https://doi.org/10.17221/17/2022-HORTSCI

LbCu/ZnSOD and *LbMnSOD* involved in drought stress tolerance induced by strigolactones of cut lily

Bowen Chi[1], Tian Xie[1], Li Liu[1], Junhui Yan[1], Zixian Zhao[1], Minghua Deng[2], Jinfen Wen[1]**

[1]*Faculty of of Architecture and Urban Planning, Kunming University of Science and Technology, Kunming, Yunnan, P. R. China*

[2]*College of Horticulture and Landscape, Yunnan Agricultural University, Kunming, Yunnan, P. R. China*

**Corresponding authors: dengminghua2013@sina.com; wenjf888@163.com*

Citatiton: Chi B., Xie T, Liu L., Yan J.H., Zhao Z.X., Deng M.H., Wen J.F. (2023): *LbCu/ZnSOD* and *LbMnSOD* involved in drought stress tolerance induced by strigolactones of cut lily. Hort. Sci. (Prague), 50: 241–251.

Abstract: In this study, *LbCu/ZnSOD*, *LbFeSOD*, and *LbMnSOD* genes were cloned, the role of strigolactones (SLs), a novel plant hormone that is ubiquitous in plants in modulating plant responses to abiotic stress, on the three superoxide dismutases (SODs) under polyethylene glycol PEG-6000 stress were researched in the petals of cut lily flowers. The results indicated that during the development of the lily bud, the expression levels of *LbMnSOD* gradually increased and those of *LbCu/ZnSOD* decreased, while the *LbFeSOD* expression remained at a very low level. When the cut lily flowers were subjected to 10% PEG-6000 stress, the relative water content (RWC) declined, the malondialdehyde (MDA) content and relative electrical conductivity (REC) dramatically increased in the petals. However, when exogenous SLs were employed, the RWC were improved, while the MDA and REC were reduced. Meanwhile, the SLs significantly increased the activities of the total SOD (T-SOD), Cu/ZnSOD and MnSOD, the expression levels of *LbCu/ZnSOD* and *LbMnSOD*, especially *LbCu/ZnSOD*, were markedly up-regulated in the petals. In conclusion, our research indicates that SOD enzymes, especially *Cu/ZnSOD* and *MnSOD*, are involved in the drought stress tolerance; the application of strigolactones can enhance the activities of the two SODs, and may increase the expression of *LbCu/ZnSOD* and *LbMnSOD* via a positive feedback mechanism in the cut lily petals.

Key words: lily; superoxide dismutase; drought stress; PEG-6000; strigolactones

Plants suffer various biotic and abiotic stresses which can induce excessive reactive oxygen species (ROS) in the cells during their growth and development. As a kind of signal molecules, ROS can trigger a series of signalling pathways to regulate the metabolic reaction and gene expression. Once ROS production and scavenging cannot keep in balance, excessive ROS will cause cell membrane lipid peroxidation, protein inactivation, DNA damage, and even worse, leading to cell dysfunction and programmed cell death (Karuppanapandian et al. 2011). Long-term adaptation has allowed plants to evolve an efficient and complex ROS scavenging system, which mainly includes an enzyme antioxidant defence system and non-enzymatic antioxidant defence system. Serving as the first line

https://doi.org/10.17221/17/2022-HORTSCI

of defence to remove ROS, superoxide dismutase (SOD) is ubiquitous in animals, plants, and microorganisms. It can catalyse the disproportionation reaction of the superoxide (Abreu, Cabelli 2009), cooperate with catalase, glutathione peroxidase and ascorbate peroxidase to effectively protect plant cells, and therefore enhance the tolerance of plants under environmental stress.

According to the binding metal co-factors, subcellular protein distribution and protein folding, SOD is mainly divided into three categories: Cu/ZnSOD, FeSOD and MnSOD (Alscher et al. 2002). Cu/ZnSOD*s* are mainly distributed in the cytoplasm of plants (Miller 2012), FeSODs are located in the plant chloroplasts and cytoplasm, while MnSOD*s* are found in the mitochondria and peroxisomes (Wang et al. 2016a). The SOD gene family has been identified in many plant species, such as Arabidopsis (Kliebenstein et al. 1998), sorghum (Filiz, Tombuloglu 2015), grapes (Hu et al. 2019), cotton (Wang et al. 2016b), rice (Dehury et al. 2013), and bananas (Feng et al. 2015). Several reports have shown that the SOD gene expression can be enhanced by environmental stresses such as drought, heavy metals, temperature stress, diseases and insect pests, pathogenic bacteria, the ozone, etc. The SOD gene expression of Broad beans was significantly up-regulated under high temperatures, (Siddiqui et al. 2015; Abdelgawad et al. 2020) found that the SOD enzyme activity of purslane increased under Cu and Cd stress. The over-expression of Cu/ZnSOD in cassavas showed enhanced drought tolerance (Xu et al. 2013).

The lily (*Lilium brownii* var. viridulum Baker) is a perennial herbaceous bulbous plant of the genus Liliaceae (scientific name: Lilium). As one of the top five cut flowers in the world, the ornamental value of cut lilies is extremely high. However, due to the long-distance dry transportation after harvesting at the green bud stage, the cut lily is extremely vulnerable to bud malformation and abnormal flower opening.

As new plant hormones, strigolactones (SLs) are ubiquitous in plants and are involved in regulating the plant growth and development (López-Ráez et al. 2017; Kramna et al. 2019; Rochange et al. 2019) engaged in the development of the root, leaf senescence, shoot branching, etc. In response to different environmental circumstances, such as salinity, drought, cold, heavy metals, heat and nutrient deprivation, SLs were studied to test their accumulation in the plant tissues (Bhoi et al. 2021).

This study aimed to investigate the involvement of the SOD family in the drought tolerance induced by SLs in the cut lily flower. Therefore, three kinds of SOD were cloned and their expression levels were analysed during the lily bud development, meanwhile their roles on the drought tolerance were researched by studying the activities and relative expression of three types of SOD under PEG-6000 stress combined with the strigolactone (SL) treatment in cut lily flower petals.

MATERIAL AND METHODS

Plant material and experimental treatment. 'Corvara' lilies were used as the experimental material, which were grown in a local greenhouse in Kunming. The fresh cut harvested lily flowers were taken to the laboratory within two hours. Flowers with uniform stem diameter and at the same flower stage were selected and randomly put into culture flasks filled with the following solutions (according to the screening results of our preliminary experiments, the concentration of the SLs was 5 μM): (1) Control: distilled water (CK); (2) PEG: 10% PEG-6000; (3) PEG+SLs: 5 μM SLs + 10% PEG-6000;

Then they were cultivated in an artificial climate room (13 hours/11 hours, light/dark, 25 °C, 20 000 lux, relative humidity 70 ± 5%). The petals were collected at fixed time points.

Images of the flowers' morphological change. In order to observe the change in the lily's morphology, each flower was photographed from above every 24 hours with the image processing software Adobe Photoshop CC2018.

Determination of the malondialdehyde (REC), relative water content (RWC) and malondialdehyde (MDA) content. The REC of the lily petals was determined according to Li's method (Li 2000); RWC = (original fresh weight − dry weight)/(saturated fresh weight − dry weight) × 100% (Zhang, Xu 2000), and each measurement was repeated six times.

The MDA content in the lily petals was tested by the thiobarbituric acid method (Li 2000), each measurement was repeated 3 times.

Determination of the SOD activity. The T-SOD, Cu/ZnSOD and MnSOD assay kits were purchased from the Nanjing Jiancheng Institute of Biological Engineering. The SOD activity was determined by the xanthine oxidase method according to the in-

https://doi.org/10.17221/17/2022-HORTSCI

structions of the kit (A001-3-2, Nanjing Jiancheng, Nanjing, P. R. China).

One-half of one gram (0.5 g) of plant tissue was accurately weighed and cut into pieces, the volume of homogenisation solution was added four times. The sample was homogenised under ice-water bath conditions and centrifuged at 3 500 rpm for 10 minutes, and the supernatant was kept for further testing. Finally, the absorbance values of the supernatants were measured by a visible light/fluorescence spectrophotometer, and the SOD (U/mgprot) activity in the sample was calculated by the Eq. (1):

$$SOD_{activityn} = \frac{A_{Contrast} - A_{Sample}}{A_{Contrast}} \div 50\% \times \frac{V_t}{V_s} \div \rho \quad (1)$$

where: $A_{Contrast}$ – the absorbance in the contrast tube; A_{Sample} – the absorbance in the assay tube, V_t is the total volume of the reaction solution; V_s – the sampling volume; ρ (mgpro/ml) – the protein concentration in the tissue homogenate.

Cloning and relative expression of *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD*. The petal samples were ground into a powder in liquid nitrogen, and the total RNA was extracted with Trizol reagent (Hui et al. 2008), and reverse transcribed with a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA), finally, the cDNA was stored at –20 °C in a refrigerator, used for the polymerase chain reaction (PCR) and quantitative polymerase chain reaction (q-PCR) analyses.

Primer 5.0 was used to design the primers. The primer sequence is shown in Table 1. A 30 μL reaction system of Reverse Transcription – Polymerase Chain Reaction (RT-PCR) was configured as follows: 3 μL of 10 × PCR buffer, 2.4 μL of dNTP, 1.5 μL of cDNA, 0.15 μL of TaKaRa Ex Taq, 0.6 μL of the PCR Forward Primer, 0.6 μL of the PCR Reverse Primer (Table 1), 21.75 μL of dd H_2O. The cloning reaction program: 94 °C pre-denaturation, 5 minutes; 94 °C denaturation for 30 second annealing for 30 seconds; 72 °C extension for 10 minutes, 35 cycles; 72 °C extension for 10 minutes. Agarose gel electrophoresis was carried out to detect the bright band so that the picked product could be sent to the company for sequencing.

The RT-PCR experiment kit (EvaGreen 2X qPCR MasterMix) was purchased from Zhenjiang Aibi Dream Biotechnology Co., Ltd., P. R. China. The reaction system of the real-time fluorescence quantitative PCR (qPCR) was as follows: 10 μL of EvaGreen 2X qPCR MasterMix, 0.6 μL of the PCR Forward Primer, 0.6 μL of the PCR Reverse Primer, 2 μL of cDNA, 6.8 μL of double distilled water (dd) H_2O. The reaction procedure was as follows: pre-denaturation at 95 °C for 10 minutes, denaturation at 95 °C for 15 s, annealing for 60 s, extension at 60 °C for 60 s, and 40 cycles. According to the experimental requirements, the reaction was run on the BIO-RAD CFX96 Optics Module system. Each material sample was repeated threetimes independently or multiple times. The $2^{-\Delta\Delta CT}$ method was used to analyse the data. The data were analysed by SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA).

Bio-informational analysis methods. The open reading frame (ORF) Finder (https://blast.ncbi.nlm.nih.gov/orffinder) in the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov) was used to find the genes open reading frame and the BLASTp function of the NCBI website (https://blast.ncbi.nlm.nih.gov/) was applied to analyse the sequence homology.

The biological software DNAMAN7.0 and ClustalX2.0.11 were employed to translate the nucleotide sequence and align the amino acid sequence of the predicted homologous gene. The phylogenetic tree system was constructed and analysed

Table 1. Sequences of the RT–PCR and qPCR primers

Gene name	QRT–PCR primer sequence	Gene name	PCR primer sequence
LbCu/ZnSOD-qF	CAGCAATAGCACCGCAGTCAG	*LbCu/ZnSOD*-F	ATCATCGTCGTTGCTCACTCG
LbCu/ZnSOD-qR	TTGGTCGTATCCCCAAAGGAA	*LbCu/ZnSOD-R*	CACATCGTACACCATGTCCTGTC
LbMnSOD-qF	CTCCCCGACCTCCCGTACGAC	*LbMnSOD-F*	CCTGCTCCCATTTCTCAC
LbMnSOD-qR	AATAGCCCAGCCAAGTGCCCC	*LbMnSOD-R*	TTCGGATAAGTTCACAAGAG
Lbaction-qF	CCCCACTCAATCCCAAGGCAA	*LbFeSOD-F*	TGAAAGGAGCCTGTTCTC
Lbaction-qR	CGGAAGTCCAGCACAATACCA	*LbFeSOD-R*	TGTGCCCTGATAGTGATT

SOD-qR and *SOD-qF* are qPCR reaction primers, *SOD-R* and *SOD-F* are PCR reaction primers, and the action is the internal reference gene of the lily

https://doi.org/10.17221/17/2022-HORTSCI

by the Neighbour-joining (NJ) calculation method in the MEGA 7.0 software.

Statistical analysis. All the experiments were repeated at least three times. All the data were analysed by an analysis of variance (ANOVA) using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was performed to assess the differences among the treatments. The differences in the means among the treatments were considered statistically significant when $P < 0.01$.

RESULTS

Cloning of *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD*. *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD* were cloned by the RT-PCR method, and products were detected by 1% agarose gel electrophoresis (Figure 1). The complete ORF frame coding regions of *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD* were 495 bp, 702 bp and 651 bp, and the coding proteins were 205, 233 and 216 amino acids, respectively.

Analysis of the phylogenetic tree of the *LbCu/ZnSOD, LbMnSOD* and *LbFeSOD* proteins. A phylogenetic tree was constructed to reveal the evolutionary relationship of the three types of SOD to other plant SOD genes. The phylogenetic tree revealed that these SODs could be classified into three groups, as shown in Figure 2, corresponding to FeSODs, Cu/ZnSODs and MnSOD*s*. The neighbour-joining phylogenetic tree showed that *LbCu/ZnSODs* and Cu/ZnSOD from *Cannabis sativa* L. were in the same clade and showed a close relationship. Likewise, *LbFeSOD* was more closely related to FeSOD from *Elaeis guineensis*, while *LbMnSOD* shared high homology with MnSOD from *Nelumbo nucifera* and *Phalaenopsis equestris*. Further analyses indicated that *LbMnSOD* and *LbFeSOD* displayed a relatively close homology.

***LbCu/ZnSOD* and *LbMnSOD* involved in the lily bud development.** In order to analyse the three *LbSOD* isoforms' role in the development of the lily bud, the gene expression in the four bud development stages was taken. As Figure 3 indicates, the *LbMnSOD* expression level gradually increased and reached the peak at the withering stage which was about 2.8 times of the green bud stage, and was also significantly higher than the other two genes expression. The expression of *LbCu/ZnSOD* decreased, increased and decreased during the bud development, with relatively low levels. The *LbFeSOD* expression kept at a very low level from the red bud period to the wilt period, and its expression could be ignored.

The PEG-6000 treatment can cause drought stress on cut lily flowers, while SLs can alleviate this damage. In the preliminary experiment, we treated the cut flowers with five different PEG-6000 concentrations (0, 5%, 10%, 15% and 20%) to observe the senescence process in the flowers. According to the vase life, the 10% PEG-6000 group was screened for the subsequent drought simulation.

In order to explore the effect of the SLs on the morphology of the cut lily under the 10% PEG-6000 stress, photographs were taken from above every 24 hours for three groups (CK, 10% PEG-6000 and 10% PEG-6000+SLs) (Figure 4). The flowers with the 10% PEG-6000 treatment presented a wilt symptom and began to senescence on day four. However, the 10% PEG-6000+SLs group started to wilt on day six. The results indicated that 10% PEG-6000 could accelerate the flowers wilting and ageing, while the SLs can alleviate the stress caused by 10% PEG-6000 on the cut flowers.

As shown in Figure 5, the RWC in the petals of all the groups showed a descreasing trend. From day one, the RWC of the three groups were as follows: CK > PEG+SLs > PEG, the RWC of the PEG+SLs group was significantly higher than that of the PEG group on day five ($P < 0.01$). It could be estimated that the PEG caused drought stress in the flowers, while the drought tolerance of the cut lily was enhanced by the SLs.

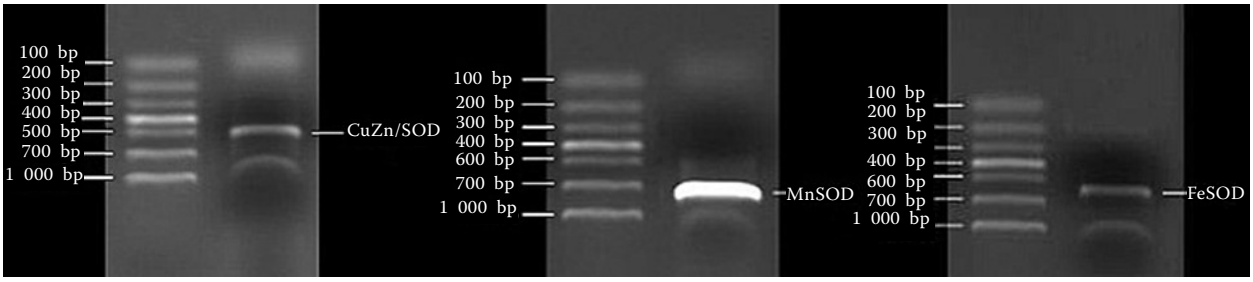

Figure 1. PCR product of *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD*. The molecular weight of the DNA maker is 1 000 bp

https://doi.org/10.17221/17/2022-HORTSCI

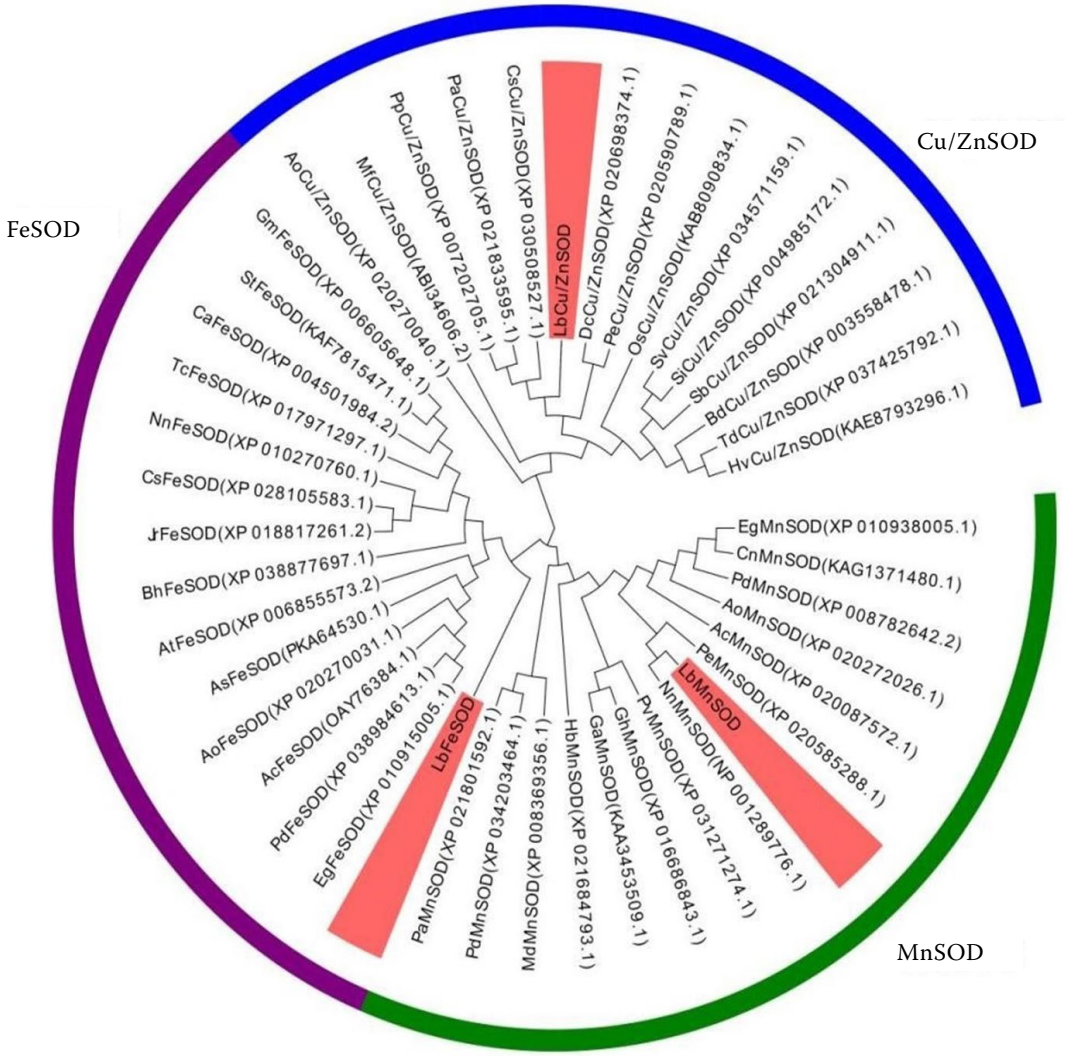

Figure 2. Phylogenetic analysis of *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD* with other SOD proteins from different species
The MnSOD proteins were as follows: *Asparagus officinalis* MnSOD (XP_020272026.1); *Nelumbo nucifera* MnSOD (NP_001289776.1); *Elaeis guineensis* MnSOD (XP_010938005.1); *Ananas comosus* MnSOD (XP_020087572.1); *Prunus dulcis* MnSOD (XP_034203464.1); *Prunus Avium* MnSOD (XP_021801592.1); *Phoenix dactylifera* MnSOD (XP_008782642.2); *Gossypium hirsutum* MnSOD (XP_016686843.1); *Phalaenopsis equestris* MnSOD (XP_020585288.1); *Malus domestica MnSOD* (XP_008369356.1); *Cocos nucifera* MnSOD (KAG1371480.1); *Gossypium australe* MnSOD (KAA3453509.1); *Pistacia vera* MnSOD (XP_031271274.1); *Hevea brasiliensis* MnSOD (XP_021684793.1).
The FeSOD proteins were as follows: *Elaeis guineensis* FeSOD (XP_010915005.1); *Phoenix dactylifera FeSOD* (XP_038984613.1); *Asparagus officinalis FeSOD* (XP_020270031.1); *Ananas comosus* FeSOD (OAY76384.1); *Nelumbo nucifera* FeSOD (XP_010270760.1); *Senna tora FeSOD* (KAF7815471.1); *Juglans regia* FeSOD (XP_018817261.2); *Cicer arietinum* FeSOD (XP_004501984.2); *Benincasa hispida* FeSOD (XP_038877697.1); *Apostasia shenzhenica* FeSOD (PKA64530.1); *Theobroma cacao* FeSOD (XP_017971297.1); *Amborella trichopoda* FeSOD (XP_006855573.2); *Glycine max* FeSOD (XP_006605648.1); *Camellia sinensis* FeSOD (XP_028105583.1).
The Cu/ZnSOD proteins were as follows: *Asparagus officinalis* Cu/ZnSOD (XP_020270040.1); *Musa formosana* Cu/ZnSOD (ABI34606.2); *Dendrobium catenatum* Cu/ZnSOD (XP_020698374.1); *Oryza sativa Cu/ZnSOD* (KAB8090834.1); *Phalaenopsis equestris* Cu/ZnSOD (XP_020590789.1); *Sorghum bicolor* Cu/ZnSOD (XP_021304911.1); *Cannabis sativa* Cu/ZnSOD (XP_030508527.1); *Setaria italica* Cu/ZnSOD (XP_004985172.1); *Triticum dicoccoides* Cu/ZnSOD (XP_037425792.1); *Setaria viridis* Cu/ZnSOD (XP_034571159.1); *Hordeum vulgare* Cu/ZnSOD (KAE8793296.1); *Prunus avium* Cu/ZnSOD (XP_021833595.1); *Brachypodium distachyon* Cu/ZnSOD (XP_003558478.1); *Prunus persica* Cu/ZnSOD (XP_007202705.1)

https://doi.org/10.17221/17/2022-HORTSCI

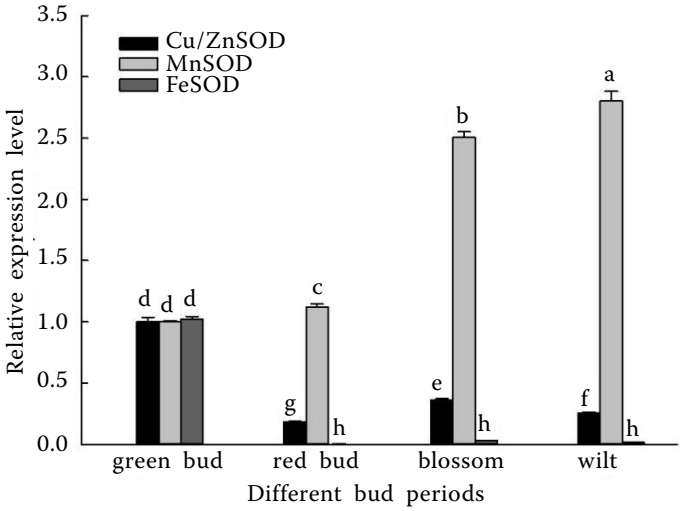

Figure 3. Expression levels of *LbCu/ZnSOD*, *LbMnSOD* and *LbFeSOD* in the different bud development stages of the lily

The total RNAs were extracted from the petals of different bud periods (green bud, red bud, blossom bud, wilt bud)

The expression levels of three SOD genes were examined by qRT-PCR. The measurement values in the green bud were set to 1 after the values in the different bud development stages were normalised using *actin*

Mean values ($n = 3$) ± SE are shown. Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

REC is the basic index that reflects the permeability of cell membranes in plants. The REC of the petals of the three groups showed increasing trends (Figure 6), among which, the 10% PEG-6000 group increased rapidly. The 10% PEG-6000+SLs group was significantly lower than that of the 10% PEG-6000 group ($P < 0.05$). It suggested that SLs can effectively reduce the damage of PEG-6000 to the membrane system of lily petals.

MDA is one of the main products of cell membrane lipid peroxidation, and is regarded as an important indicator for the lipid peroxidation degree of the plant cell membrane. Figure 7 indicates that the MDA content in the petals of the lily were extremely increased after the 10% PEG-6000 stress. The MDA content of the PEG+SLs treatment was significantly lower than that of the PEG treatment ($P < 0.05$), suggesting that the SL application can reduce the MDA content under PEG-6000 stress, thereby effectively protecting the cytoplasmic membrane structure.

***Cu/Zn-SOD* and *Mn-SOD* activities contributed to the drought tolerance induced by the SLs of the cut lily flowers.** As shown in Figure 8, the T-SOD activities of the three groups increased and then decreased from day 1 to day 5. Compared with the CK group, the T-SOD activities of the petals under the PEG treatment increased by 24.71% on day three. On day 5, the enzyme activities of the

Figure 4. Effect of the PEG and strigolactones (SLs) treatment on appearance of the lily

The images of the cut lilies were photographed from above which were treated with 10% PEG-6000 and SLs for six days

The 0% PEG-6000 treatment was the control

https://doi.org/10.17221/17/2022-HORTSCI

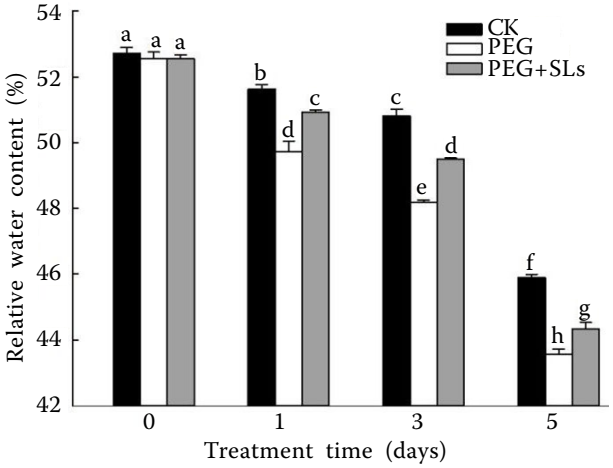

Figure 5. Effects of the different treatments on the relative water content of the lily petals

The relative water content of the lily petals = (original fresh weight-dry weight)/(saturated fresh weight-dry weight) × 100%. Mean values (n = 6) ± SE are shown. Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

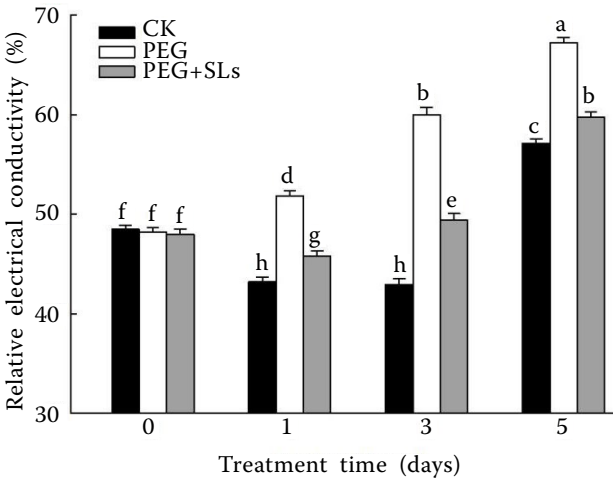

Figure 6. Effects of the different treatments on the relative electrical conductivity of the lily petals

Mean values (n = 6) ± SE are shown

Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

10% PEG-6000+SLs group were significantly higher than those of the 10% PEG-6000 group. Therefore, as an important part of the antioxidant defence system, SOD participated in the anti-drought stress of the cut flowers under 10% PEF-6000. The SLs enhanced the activity of T-SOD, indicating that the SLs contributed to the drought tolerance.

Figure 9 indicates that the Cu/ZnSOD activity first decreased and then increased after the 10% PEG-6000 treatment, which was higher than that of control, indicating that the Cu/ZnSOD activity increased in response to the drought stress. The Cu/ZnSOD activity of the 10% PEG-6000 + SLs group was higher than that of the 10% PEG-6000 group, implying that the SL treatment increased the Cu/ZnSOD activity. Combining Figure 8 with Figure 9, one could estimate that the Cu/ZnSOD activity accounted for about 80% of the T-SOD activity, showing that the Cu/ZnSOD activity played the main role of the T-SOD activity.

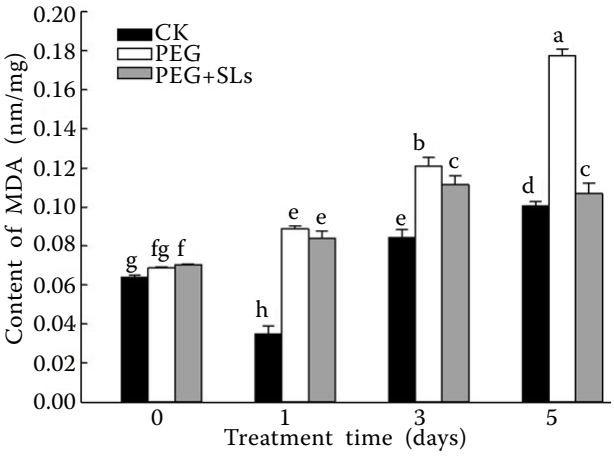

Figure 7. Effects of the different treatments on the MDA content in the lily petals

The MDA content is measured by the thiobarbituric acid method. Mean values (n = 3) ± SE are shown. Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

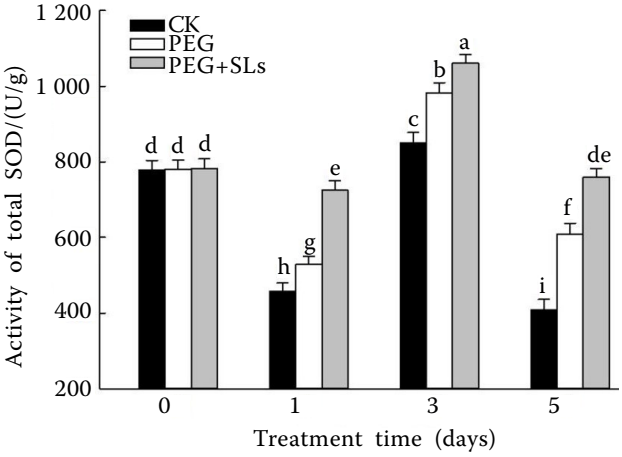

Figure 8. Effects of the different treatments on the T-SOD activity in the lily petals

Mean values (n = 3) ± SE are shown. Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

https://doi.org/10.17221/17/2022-HORTSCI

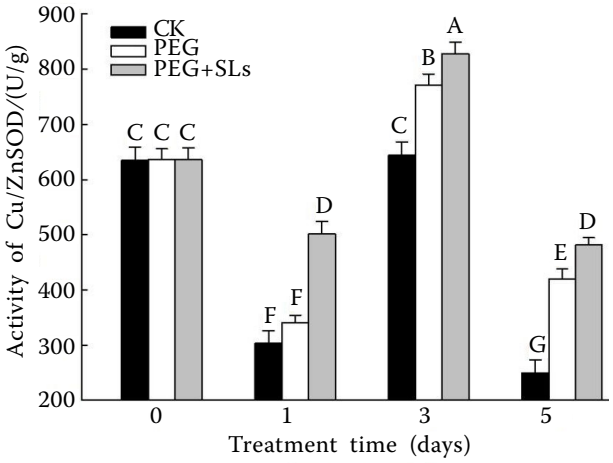

Figure 9. Effects of the different treatments on the *Cu/ZnSOD* activity in the lily petals

Mean values (n = 3) ± SE are shown

Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

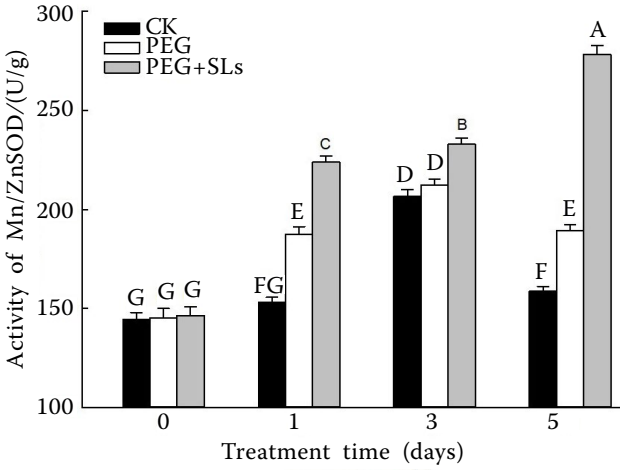

Figure 10. Effects of the different treatments on the MnSOD activity in the lily petals

Mean values (n=3) ± SE are shown

Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

Figure 10 shows that the MnSOD activities of the CK and 10% PEG-6000 groups displayed the same trend as the Cu/ZnSOD and T-SOD, while the *Mn-SOD* activity of the 10% PEG-6000+SLs group in the petals kept increasing and were significantly higher than those of the control and the 10% PEG-6000 group, which suggested that this could enhance the MnSOD activity under drought stress.

The activities of T-SOD include that of the Mn-SOD, Cu/ZnSOD and FeSOD. Combining the expression of *LbFeSOD* during the lily bud development (Figure 3) with the activity changes of T-SOD, Cu/ZnSOD and MnSOD under the PEG-6000 treatment, the role of FeSOD on the cut lily petals could be ignored, so the *LbFeSOD* gene was not in the list of the following gene expression analyses.

PEG-6000 up-regulated the *LbCu/ZnSOD* and *LbMnSOD* expression, and the SLs elevated their levels in the lily petals under 10% PEG-6000. As exhibited in Figure 11, the expression trends of the two genes in the three groups were roughly the same, both showing an increasing and then decreasing trend. Under the 10% PEG-6000 treatment, the *LbMnSOD* expression (Figure 11B) reached the highest value on day three while the expression levels of *LbCu/ZnSOD* (Figure 11A) reached the peak on day one and

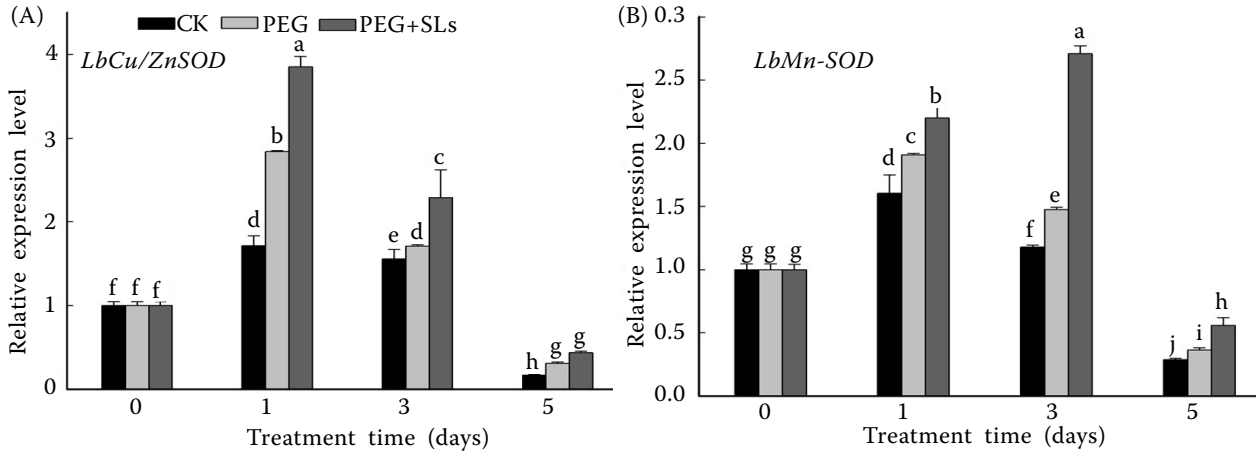

Figure 11. Expression levels of *LbCu/ZnSOD* (A) and *LbMnSOD* (B) with the PEG and SLs treatment

The expression levels of two SOD genes were examined by qRT-PCR. The measurement values on the 0th day were set to 1 after the values in the different treatment times were normalised using *actin*

Mean values (n = 3) ± SE are shown. Significant differences (ANOVA, $P \leq 0.001$, Holm-Sidak test) are indicated with letters

https://doi.org/10.17221/17/2022-HORTSCI

then decreased; the results also indicate that under the 10% PEG-6000 treatment, the two gene expression levels were significantly up-regulated by the SLs ($P < 0.05$). On the third day, the expression of *LbMnSOD* in 10% PEG-6000+SLs was almost twice as high as that of the 10% PEG treatment, thus, the SLs had a positive effect on the expression of the two genes.

DISCUSSION

SLs alleviated the damage induced by the 10% PEG-6000 treatment in lily flowers. Commercially, cut lilies are usually harvested at the green bud stage, and are easily dehydrated during the long-distance dry transportation after harvesting, resulting in abnormal flower opening, flower wilting, pedicel wilting (curved neck), an inability to open, and flower deformities.

In this study, the PEG-6000 treatment resulted in premature senescence, a shortened vase period, and a decreased RWC of the cut flowers. Under drought stress, the plant cell membrane permeability will change greatly, and our research results showed that the MDA and REC content was increased. However, an exogenous SL treatment effectively delayed the senescence of the cut flowers, and the MDA and REC content decreased. In maize seedlings, SLs improved the plant growth under drought stress (Sattar et al. 2021). The application of SLs decreased the H_2O_2 and MDA content in *Triticum aestivum* (Sedaghat et al. 2017). All these studies imply that SL may serve as a ROS scavenger and reduce the lipid peroxidation under drought conditions.

SLs enhanced the *LbCu/ZnSOD* and *LbMnSOD* activities under the PEG-6000 stress. Various environmental stresses, especially drought stress, can disturb the oxidative balance of plant cells. A plant's adaptation mechanism is usually related to the antioxidant enzyme activity (Sun et al. 2010). SOD is an important member of the intracellular ROS scavenging system, where its activity reflects the intracellular ROS scavenging ability. Our research indicated that the T-SOD, Cu/ZnSOD and Mn/SOD activities under the 10% PEG-6000 treatment showed the same trend: first an increase and then a decrease. Reddy et al. (2004) found that, in mulberry leaves under various water stress factors, the activities of various SOD enzymes were significantly improved; while during the period of water shortage in Pisum sativum, the Cu/ZnSOD activity strongly increased (Mascher et al. 2005).

Regarding the research on the role of three kinds of SOD in resistance to adverse stress, previous reports showed that, in some plant species, FeSOD or MnSOD was found to be absent, or their proportion in the total SOD activity was extremely low (Scandalios 1993). In the narrow-leaf lupin (Yu, Rengel 1999), Cu/ZnSOD and FeSOD, but not MnSOD, might participate in the anti-oxidative stress induced by drought. However, it was found that the activity of MnSOD, Cu/ZnSOD and FeSOD accounted for 60%, 30% and 10% of the T-SOD activity, respectively, in an analysis of *Lotus corniculatus* with specific inhibitors (Borsani et al. 2001). Our research found that Cu/ZnSOD and A, especially Cu/ZnSOD whose activity accounted 80% of the T-SOD activity, might be involved in the drought-induced oxidative stress in lily petals.

The SLs+PEG group achieved higher SOD activity than the PEG group, indicating that the SLs enhanced the drought tolerance of the lily flower. Sattar et al.(2021) also reported an exogenous application of SL alleviates drought stress in maize seedlings by enhancing the SOD activity.

SLs might increase the Cu/ZnSOD and MnSOD activities at the transcription stage. The report on the response of three types of *SOD* to stress found that Cu/ZnSOD showed an irregular expression level pattern under drought stress in *L. corniculatus*. (Borsani et al. 2001). When *Hordeum vulgare* barley was subjected to drought stress, the expression levels of all the *SOD* sub-types increased, in which the Cu/ZnSOD mRNA level was strongly up-regulated (Mascher et al. 2005). Another study also showed that drought stress can induce an increase in the expression of Cu/ZnSOD genes in *H. vulgare* (Mascher et al. 2005). However, a report taken in the cut rose indicated that dehydration can significantly inhibit the expression level of four types of *RhSOD (RhMnSOD1, RhCu/ZnSOD2, RhCu/ZnSOD1* and *RhCu/ZnSOD3)* (Jiang et al. 2015). Our results showed that *LbCu/ZnSOD* and *LbMnSOD* first increased and then decreased after the 10% PEG-6000 treatment, where they reached their peaks on days one and three, respectively, among them the *LbCu/ZnSOD* expression level was higher and also more sensitive to drought.

In addition, our results also indicated that the expression levels of *LbCu/ZnSOD* and *LbMnSOD* were up-regulated by the SLs, especially the *LbCu/*

https://doi.org/10.17221/17/2022-HORTSCI

ZnSOD expression level peaked on day three. Therefore, it was speculated that the SLs might increase the Cu/ZnSOD and MnSOD activity at the transcription stage.

CONCLUSION

Three kinds of *LbSOD* were cloned from lily petals. During the development of the lily buds, the *LbMnSOD* expression continuously increased, the *LbCu/ZnSOD* expression was moderate, and the *LbFeSOD* expression was low. Our results showed that under the 10% PEG-6000 treatment, the cut lily flowers suffered from drought stress resulting in morphological and physiological changes, and the SL application effectively alleviates the injuries suffered from PEG-6000. The PEG-6000 treatment increased the T-SOD activity significantly, in which the Cu/ZnSOD and MnSOD activities were enhanced dramatically, while the *FeSOD* activity was almost undetectable. SLs enhanced the T-SOD activity under the PEG-6000 stress, mainly Cu/ZnSOD and MnSOD. SLs can improve the expression level of *LbCu/ZnSOD* and *LbMnSOD*. Therefore, *LbCuZn/SOD* and *LbMnSOD*, especially *LbCuZn/SOD*, played a crucial role in enhancing the tolerance of cut lily flowers under drought stress.

REFERENCES

Abdelgawad H., Zinta G., Badreldin A. H., Selim S., Abuelsoud W. (2019): Maize roots and shoots show distinct profiles of oxidative stress and antioxidant defense under heavy metal toxicity. Environmental Pollution, 258: 113705.

Abreu I.A., Cabelli D.E. (2009): Superoxide dismutases a review of the metal-associated mechanistic variations. BBA-Bioenergetics, 1804: 263–274.

Alscher R.G., Erturk N., Heath L.S. (2002): Role of superoxide dismutases (SODs) in controlling oxidative stress in plants, Journal of Experimental Botany, 372: 1331–1341.

Bhoi A., Yadu B., Chandra J., Keshavkant S. (2021): Contribution of strigolactone in plant physiology, hormonal interaction and abiotic stresses. Planta, 254: 28.

Borsani O., Díaz P., Agius M.F., Valpuesta V., Monza J. (2001): Water stress generates an oxidative stress through the induction of a specific Cu/Zn superoxide dismutase in *Lotus corniculatus* leaves. Plant Science, 161: 757–763.

Dehury B., Sarma K., Sarmah R., Sahu J., Sahoo S., Sahu M., Sen P., Modi M.K., Sharma G.D., Choudhury M.D., Barooah M. (2013): In silico analyses of superoxide dismutases (SODs) of rice (*Oryza sativa* L.). Journal of Plant Biochemistry and Biotechnology, 22: 150–156

Feng X., Lai Z., Lin Y., Lai G.T., Lian C.L. (2015): Genome-wide identification and characterization of the superoxide dismutase gene family in *Musa acuminata* cv. *Tianbaojiao* (AAA group). BMC Genomics, 16: 823.

Filiz E., Tombuloğlu H. (2015): Genome-wide distribution of superoxide dismutase (SOD) gene families in *Sorghum bicolor*, Turkish Journal of Biology, 39: 49–59.

Hu X.X., Hao C.Y., Cheng Z.M., Zhong Y. (2019): Genome-wide identification, characterization, and expression analysis of the grapevine superoxide dismutase (SOD) family. International Journal of Genomics, 2019: 1–13.

Hui Y., Chen L., Li X.Y., Chen Q.M., Yi M.F. (2008): Comparison and optimization of total RNA extraction methods from lily leaves. Journal of China Agricultural University, 13: 41–45.

Jiang Y.D., Khan M.A., Wang Z., Liu J., Zhang C. (2015): Cu/ZnSOD involved in tolerance to dehydration in cut rose (*Rosa hybrida*). Postharvest Biology & Technology, 100: 187–195.

Karuppanapandian T., Moon J.C., Kim C., Manoharan K., Kim W. (2011): Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Australian Journal of Crop Science, 5: 709–725.

Kliebenstein D.J., Monde R.A., Last R.L. (1998): Superoxide dismutase in *Arabidopsis*: an eclectic enzyme family with disparate regulation and protein localization. Plant Physiology, 118: 637–650.

Kramna B., Prerostova S., Vankova R. (2019): Strigolactones in an experimental context. Plant Growth Regulation, 88: 113–128.

Li H.S. (2000): Experimental principles and techniques of plant physiology and biochemistry [M]. Beijing: Higher Education Press.

López-Ráez J.A., Shirasu K., Foo E. (2017): Strigolactones in plant interactions with beneficial and detrimental organisms: the Yin and Yang. Trends in Plant Science, 22: 527–537.

Mascher R., Nagy E., Lippmann B., Hörnlein S., Fischer S., Scheiding W., Neagoe A., Bergmann H. (2005): Improvement of tolerance to paraquat and drought in barley (*Hordeum vulgare* L.) by exogenous 2-aminoethanol: effects on superoxide dismutase activity and chloroplast ultrastructure. Plant Science, 168: 691–698.

Miller A.F. (2012): Superoxide dismutases: ancient enzymes and new insights, FEBS Letters, 586: 585–595.

Reddy A.R., Chaitanya K.V., Jutur P.P., Sumithra K. (2004): Differential antioxidative responses to water stress among five mulberry (*Morus alba* L.) cultivars. Environmental & Experimental Botany, 52: 33–42.

Rochange S., Goormachtig S., Lopez-Raez J.A., Gutjahr C. (2019): The role of strigolactones in plant–microbe interactions. Strigolactones : Biology and Applications, 121–142.

https://doi.org/10.17221/17/2022-HORTSCI

Sattar A., Ul-Allah S., Ijaz M. Sher A., Butt M., Abbas T., Irfan M., Fatima T., Alfarraj S., Alharbi S.A. (2021): Exogenous application of strigolactone alleviates drought stress in maize seedlings by regulating the physiological and antioxidants defense mechanisms. Cereal Research Communications, 2021: 1–10.

Scandalios J.G. (1993): Oxygen stress and superoxide dismutases. Plant Physiology, 10: 7–12.

Sedaghat M., Sarvestani Z.T., Emam Y., Bidgoli A.M. (2017): Physiological and antioxidant responses of winter wheat cultivars to strigolactone and salicylic acid in drought. Plant Physiology & Biochemistry, 119: 59–69.

Siddiqui M.H., Al-Khaishany M.Y., Al-Qutami M.A., Al-Whaibia M.H., Groverb A., Alia H.M., Al-Wahibia M.S. (2015): Morphological and physiological characterization of different genotypes of faba bean under heat stress. Saudi Journal of Biological Sciences, 22: 656–663.

Sun W.H., Duan M., Shu D.F., Yang S., Meng Q.W. (2010): Over-expression of *StAPX* in tobacco improves seed germination and increases early seedling tolerance to salinity and osmotic stresses. Plant Cell Reports, 29: 917–926.

Wang W., Xia M.X., Chen J., Yuan R., Deng F.N., Shen F.F. (2016a): Gene expression characteristics and regulation mechanisms of superoxide dismutase and its physiological roles in plants under stress. Biochemistry (Mosc), 81: 465–480.

Wang W., Xia M.X., Chen J., Deng F.N., Yuan R., Zhang X., Shen F.F. (2016b): Genome-wide analysis of superoxide dismutase gene family in *Gossypium raimondii* and *G. arboreum*. Plant Gene, 6: 18–29.

Xu J., Duan X., Yang J., Beeching J.R., Zhang P. (2013): Coupled expression of Cu/Zn-superoxide dismutase and catalase in *Cassava* improves tolerance against cold and drought stresses. Plant Signaling & Behavior, 8: e24525.

Yu Q., Rengel Z. (1999): Drought and salinity differentially influence activities of superoxide dismutases in narrow-leafed lupins. Plant Science, 142: 1–11.

Zhang X.R., Xu X.Y. (1992): Comparative experiment on drought resistance and wind erosion resistance among different species of Shaguai. Journal of Arid Land Resources and Environment, 6: 55–62.

Received: February 24, 2022
Accepted: April 6, 2023