The plants were allowed to grow for at least 60 d in a greenhouse (30/20°C day/night) under natural light, with daily irrigation, biweekly mowing at 4 cm, and biweekly fertilization with 0.3% solution of 15 : 6.6 : 12.5 NPK fertilizer as previously described[8]. For chilling treatment, SG and Common plants were moved to a growth chamber with a 12-h photoperiod under an irradiance of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD at 8°C for 30 d until the plants showed severe chilling injury as previously described [8]. For treatment with Put or Spd, the additional Common plants were irrigated with 50 mL of 0.1 $mmol \cdot L^{-1}$ Put or Spd solution per pot, because this concentration showed the best effect for increasing chilling tolerance of centipedegrass in preliminary experiments. The third leaf from the top was sampled for measurements. Several individual leaf samples were collected from the same pot, and three pots were used as replicates for each physiological measurement.

2.2 Determination of ion leakage and chlorophyll

Ion leakage was measured as previously described[8]. Leaf samples were immersed in 10 mL of distilled water until the conductivity reached a constant level. The conductivity of the solution (C_1) was measured using a conductivity meter (Model DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China), and was measured again (C_2) after boiling for 20 min and cooling to room temperature. Ion leakage was calculated as $(C_1/C_2) \times 100$. Chlorophyll (Chl) concentration was determined as described by Arnon[25]. Leaves (0.1 g) were ground with a mortar and pestle, and extracted with 10 mL of 80% acetone. Absorbance was measured at 663 nm and 645 nm using spectrophotometer (Model UV-2010, Hitachi, Japan) after filtration.

2.3 Determination of photosynthetic parameters

Net photosynthetic rate (A) was measured as described previously[8], using a LI-6400P Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA) according to the manufacturer's instructions. The measurement was conducted after 10 min equilibration to achieve steady-state conditions in the leaf chamber: photosynthetic active radiation was maintained at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, CO_2 concentration was controlled at 360 $\mu mol \cdot mol^{-1}$ at 80% relative humidity. Chl a fluorescence index was

measured by using a pulse-modulated fluorometer (Model FMS-2, Hansatech Instruments Ltd., Norfolk, UK) according to the manufacturer's instructions as previously described[7]. Maximal photochemical efficiency of photosystem II (PSII) (F_v/F_m) was calculated as (F_m–F_o)/F_m. The actual PSII efficiency (Φ_{PSII}) was calculated as (F_m'–F_s)/F_m'. Photochemical quenching (q_p), which represents the fraction of open PSII reaction centers, was calculated as (F_m'–F_s)/(F_m'–F_o'). Non-photochemical quenching (*NPQ*) was estimated as (F_m–F_m')/F_m'. The above indices were calculated automatically by the fluorometer.

2.4 Determination of enzyme activities and protein concentration

Antioxidant enzyme activities were determined as previously described[8]. Leaves (0.5 g) were ground in 5 mL of 50 mmol·L^{-1} phosphate buffer solution (pH 7.8) for extraction of SOD and CAT, or in 5 mL of 50 mmol·L^{-1} phosphate buffer (pH 7.0, containing 1 mmol·L^{-1} ascorbic acid and 1 mmol·L^{-1} EDTA) for extraction of APX[5,6]. After centrifugation at 15000 *g* for 15 min at 4°C, the supernatants were recovered for determinations of SOD, CAT, GR and APX as previously described[6]. One unit of SOD activity was defined as the amount of enzyme required for inhibition of photochemical reduction of ρ-nitro blue tetrazolium chloride by 50%. One unit of CAT, APX and GR was defined as the amount of enzyme required for catalyzing the conversion of one μmol H_2O_2 (extinction coefficient 0.0394 m·M^{-1}·cm^{-1}), AsA (extinction coefficient 2.8 m·M^{-1}·cm^{-1}), and oxidation of NADPH (extinction coefficient 6.22 m·M^{-1}·cm^{-1}) within 1 min, respectively. Protein concentration was determined using Coomassie Brilliant Blue G-250 according to the method of Bradford[26], using albumin from bovine serum as a standard.

2.5 Determination of ascorbic acid and glutathione

Fresh leaves (1 g) were ground in mortar and pestle in 5 mL of 5% trichloroacetic acid at 4°C. After centrifugation at 13000 *g* for 15 min, the supernatant was used for determinations of AsA and GSH as previously described[7]. AsA was measured using bipyridyl, while GSH was determined using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). Concentrations of AsA and GSH were calculated by using standard curves.

2.6 Determination of polyamine concentrations

PAs were extracted from leaves (0.5 g) with 4 mL of freshly prepared 5% (v/v) perchloric acid and extracted at 4°C, followed by centrifugation at 15000 *g* for 30 min. The supernatants were used for detection of free PAs using high-performance liquid chromatography (HPLC) as previously described[8]. Aliquots (0.5 mL) were benzoylated as described by Flores and Galston[27]. The benzoylated PAs were re-suspended in 1 mL of mobile phase solution and filtered (4.5 μm filter) before HPLC analysis. 20 μL of sample was injected into a Waters chromatographic system (Waters, Milford, MA, USA), supplied with a C18 column (Dalian Elite Analytical Instruments Co., Ltd., Dalian, China, 4.6 mm × 250 mm). The mobile phase was 64% methanol in an isocratic elution, at a flow rate of 0.7 mL·min^{-1} and ambient temperature. Identification and quantification of Put, Spd and Spm in each sample were achieved by comparing each peak retention time and peak area with the standard PAs, detected at 254 nm using a 2487 dual UV detector (Waters, Mildford, MA, USA). Put, Spd and Spm concentrations were calculated using standard curves with commercial standards and a correction for recovery after the extraction procedure.

2.7 Statistical analysis

All treatments in the study were arranged in a completely randomized design with three replicates. All data were subjected to analysis of variance according to the model for completely randomized design using an SPSS program (IBM, Armonk, NY, USA). Differences among means of plants from three pots as replicates and treatments were evaluated by Duncan's test at the 0.05 probability level.

3 Results

3.1 Differential tolerance to chilling in SG and Common

Ion leakage is usually used for physiological evaluation of plant chilling tolerance. Chilling treatment resulted in increased ion leakage in both SG and Common, with lower levels in SG than in Common (Fig. 1a). Compared to the response prior to chilling, for example, ion leakage increased 9.5 times in Common (from 5.9% to 61.2%), and 6.2 times in SG (from 5.7% to 40.8%) when the plants were treated at 8°C for 30 d (Fig. 1a). Chl concentration decreased in both cultivars under chilling treatment, with significantly higher levels remaining in SG than Common when treated from 15 to 30 d (Fig. 1b).

3.2 Responses of photosynthesis to chilling

Measurements of PSII activity in SG and Common using Chl a fluorescence assay found that the maximum photochemical efficiency of PSII (F_v/F_m) decreased. There was no significant difference between the two genotypes until 20 d of chilling treatment, with SG being significantly higher (42% and 57%, respectively) than Common at day 25 and day 30 (Fig. 2a). Likely, Φ_{PSII} and q_p decreased in both cultivars after chilling treatment, with significantly higher levels in SG than in Common from 20

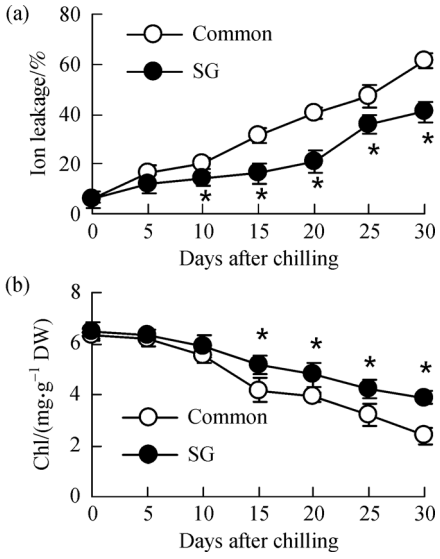

Fig. 1 Ion leakage (a) and chlorophyll (Chl) (b) in a chilling-tolerant accession (SG) and the commercial cultivar Common of centipedegrass in response to chilling at 8°C. Values are means ± SE ($n = 3$); *, $P < 0.05$.

to 30 d (Fig. 2b–2c). No significant difference in *NPQ* was observed between SG and Common except for 30 d after chilling treatment (data not shown). Net photosynthetic rate (*A*) decreased with chilling treatment and was higher in SG than in Common (Fig. 2d), whereas no significant difference in *A* was observed under normal growth conditions (Fig. S1).

3.3 Responses of antioxidants to chilling stress

Activities of SOD, CAT, APX and GR, and concentrations of AsA and GSH showed no significant difference between the two genotypes under normal growth conditions (Fig. S2). Chilling treatment resulted in an increased SOD activity in SG, but not in Common for the first 5 d. SOD activity decreased from 15 to 30 d in both SG and Common, but remained 26% to 62% higher in SG than in Common (Fig. 3a). CAT, APX and GR activities increased in both genotypes after 5 d of chilling, followed by gradual decrease from 5 to 30 d; however, they remained 23% to 88%, 32% to 59%, and 23% to 84% higher in SG than in Common, respectively, from 10 to 30 d of chilling treatment (Fig. 3b–3d).

AsA was induced to a peak at 5 d, followed by a gradual decrease, but SG was still significantly higher (16% to 33%) than Common from 5 to 30 d (Fig. 3e). GSH showed a slight increase in SG, but not in Common, from 0 to 5 d of chilling stress. GSH in SG was significantly higher (by 23% to 40%) at 15 and 30 d than in Common (Fig. 3f).

3.4 Responses of polyamines to chilling

The data indicate that SG has significantly higher PAs than Common even without the chilling treatment. Put concentration was 22% higher in SG than in Common prior to chilling. It increased and reached a peak at 5 and 10 d under chilling stress, followed by a gradual decrease after 15 d in both genotypes. Put concentration increased more in SG (49% and 54%) than in Common (29% and 27%) at 5 and 10 d, respectively. The Put concentrations were

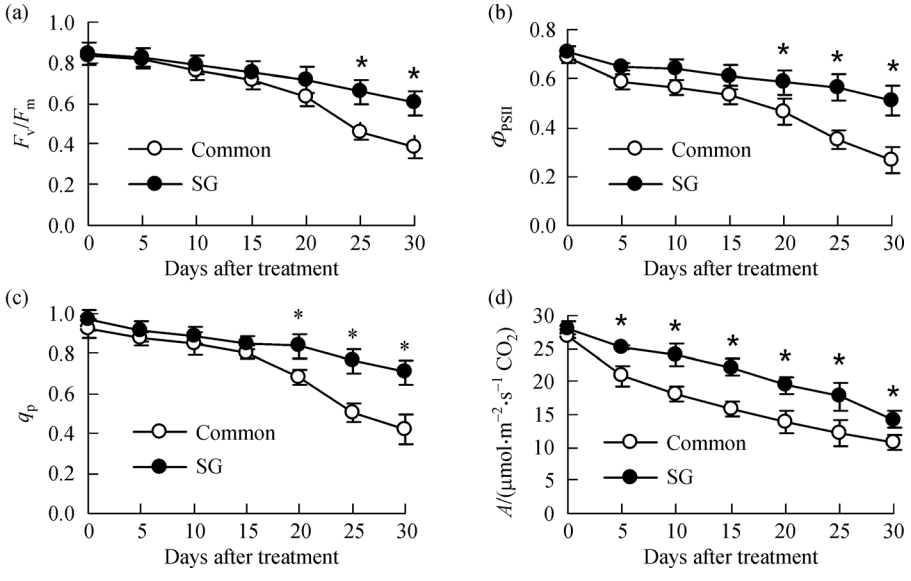

Fig. 2 Maximal photochemical efficiency of PSII (F_v/F_m) (a), the actual PSII efficiency (Φ_{PSII}) (b), photochemical quenching (q_p) (c), and net photosynthetic rate (*A*) (d) in two centipedegrass genotypes, SG and cv. Common, in response to chilling at 8°C. Values are means ± SE ($n = 3$); *, $P < 0.05$.

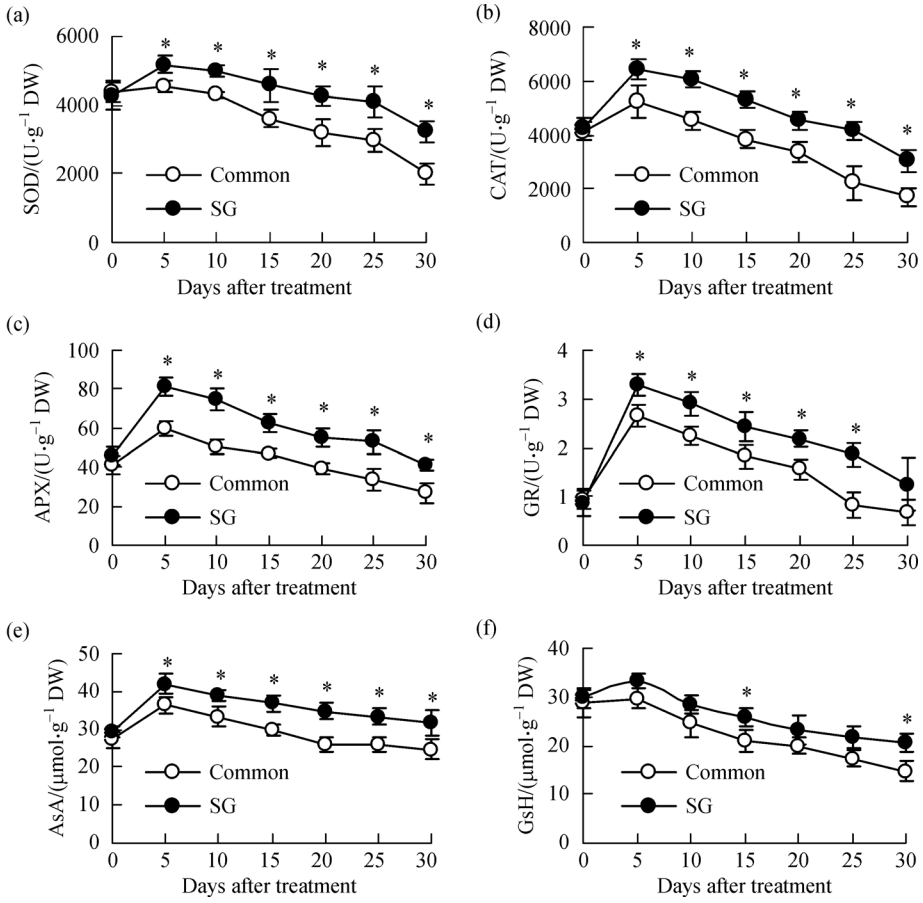

Fig. 3 Activities of superoxide dismutase (SOD) (a), catalase (CAT) (b), ascorbate-peroxidase (APX) (c), glutathione reductase (GR) (d), concentrations of ascorbate (AsA) (e) and reduced glutathione (GSH) (f) in two centipedegrass genotypes, SG and cv. Common, in response to chilling at 8°C. Values are means ± SE ($n = 3$); *, $P < 0.05$.

always significantly higher in SG than in Common throughout the chilling treatment (Fig. 4a).

Spd concentration was 51% higher in SG than in Common before chilling treatment (Fig. 4b), increased to a peak at 10 d chilling in both genotypes and was 68% higher in SG than in Common. Although Spd decreased from 15 d in both genotypes, SG still had significantly higher concentrations of Spd. For example, SG Spd concentration was 90% and 76% higher than Common at 15 and 30 d after chilling (Fig. 4b), respectively. Similarly, Spm concentration was higher in SG than in Common by 35% before chilling, and significantly higher concentrations were observed in SG compared to Common at most time points during the chilling treatment (Fig. 4c).

3.5 Correlations between polyamines and antioxidants

Correlations between PAs and antioxidants in both SG and Common plants were analyzed. Put showed a significant correlation with SOD, APX and GR activities and AsA and GSH concentrations (Fig. 5a–5e); Spd had significant correlation with APX and GR activities and AsA concentration (Fig. 5f–5h); and Spm was significantly correlated with GR activity (Fig. 5i).

3.6 Effect of exogenously applied Put and Spd on chilling tolerance and antioxidant enzyme activities

To evaluate chilling tolerance affected by PAs, ion leakage, F_v/F_m, and Chl were measured in Common during chilling stress after plants were irrigated with 0.1 mmol·L^{-1} of Put or Spd. Although ion leakage was increased as a result of the chilling treatment, significantly lower ion leakage was observed in the Put or Spd treated plants than in control plants (Fig. 6a). Similarly, higher levels of F_v/F_m and Chl concentration were observed in the Put or Spd treated plants than in the control during the chilling treatment (Fig. 6b–6c), indicating that higher Put and Spd could reduce chilling damage to centipedegrass. In addition, significantly higher activities of SOD, CAT, and APX were

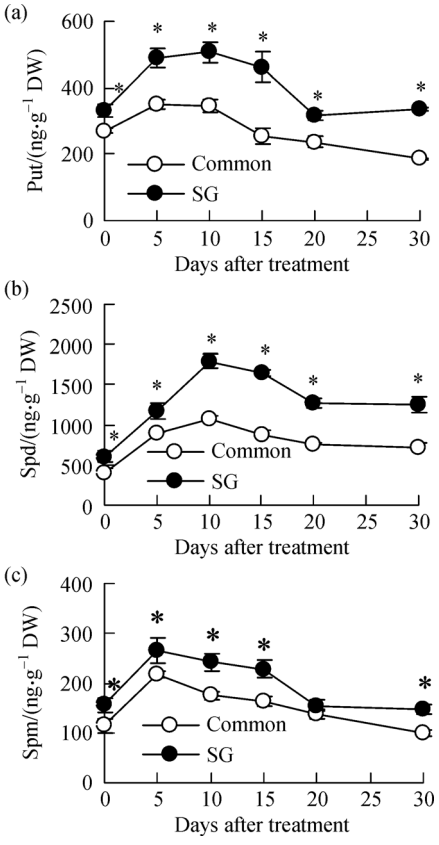

Fig. 4 Putrescine (Put) (a), spermidine (Spd) (b), and spermine (Spm) (c) concentrations in two centipedegrass genotypes, SG and cv. Common, in response to chilling at 8°C. Values are means ± SE ($n = 3$); *, $P < 0.05$.

observed in Put or Spd treated plants than in control plants prior to or under the chilling treatment (Fig. 7).

4 Discussion

Ion leakage can indicate plasmalemma damage. Ion leakage increased in both SG and Common centipedegrass during chilling stress, indicating a chilling injury; lower levels of ion leakage were observed in SG than in Common, indicating that SG had higher chilling tolerance than Common. This was further demonstrated by the higher Chl concentration remaining in SG than in Common after chilling treatment. Chilling has a direct impact on the photosynthetic apparatus by disrupting all the major components of photosynthesis, including the thylakoid membranes and subsequently the Chl pigments[28]. F_v/F_m is used as an indicator of stress damage to PSII[6–8]. Chilling resulted in a decreased F_v/F_m in centipedegrass plants, with higher F_v/F_m remaining in SG than in Common, suggesting that SG was less damaged by chilling. Φ_{PSII} indicates quantum yield of PSII linear electron transport and is positively related to linear electron transport rate (ETR)[29], while q_p is the PSII efficiency factor[30]. Φ_{PSII} and q_p decreased in all centipedegrass plants with chilling treatment, indicating that chilling resulted in decreased ETR and PSII efficiency. Higher levels of Φ_{PSII} and q_p were maintained in SG than in Common under chilling, suggesting that SG had greater capacity to use the absorbed light energy due to a higher rate of electron transport for photochemical reactions than Common. The maintenance of a greater rate of electron flow for enhancing photochemical light energy consumption can decrease Q_A reduction for protection of PSII against chilling injury[29]. Thermal energy dissipation, which is usually measured by *NPQ*, is one of the major protection mechanisms against chilling[31]. However, there was no significant difference in *NPQ* between SG and Common at most time points during chilling stress, suggesting that the differential chilling tolerance between SG and Common was not associated with thermal energy dissipation.

A balance between production and scavenging of ROS is essential for plant survival under stressed conditions. Low temperature-inhibited enzyme activities in the Calvin–Benson cycle reduces the utilization of absorbed light energy for CO_2 assimilation and results in an increased photosynthetic electron flux to O_2 for production of ROS[1]. The antioxidant defense system protects plants against chilling injury through scavenging ROS. Most of the antioxidants had increased in both SG and Common after 5 and/or 10 d of chilling, which indicates an adaptation mechanism of centipedegrass to chilling, avoiding accumulation of ROS under low temperature conditions. In addition, higher activities of antioxidant enzymes (SOD, CAT, APX and GR) and concentrations of non-enzyme antioxidants (AsA and GSH) were observed in SG as compared with Common under chilling conditions. This result is consistent with previous observations that higher concentrations of antioxidants were maintained in a chilling-tolerant mutant of centipedegrass and in chilling-tolerant cultivars of other crops during chilling when compared to chilling-sensitive cultivars[6–8]. Our results suggest that upregulation of antioxidant defense system has an important role in chilling tolerance in centipedegrass.

Polyamines may regulate plant adaptation to abiotic stresses. An improved tolerance to chilling and/or freezing was observed in the transgenic plants upregulating Spd or Put synthesis[14–16], although the molecular mechanism of PAs action is still not understood. In contrast, knockout or downregulation of *ADC*, *SAMDC*, *SPDS* and *SPMS* genes decreases concentrations of polyamines, and tolerance to cold, drought and salinity stress[14,17,31]. The decreased Spm concentration in the *spms* mutant results in decreased tolerance to drought and salinity by impairing Ca^{2+}

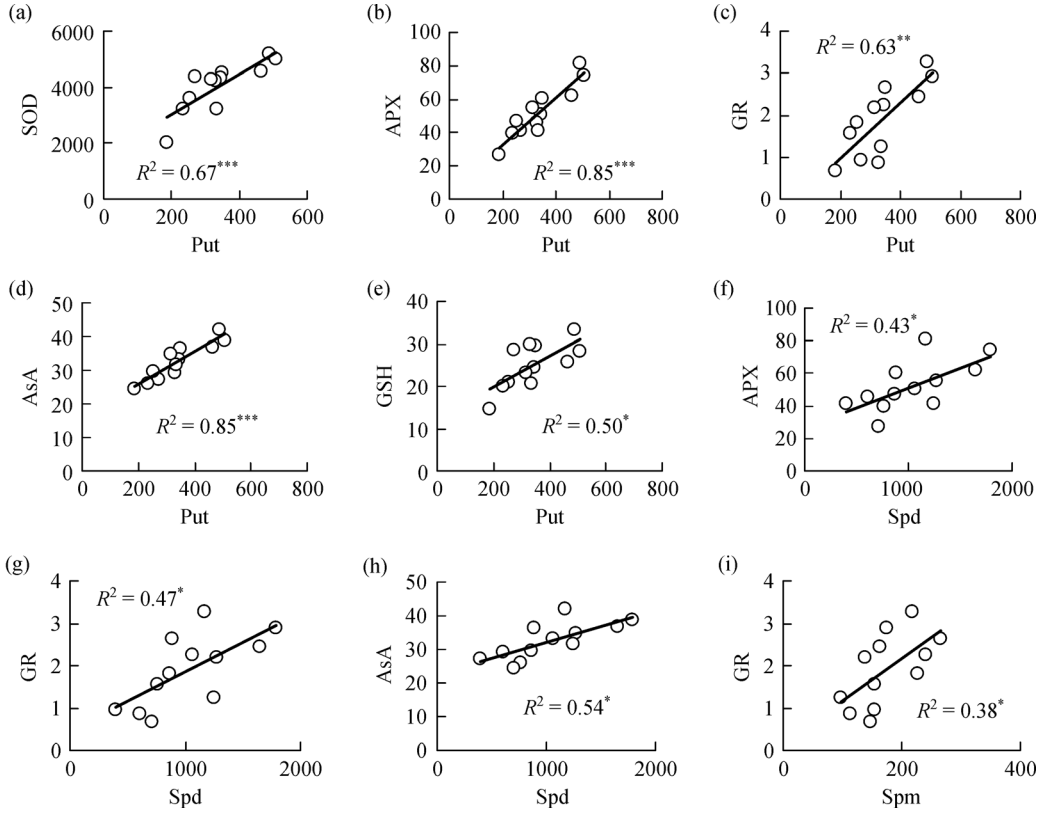

Fig. 5 Analysis of correlation of antioxidants with putrescine (Put) (a–e), spermidine (Spd) (f–h), and spermine (Spm) (i) in centipedegrass under chilling stress conditions. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

homeostasis[32,33]. Higher concentrations of Put, Spd and Spm were observed in SG in the present study. The results are consistent with the previous report that higher concentrations were maintained in a chilling-tolerant mutant than in the wild type of centipedegrass[8] Spd is accumulated in the apoplast under salinity, where PAO catalyzes its conversion to H_2O_2. The accumulated H_2O_2 results in induction of either stress tolerance responses or PCD, depending on the concentration of intracellular PAs[34]. Put accumulates under cold stress, which is essential for proper cold acclimation and survival at freezing in *Arabidopsis* by increasing ABA concentration through inducing gene expression involved in ABA biosynthesis[14]. ABA and H_2O_2 induce expression of antioxidant enzyme genes and result in increased activities of these enzymes in plants[35,36]. Correlation analysis of PA concentrations and antioxidants showed that Put concentrations were highly correlated with most of the determined antioxidants, while Spd concentrations correlated with APX, GR and AsA, indicating that the antioxidant system might be regulated by Put and Spd in centipedegrass. It has been reported that treatment with PAs increases activities of antioxidant enzymes and reduces oxidative damage in chickpea (*Cicer arietinum*)[37] and *Brassica juncea*[38]. Induced activities of SOD, CAT and APX and enhanced chilling tolerance of centipedegrass were observed in the present study. Our results suggest that the differential accumulation of Put, Spd and Spm under chilling, which leads to the differential antioxidant defense capacity, was associated with the differential tolerance to chilling between SG and Common. It seems that SG has a variation/mutant with causes altered polyamine metabolism leading to elevated concentrations of polyamines. Our data points to the potential for future upregulation of polyamine biosynthesis using a transgenic approach to improve centipedegrass chilling tolerance.

5 Conclusions

In conclusion, higher concentrations of antioxidants and PAs maintained in SG under chilling improved protection of photosynthesis against oxidative damage and were associated with higher chilling tolerance as compared with Common. Antioxidant responses to chilling were correlated with PAs, especially with Put and Spd in

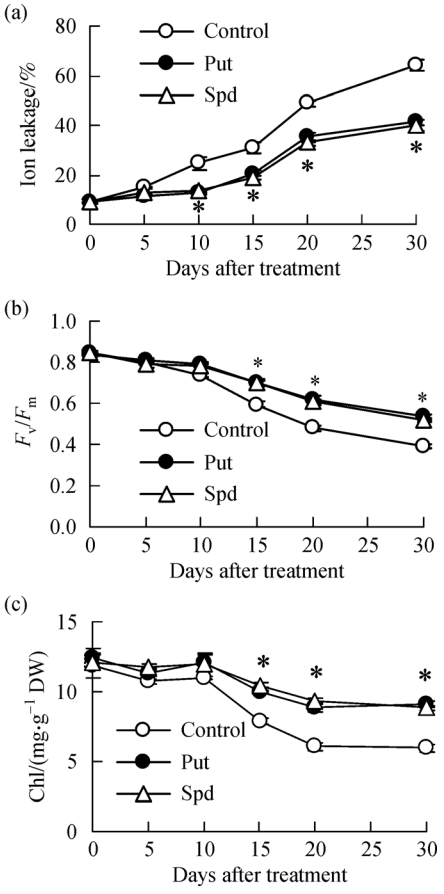

Fig. 6 Effects of exogenous putrescine (Put) and spermidine (Spd) on chilling tolerance. Centipedegrass cv. Common was irrigated with 50 mL of 0.1 mmol·L^{-1} Put or Spd solution per pot, followed by chilling at 8°C. Ion leakage (a), maximal photochemical efficiency of PSII (F_v/F_m) (b), and chlorophyll (Chl) concentration (c) were measured during the chilling treatment. *, $P < 0.05$.

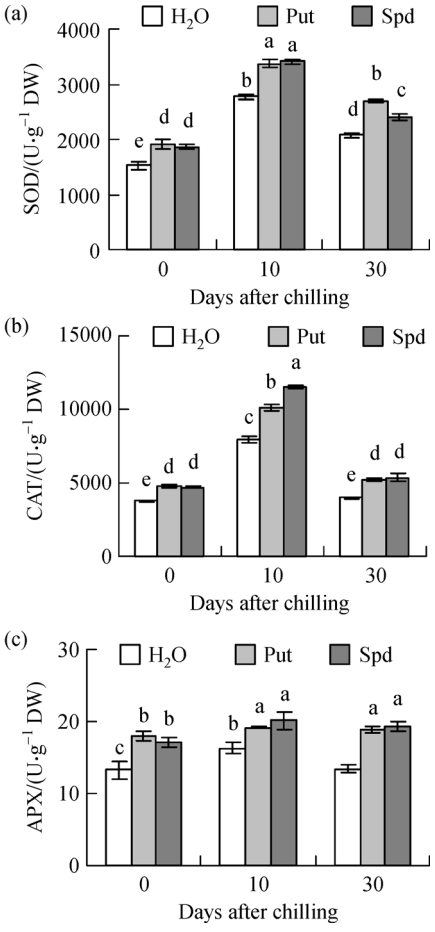

Fig. 7 Effects of exogenous putrescine (Put) and spermidine (Spd) on antioxidant enzyme activities in centipedegrass. Centipedegrass cv. Common was irrigated with 50 mL of 0.1 mmol·L^{-1} Put or Spd solution per pot, followed by chilling at 8°C. Superoxide dismutase (SOD) (a), catalase (CAT) (b), and ascorbate-peroxidase (APX) (c) activities were measured during the chilling treatment. The same letter above the column indicates no significant difference at $P < 0.05$.

centipedegrass and application of Put or Spd increased antioxidant enzyme activities and chilling tolerance. It is suggested that PA-regulated antioxidant protection is an important mechanism for chilling tolerance in centipedegrass.

Supplementary materials The online version of this article at https://doi.org/10.15302/J-FASE-2017197 contains supplementary materials (Fig. S1; Fig. S2).

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This article does not contain any studies with human or animal subjects performed by any of the authors.

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