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2,4-EPIBRASSINOLIDE RELIEVES THE DAMAGE OF DROUGHT STRESS ON FRESH-CUT LILIES BY INCREASING ANTIOXIDANT ENZYME ACTIVITIES

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Abstract

Fresh-cut flowers are often suffered from drought stress after they are harvested, which results in quality loss and vase life shorten during marketing of fresh-cut flowers. In this study, the lilies gathered from the greenhouse were treated with 10^{-7} M 2,4-epibrassinolide (EBR), and then subjected to drought treatment to assess the changes in wilting time, malondialdehyde (MDA) contents, relative conductivity, the total phenol (TP), antioxidant enzyme activities and these enzyme genes expression levels. Drought considerably accelerated the flowers wilting compared to the control. Moreover, extensive increase by drought in MDA content, and relative conductivity were also recorded. While the TP contents and the activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD); ascorbate peroxidase (APX) were firstly increased and then dropped in the later period. In which it seemed that SOD and POD played more important roles when the flowers were suffered drought stress. The expression of *LbAPX*, *LbCu-ZnSOD*, *LbMn-SOD* and *LbCAT* showed a rise and then decline, *LbFe-SOD* kept decreasing, while *LbPOD* maintained increasing. However, exogenous application of EBR remarkably delayed the flowers wilting time by 2 days and decreased the MDA content, relative conductivity compared to the drought group. These meant EBR treatment could maintain the membrane integrity of fresh-cut lilies. Furthermore, pre-treatment with EBR could significantly improve the TP contents and the four antioxidant enzymes activities and their genes expression levels. On the basis of these findings, it can be concluded exogenous application of EBR could effectively alleviate the damage of drought stress by reducing MDA contents and relative conductivity, improving TP content, antioxidant enzymes' activities and their genes expressions, and finally extending the flowers' post-harvest lives.

Key words: Fresh-cut lily; Drought; 2,4-Epibrassinolide; Antioxidant enzyme.

Introduction

Oriental hybrid lilies (*Lilium* spp.), which belong to bulbous plants, derive from interspecific crosses of species belonging to the Archelirion section (Ren *et al*., 2017). Due to large, trumpet-shaped, especial fragrant flowers and long vase life, oriental hybrid lilies are among the most economically important cut-flower plants.

Fresh-cut flowers are living organisms detached from the mother body. During thier long dry transportation, their respiratory consumption and transpiration still proceed, they are suffered hunger and drought stress which may result to abnormal flower opening and flower deformities. Under drought stress, the plant cells will produce a large amount of reactive oxygen species (ROS) which react with protein and plasma membrane, thereby destroying the cell structure, metabolic disorder, and eventually leading to oxidative stress. In order to cope with oxidative stress, the plant cell take advantage of antioxidant system to alleviate the damage of ROS. These antioxidant systems include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD); ascorbate peroxidase (APX), etc., as well as non-enzymatic antioxidant. Increasing antioxidant capacity is an effective adaptation mechanism resistance to drought stress, and studies in mulberry leaves and quinoa have shown that elevated antioxidant enzyme activity are observed under water shortage conditions (Othmani *et al*., 2021; Lalarukh *et al*., 2020).

Brassinolide is a kind of steroidal hormone in plants, which plays a very important role in the growth and development of plants (Fujioka & Yokota, 2003). It is involved in the regulation of gene expression, which causes various reactions of cells such as cell division, cell elongation, xylem differentiation, hypocotyl elongation, leaf enlargement and pollen germination (Müssig, 2005; Asahina *et al*., 2014; Singh & Shono, 2005). BR also participates biotic and abiotic resistance, such as cold stress, temperature stress, salt stress and disease (Clouse & Sasse, 1998; Krishna, 2003). A number of studies have shown that the application of exogenous brassinolide increases the antioxidant enzyme activity, soluble matter content, thereby increase plant resistance to drought stress and enhance crop yield (Dhayal *et al*., 2012; Morinaka *et al*., 2006). In addition, BR can activate the expression of genes involved in environmental stimuli (Xia *et al*., 2011). All these studies have shown that BRs play an important role in alleviating the damage of plants caused by stress. However, there are few studies of BRs effects on ornamental horticulture, especial fresh-cut flowers. In this study, the fresh-cut lily was used to study the affection of exogenous EBR, on the physiological, antioxidant enzyme activities and antioxidant enzyme gene expression under drought, lay the foundation for delaying the fresh-cut flowers' senescence.

Materials and Methods

Experimental design: The fresh-cut Lily flowers from a local greenhouse in Kunming were cut into 60-70 cm length, and then brought back to the laboratory in 2 h; Using a blade (the blade was sterilized with 95% ethanol), the flowers were cut the branch length to 30 cm with 2-3 leaves in distilled water, and then were put in a container filled with distilled water (the container was also sterilized with 95% ethanol), finally cultivated in an incubator. Two days later, the flowers at the same flower opening stage were selected, cut the branches length to 4-5 cm long in distilled water. The flowers were randomly subjected to different treatments: (1) control group was treated with distilled water; (2) drought group (‘drought’) was placed in empty containers; (3) EBR group (‘EBR’) was treated with 10^{-7} M EBR for 4 h, after the end of the branch was naturally dried in the air, the flowers were placed in containers filled with distilled water; (4) EBR+drought group (‘EBR+drought’) was treated with 10^{-7} M EBR for 4 h and then placed in empty containers. The flowers in all groups were placed in the incubator maintained at 20-23℃, with 40-50 % relative humidity (RH), and 12 h of illumination at a light intensity of 140 mol·m^{-2}·s^{-1}, and distilled water was changed daily for the control group and ‘EBR’.

Methods: Malondialdehyde (MDA) content was carried out by thiobarbituric acid (TBA) method (Liu *et al*., 2016); The relative conductivity was determined according to the method of Saeed *et al*., (2016); The total phenol (TP) content was examined using a reagent kit (NanJing JianCheng Bio Inst, Nanjing, China) according to the manufacture’s instruction; Determination of SOD activity was carried out by the method of Kono (1978); CAT activity was assayed according to Dhindsa *et al*., (1981); APX activity was measured according to the method of Nakano & Asada (1981), POD activity was determined using the method of Kochba *et al*., (1997).

Quantitative analysis of gene expression was performed by qPCR. Total RNA was extracted from Lily petals using Trizol according to the supplier’s recommendation. The cDNA was synthesized using the PrimeScriptTM RT reagent Kit with gDNA Eraser (Perfect Real Time) kit. qPCR was performed on a Roche LightCycler 480 system using the SYBR® Premix Ex TaqTM II (Tli RNaseH Plus) kit following the supplier’s recommendation. The gene-specifc primers are used for amplification (Table 1).

Data processing: The experimental data were analyzed by SPSS 16.0 and plotted with Sigmaplot 10.0, and each set was repeated three times and average values with standard errors were reported.

Results and Analysis

EBR delays flower senescence under drought stress: In the preliminary experiment, flowers were pretreated with 0 M, 10^{-5} M, 10^{-7} M, 10^{-9} M, 10^{-11} M EBR solution for 4 h, and then placed in distilled water. An ideal EBR concentration was screened according to senescence speed of the flowers. When the concentration of EBR was 10^{-7} M, it could effectively delay the senescence of cut flowers without causing phyto-toxicity, so 10^{-7} M was chosen for the following treatments.

To investigate the effects of drought and EBR pretreatments on the cut flowers, the color change and flower diameter were watched to judge the flower wilting. As shown in Fig. 1, the ‘drought’ began to wilt at day 2, while the ‘EBR+drought’ began at day 4. At day 6, the ‘drought’ totally wilted and all the petals fell, but most of the ‘EBR+drought’ flowers did not fell. Drought accelerated the flowers wilting and senescence, and EBR could attenuate the flowers death.

Fig. 1. The flower opening status of the control, ‘drought’, ‘EBR’, ‘EBR+drought’ within 6 days.

EBR declines the MDA content and relative conductivity, and increases TP content of the flowers: The MDA content of the four groups showed upward trends, in which the ‘drought’ increased severely (Fig. 2). However, the ‘EBR +drought’ MDA contents were far lower than those of the ‘drought’ ($p<0.01$). The order of the MDA content of the four groups was: drought>‘EBR+drought’>control>‘EBR’. The change in relative conductivity was similar to the change of MDA content (Fig. 3). EBR significantly reduced the relative conductivity at day 3, 4, 5 ($p<0.01$) under drought stress and prolonged the lives of fresh-cut lilies. The changes of TP showed a trend of increasing first and then decreasing (Fig. 4). The most significant change was observedin the ‘EBR+drought’, which peaked on the fourth day, and the difference was significant compared with the other three groups ($p<0.01$).

Table 1. Primers of antioxidase genes.

Gene	F	R
LbCu/Zn-SOD	5’-CAGCAATAGCACCGCAGTCAG-3’	3’-TTGGTCGTATCCCCAAAGGAA-5’
LbMn-SOD	5’-CTCCCCGACCTCCCGTACGAC-3’	3’-AATAGCCCAGCCAAGTGCCCC-5’
LbFe-SOD	5’-GCTTCAGCAAATCGAAAAGGA-3’	3’-GAGAATCGGAATATCACCCCA-5’
LbCAT	5’-CCAACCCGAAATCCCACATCC-3’	3’-ATTCAGCACCAGCCTCCCCAC-5’
LbAPX	5’-GGTGGGGATGGTTCCTCTTTA-3’	3’-CCAATTTGTGTCGGACGGTGT-5’
LbPOD	5’-GGAGGACAAGCCTGAACCCCC-3’	3’-GTGCCCACCAGACAGAGCAAC-5’
actin	5’-CCCCACTCAATCCCAAGGCAA-3’	3’-CGGAAGTCCAGCACAATACCA-5’

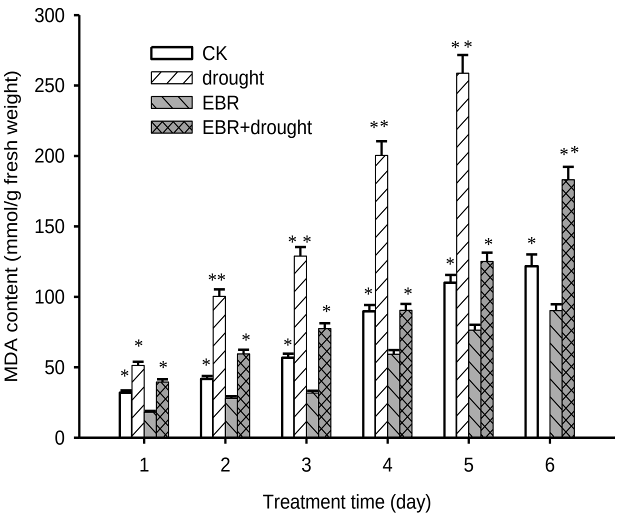

Fig. 2. MDA contents of Lilies petals in the control, 'drought', 'EBR', 'EBR+drought' within 6 days.The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

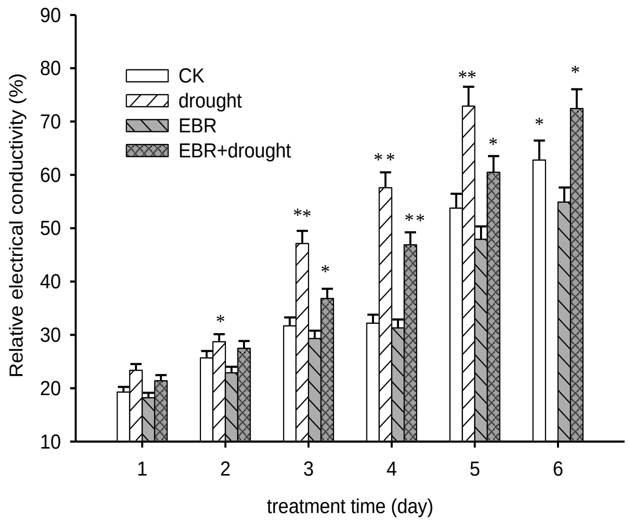

Fig. 3. Relative electrical of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 6 days. The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

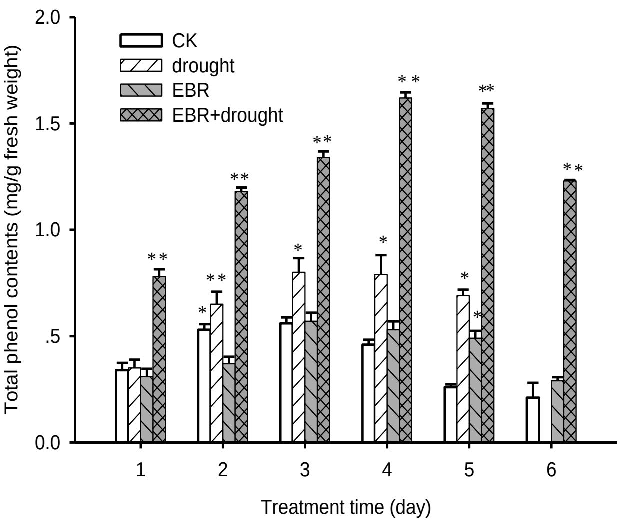

Fig. 4. TP contents of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 6 days.The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

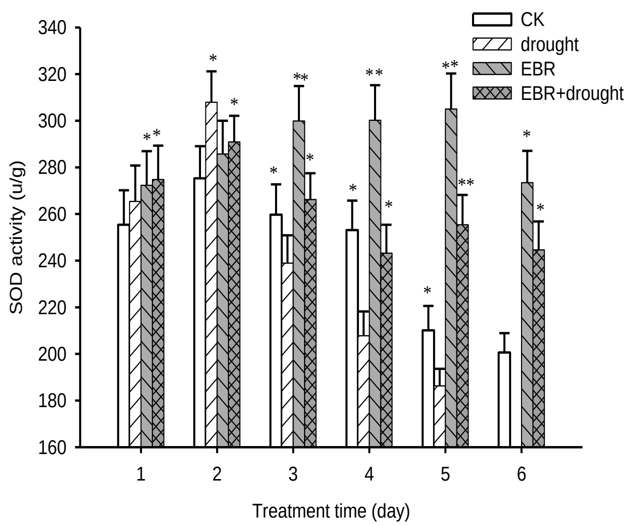

Fig. 5. SOD activities of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 6 days. The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

EBR increased the antioxidant enzymes' activities under drought stress: The SOD activities were as shown in the Fig. 5. The activities of the control, 'drought' and 'EBR+ drought' reached the peak on the second day, and then decreased. The 'drought' was the highest on the second day, and then followed by a linear decline; The 'EBR' had been maintained at high activity; The EBR+ drought group was higher than the 'drought' from the second day.

The CAT activity demonstrated a low-high-low trend (Fig. 6). The peaks of the control, 'EBR' and 'EBR+ drought' occurred on the third day, and the 'drought' was on the fourth day; The 'EBR+ drought' maintained at a high level during the whole experiment.

The activity changes of APX showed a single-peak curve (Fig. 7). The 'EBR' and 'EBR+ drought' showed the largest changes, and the peaks appeared on the fourth and third days, respectively. The difference betweenthe control and 'drought' reached a significant level (p<0.01).

The POD activities of the control and 'EBR' kept increasing, while the 'drought' and 'EBR+drought' firstly raised and then declined. But the former two were significantly lower than the latter two (Fig. 8).

EBR increased the expression levels of the antioxidant enzyme genes: The expression of antioxidant enzyme genes of the 'drought' and 'EBR+drought' was further studied as shown in the Fig. 9. The results showed that the expression levels of *LbCu-ZnSOD* and *LbMnSOD* genes increased first and then decreased under drought stresses. They peaked on the third day and the fourth day respectively. However the expression level of the *LbFeSOD* kept decreasing; the expressions of *LbCAT* and *LbAPX* also increased first and then declined. *LbCAT* expressed the largest amount on the third day, and *LbAPX* peaked on the second day; while the expression of *LbPOD* maintained rinsing during the whole research. Treatment with EBR increased most of these antioxidant enzyme gene expressions.

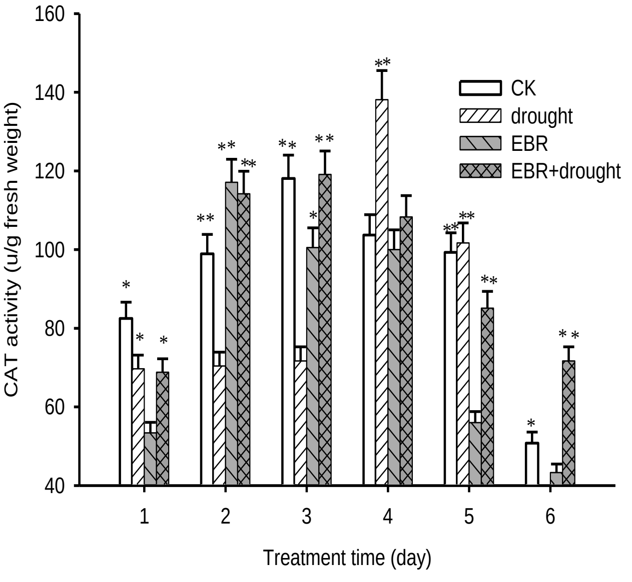

Fig. 6. CAT activities of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 6 days. The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

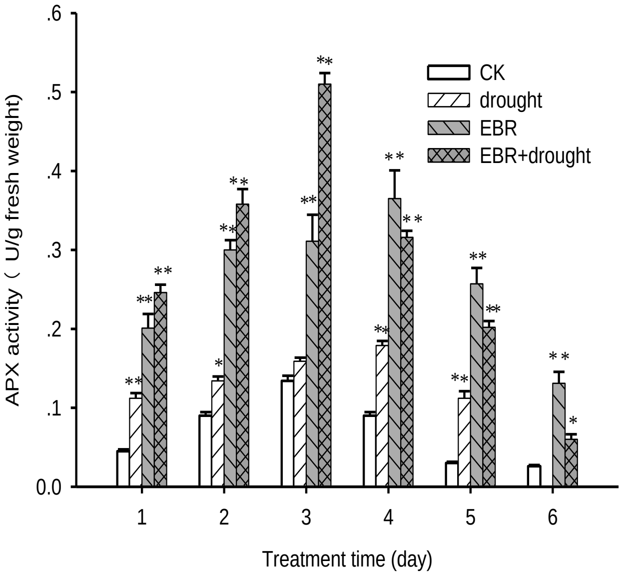

Fig. 7. APX activities of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 6 days.The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

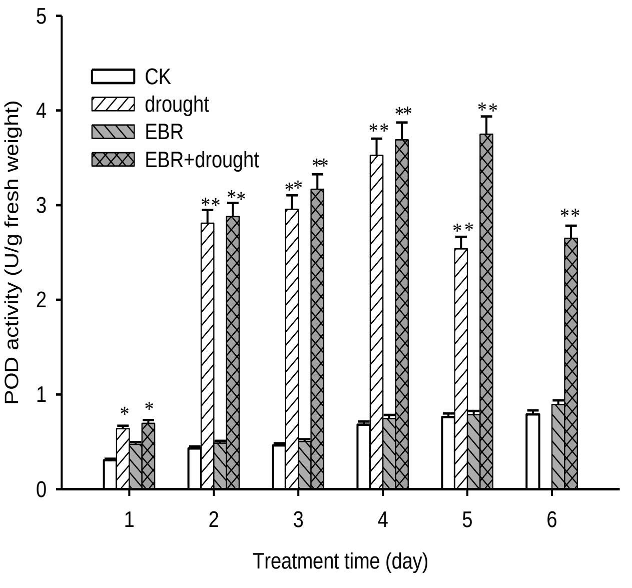

Fig. 8. POD activities of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 6 days. The data are presented as means ± standard deviation (n=3). * and ** indicate significant differences at p<0.05 and 0.01 by treatments within the same day.

Discussion

After fresh-cut flowers are separated from mother bodies, the supply of nutrients and water stop. For they are still living, various metabolisms continue. The aging of cut flowers is accelerated due to drought, which affects the quality of the flowers and shortens the vase life in transportation. BR is a kind of natural plant hormone, which has the function of regulating plant growth and development and improving plant resistance. In this study, EBR was used to study the alleviation damage of drought stress on fresh-cut lily. The beginning wilting time of the 'EBR+ drought' was delayed by two days compared with the control, which indicated EBR could delay senescence of lilies under drought stress (Fig. 1).

MDA is a product of plasma membrane oxidation, which content can better reflect the degree of plant cell membrane damage (Liu *et al*., 2016). In this study, compared with the control, the MDA content of 'drought' increased rapidly under drought stress, which indicated that the production of MDA was directly related to drought stress; When treated with EBR, the MDA content of 'EBR+drought' could be significantly reduced (Fig. 2). Gill *et al*., (2017) used PEG to simulate drought, studied the alleviation effect of EBR on barley seedlings under drought stress, and found that EBR could significantly reduce MDA content in seedlings.

Phenols can postpone the oxidation of lipid via inhibiting the initiation or propagation of oxidizing chain reactions (Rivero *et al*., 2005). In plants, phenols have anti-UV radiation, anti-oxidation and disease resistance, and insect resistance (Wang *et al*., 2014), cold resistance (Xiao *et al*., 2015) and other functions, as well as the ability to present flower color. Studies have shown that plants under stress often accumulate phenol substances to protect themselves (Yang *et al*., 2015). In our study, TP of lily was found to increase rapidly under drought stress; Compared with the control, EBR treatment could enhance TP contents; Overall, TP contents in each group were followed as: 'EBR +drought' > 'drought' > 'EBR' > control. Gao *et al*., (2017) studied the fresh-cut lotus root slices and found that EBR can reduce the total phenolic content in the slices and lower the browning degree. Maybe because of different plants, their results disagreed with ours.

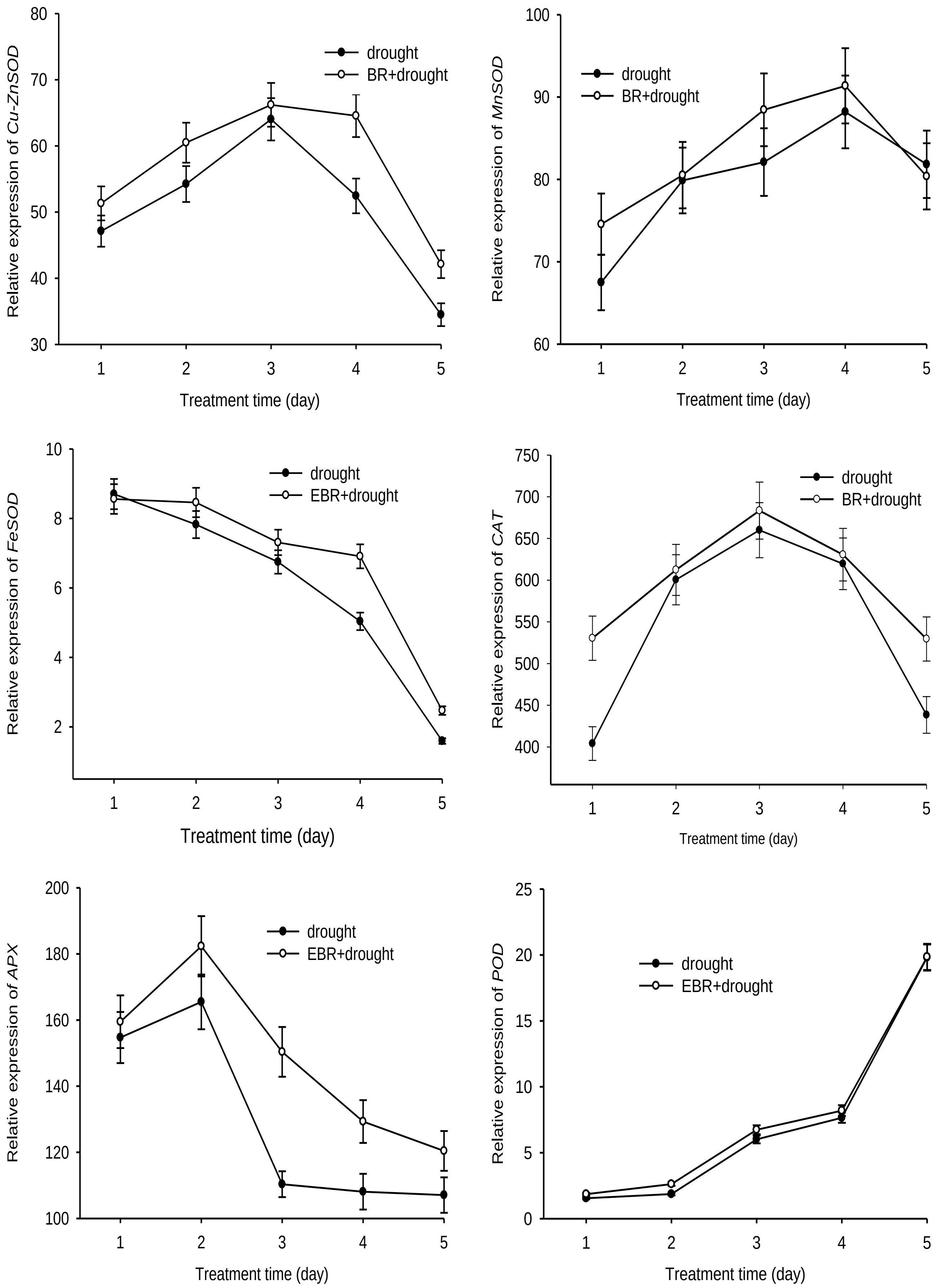

Fig. 9. The antioxidant enzyme genes' relative expression of Lilies flower petals in the control, 'drought', 'EBR', 'EBR+drought' for 5 days. The data are presented as means ± standard deviation (n=3).

When plants are suffered stress, cells often produce large amounts of ROS, which lead to oxidative stress, and drought is no exception to this rule. Plants have a variety of antioxidant enzymes that remove excess ROS from the cells. Our research showed that when the fresh cut lilies were suffered drought stress, the activities of SOD, CAT, APX, and POD increased, among which SOD was the most sensitive, reaching the peak on the second day and then decreasing; CAT, APX and POD firstly rose and then fell, and all these three reached the maximum on the fourth day; It seemed that drought dramatically increased POD activities compared to the contrast.

When treated with EBR, the four enzymes' activities were improved. The effect of EBR on SOD activity appeared in the later period, while the effect on CAT took place in the early stage. That of APX occurred significantly during the whole period; from the fifth day EBR could significantly enhance POD activities compared to the 'drought'. These results confirm the reports in daylily flower buds and fresh-cut lotus root slices previously reported (Yao *et al*., 2017; Gao *et al*., 2017). So when the fresh-cut lilies were under drought stress, the activities of antioxidant enzymes were enhanced to scavenge ROS, while exogenous EBR can further strengthen the ability and improve the drought resistance.

The expression of these enzymes' genes was also analyzed in the petals. Among the three *SOD* genes, *LbCu-ZnSOD*, *LbFeSOD* and *LbMnSOD*, drought reduced expression of *LbFeSOD*, while *LbCu-ZnSOD* and *LbMnSOD* firstly rose and then decreased. Jiang *et al*., (2015) research suggested *SOD*, especially *Cu-ZnSOD* are involved in the dehydration tolerance of cut rose flowers. The highest-level expression of *LbAPX* was observedon the second day, *LbCAT* peaked was on the third day, while *LbPOD* maintained increasing. Form the comparison between the dynamic of enzyme activity and related gene expression, it seemed that drought fortifying antioxidase activities might take place at the transcription level. Treatment with EBR could increase most of all the antioxidant enzyme gene expressions.

In conclusion, our study showed that exogenous EBR can effectively reduce the damage of drought stress on fresh-cut lily by lowering MDA content and electrolyte leakage, increasing TP content, the activities of SOD, CAT, POD, APX, PAL, and these genes' expression. It seemed that SOD and POD played more important roles in drought resistance. Our results indicated that EBR treatment could benefit in postponing the senescence of fresh-cut lily under drought stress.

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