Research article

24-Epibrassinolide-alleviated drought stress damage influences antioxidant enzymes and autophagy changes in peach (*Prunus persicae* L.) leaves

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A B S T R A C T

Drought stress is a serious threat to agriculture and the environment. Brassinosteroids (BRs) increase tolerance to drought stress of plant. Autophagy plays important roles in plant responses to drought stress; however, there are few reports on autophagy in peach (*Prunus persica*). In total, 23 putative autophagy-related genes (*ATGs*) in peach were identified using *ATGs* from the *Arabidopsis thaliana* genome as query in BLASTx algorithm-based searches. Under drought stress, the photosynthetic abilities of peach leaves decreased, while antioxidant enzyme activities, autophagy and *ATG* expression increased. A correlation analysis showed that antioxidant enzyme activities are inversely correlated to the expression levels of the *PpATGs*. During drought, the *PpATG8s* and some *PpATG18s* had the strongest responses. To investigate enhanced drought-stress tolerance, peach was treated with water, 100 nM 24-epibrassinolide (EBR), 1 μM EBR, 10 μM EBR and 1 μM voriconazole. Exogenous EBR at 1 μM decreased the malondialdehyde (MDA) content under drought stress when compared with water-, 1 μM vor-iconazole-, 100 nM EBR- and 10 μM EBR-treated peach leaf. The 1-μM EBR application increased superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione peroxidase (GR) activities during drought stress. In addition, the expression levels of *PpATGs* were inhibited by EBR. Thus, the 1-μM EBR treatment alleviated drought-stress damage to peach leaves, decreased *PpATG* expression levels and reduced the number of autophagosomes.

1. Introduction

Plants are inevitably challenged by various environmental stresses, in particular, salt, heat, intense irradiance and drought conditions. Abiotic stress can reduce crop growth, plant leaf area and photo-synthesis rates (Ma et al., 2016). Owing to climate changes, that include warming temperatures, water supplies are becoming limited drought stress is a significant threat to future crop production on a global scale (Zhao and Running, 2010). Peach (*Prunus persica* L.) is an important commercial fresh fruit worldwide. Peach fruit growth largely depends on an adequate water supply; and drought stress limits fleshy fruit production and quality (Eldem et al., 2012). 'Lumi 1', which is a bud mutation of the US peach variety Snow Kist, was bred in our laboratory, and has a soluble solids content that can reach 13%. It also has excellent storage and transportation capacities (Wang et al., 2018). The physio-logical condition of peach leaves has a great influence on fruit quality (Wang et al., 2018). Therefore, it is necessary to study the physiological

and biochemical changes in peach leaves under drought stress condi-tions.

Reactive oxidative species (ROS) levels are increased by drought stress and have a negative impact on plant survival, leading to mem-brane disruption, enzyme dysfunction and protein oxidation and ag-gregation (Tsugane et al., 1999). In plant cells, ROS can be scavenged through enzymatic and nonenzymatic pathways (Apel and Hirt, 2004). These ROS-scavenging systems are crucial to the plant's stress tolerance levels, such as salt, high temperature and drought that are affected by environmental factors, which have substantial effects on plant growth and yield worldwide (Foyer and Noctor, 2009). Crucial enzymes and autophagy are involved in enzymatic detoxification systems, and they play important roles in signaling and cellular adaptation to biotic and abiotic stresses (Li et al., 2016; Pérezpérez et al., 2012; Shangguan et al., 2018; Zhou et al., 2013). Autophagy involves a double-membrane structure termed an autophagosome that delivers the damaged proteins or organelles to a vacuole for degradation by hydrolases in animal,

plant and yeast cells (Shangguan et al., 2018; Ryabovol and Minibayeva, 2016). Autophagy is a recycling of cellular cytoplasmic contents and the removal of damaged proteins or organelles, and under unfavorable growth conditions, it is activated and involved in cell death, along with the cathepsin degradation (Bárány et al., 2018; Liu et al., 2012; Zeng et al., 2017). Autophagy-defective mutants are hypersensitive to abiotic stress conditions (Han et al., 2011; Zhou et al., 2014). In tobacco, under both nutrient starvation and optimal growth conditions, a great fraction of the peroxisome pool is subject to extensive autophagy-mediated turnover (Voitsekhovskaja et al., 2014).

Autophagy-related genes (*ATGs*) have been found in the genomes of plants, including *Arabidopsis*, rice, tobacco and barley (Kwon and Park, 2008; Zhou et al., 2015; Avila-Ospina et al., 2016). *ATG2* is involved in the early steps of autophagosome biogenesis. In autophagosome formation, the *atg2-2* mutant exhibits typical autophagy defects and has reduced resistance to powdery mildew of *Arabidopsis* (Wang et al., 2011). The overexpression of *MdATG3s* in callus lines of 'Orin' apple can increase the tolerance to multiple abiotic stresses (Wang et al., 2017). The *atg2* and *atg5* mutants display enhanced pathogen-induced cell death and early senescence in *Arabidopsis* (Yoshimoto et al., 2009). *HvATG6* of barley is involved in responding to abiotic stresses, such as drought, dark, salinity, nitrogen deficiency and hydrogen peroxide exposure, and in regulating stress resistance (Zeng et al., 2017). ATG11 can selectively induce the clearance of mitochondria and the production of a multifunctional scaffold required for autophagy. It acts as an important modulator of the ATG1–13 complex, which is an upstream regulator of an autophagy-related kinase complex (Li and Vierstra, 2014). The *Arabidopsis* ATG1/13 kinase complex is essential for autophagic transport, senescence and plant survival under nutrient-limited conditions (Suttangkakul et al., 2011). Macroautophagy-defective RNAi-*AtATG18a* transgenic *Arabidopsis thaliana* plants are more sensitive to hydrogen peroxide and high-salt, and accumulate more oxidized proteins than wild-type plants due to a lower degradation rate, indicating that autophagy is important in removing oxidized proteins under oxidative and salt stresses (Liu et al., 2009; Xiong et al., 2007).

Brassinosteroids (BRs) are a group of plant steroid hormones that can regulate seedling development, adult shoot and root growth, flowering, fruit development and senescence. They exhibit high physiological activity levels at low concentrations (Sun et al., 2015; Unterholzner et al., 2015). BRs can improve plant tolerance levels to abiotic stresses, such as drought, salt, high temperature and heavy metals (Krishna et al., 2017). In *A. thaliana*, BIN2 is inhibited by a high-concentration of BR, but BIN2 can phosphorylate DSK2, which enhances DSK2's interaction with ATG8 (Wang et al., 2012; Nolan et al., 2017).

In peach, the *ATG* genes may have roles in responses to drought stress, but the effects of drought stress on the physiological mechanism and autophagy activities in peach leaves treated with 24-epibrassinolide (EBR) are still unclear. In this study, the gene expression levels of *ATGs* in peach and correlations between autophagosomes and the enzymatic detoxification system were investigated. In peach, autophagy resisted disturbances caused by drought stress through the upregulation of genes and the production of greater numbers of autophagosomes. After EBR relieved the damage caused by drought stress in peach, we measured changes in the physiological mechanism and autophagy levels. The objective of this study was to investigate the *ATGs* gene expression levels and autophagy-related responses to EBR treatments under drought-stress conditions in peach.

2. Materials and methods

2.1. Plant materials

Two-year-old peach trees (*P. persica* L. cv. Lumi 1) were grown in an greenhouse with automated polycarbonate covers at the Horticultural Science Experimental Station of Shandong Agriculture University,

located in Tai'an, China (117°06′ E, 36°15′ N) to study the expression profiles of peach *ATGs* under drought stress.

2.2. Drought treatments

Leaves were sampled at 2, 4, 6, 8, 10, 11 and 12 day (d) after exposure to a natural drought-treatment. Watering was restored at the 13th d, and samples were taken on the 14th d. Samples were frozen in liquid nitrogen and immediately stored at $-75\,°C$ for RT-qPCR, antioxidant enzyme, malondialdehyde (MDA) and proline content analyses. Fresh samples were used for chlorophyll and photosynthetic parameter analyses, electron microscopy, and cell morphology and autophagosome monitoring.

2.3. EBR treatments

As a highly active synthetic analog of the BRs (Wu et al., 2017), EBR was used in this study. The voriconazole, a chemical that reduces sterol and BR contents (Rozhon et al., 2013). After fully watering, the one-year-old peach branches in similar growth status with 30 ± 5 leaves were subjected to five treatments: (1) Control: normal water; (2) 100 nM EBR; (3) 1 μM EBR; (4) 10 μM EBR; and (5) 1 μM voriconazole. The EBR and voriconazole solutions were applied at 20 mL per pot using a hand-held sprayer per branch.

Leaf samples were collected at 2, 4, 6, 8, 10, 11 and 12 d, and a part of each sample was stored at $-75\,°C$ for RT-qPCR, antioxidant enzyme, MDA and proline content analyses. Fresh samples were used for autophagosome monitoring.

2.4. Relative soil moisture and peach tree growth measurements

The relative soil moisture was measured using a JL-01 intelligent environment system's data acquisition instrument (Qingyi Electronic Technology Co., Ltd., Handan, China). The new tip lengths of peach trees were measured during the sampling process.

2.5. Chlorophyll determination and photosynthetic parameter measurements

The chlorophyll content was determined using a TYS-A chlorophyll meter (Top Instrument, Zhejiang, China) from 10 pieces of functional leaves having the same growth level and located in the middle of a new tip of the tree crown.

A CIRAS-3 portable photosynthesis instrument (PP-Systems, MA, USA) was used to measure the photosynthetic parameters. The analyses were performed between 09:00 h and 11:00 h during sunny, cloudless weather using central leaves that grew uniformly on the outside of the canopy and received light from a uniform direction. The photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and water utilization efficiency were determined. An LED red–blue light source was used for the measurements. The light intensity was set to 1200 μmol m^{-2}·s^{-1}. An open air system was used. The chamber temperature was 25 °C.

2.6. Leaf MDA contents

The method of Hodges et al. (1999), with modifications, was used for MDA measurements. Leaf samples of 50 mg were homogenized in 1.8 mL 10% trichloroacetic acid and then centrifuged for 20 min at 12,000 × g. Then, 1 mL 10% trichloroacetic acid with 0.6% thiobarbituric acid was added to 1 mL supernatant. The mixture was heated in boiling water for 30 min and then quickly cooled in an ice bath. After centrifugation for 10 min at 1600 × g, the mixture's absorbance was determined at 532 and 600 nm. The nonspecific absorbance at 600 nm was subtracted from that at 532 nm. The MDA concentration was calculated using this adjusted absorbance and the MDA extinction

Fig. 1. Drought stress effect the plant growth and leaf morphology of peach. A, Relative soil moisture. B, Growth increment. C, Leaf morphology of peach. D, Transmission electron micrographs of starch granule present in peach leaves. E, Transmission electron micrographs of vacuole in peach leaves. CW: cell wall; V: vacuole; S: starch granule. The data are from three replicated experiments, and represent means ± SE. Different lower-case letters in each analyzed indicate significant differences at $p < 0.05$.

was involved in drought-stress responses, and *PpATGs* were differentially expressed in peach leaves during the drought (Fig. 4). The *PpATG3, 8a, 8c, 8f, 8h, 18a, 18b, 18f, 18g* and *101* genes were expressed when the soil moisture level was suitable (0–4 d). The gene expression levels of *PpATG5, 8a, 8c, 8f, 8h, 11, 18a, 18f, 18g* and *18h* were extremely high under severe drought conditions (11–12 d). Of the 23 genes, the expression levels of the ATG8 (*PpATG8a, 8c, 8f* and *8h*) and

some of the ATG18s (*PpATG18a, 18f* and *18g*) were high under both suitable and drought conditions. However, in this experiment, the *PpATG7, 10* and *18c* gene were hardly expressed during drought stress. Except for the up-regulated expression of *PpATG8a*, the expression levels of the other *ATGs* were down-regulated after rehydration (14 d).

The Pearson correlation coefficients between antioxidant enzyme activity levels and the autophagy activities in peach leaves are shown in
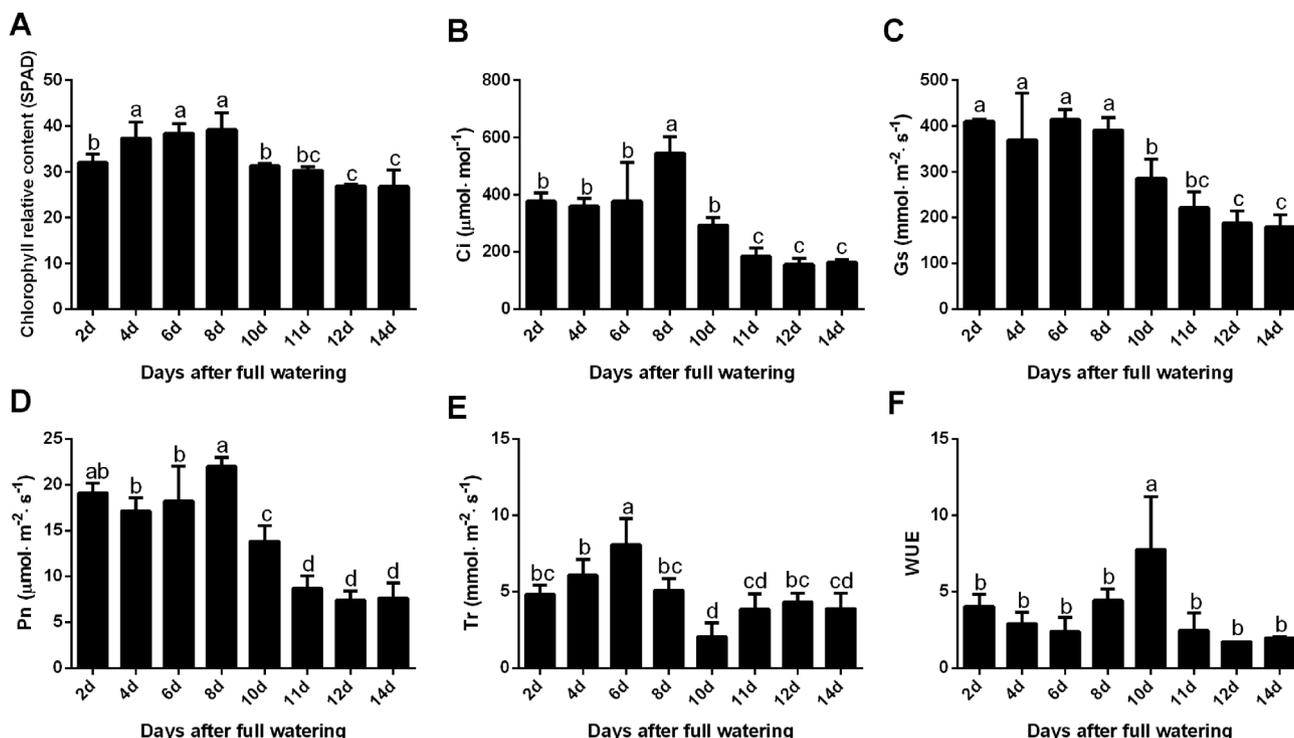
Fig. 2. Effects of drought stress on leaf chlorophyll content and photosynthesis. A, Chlorophyll relative content. B, Intercellular CO2 concentration (Ci). C, Stomatal conductance (Gs). D, Photosynthetic rate (Pn). E, Transpiration rate (Tr). F, Water utilization efficiency (Wue). The data are from three replicated experiments, and represent means ± SE. Different lower-case letters in each analyzed indicate significant differences at p < 0.05.

Table 1. SOD was negatively correlated with the expression levels of seven of the *PpATG* genes, *PpATG5, 8a, 8h, 11, 13, 13b* and *18b*. POD was negatively correlated with the expression levels of *PpATG6, 9, 10, 11, 13b, 14a, 18f* and *18g*. The expression levels of *PpATG18b* and *101* were negatively correlated with CAT enzyme activity. APX was negatively correlated with the gene expression levels of *PpATG3, 6, 8a, 8c,*

8f, 9, 10, 11, 13b, 14a, 18a, 18b, 18c, 18f, 18g and *18h*. GR was negatively correlated with the number of autophagosomes and the gene expression levels of *PpATG2, 5, 6, 8a, 8c, 8f, 9, 10, 11, 13b, 14a, 18a, 18c, 18f, 18g* and *18h*. The level of autophagy was inversely proportional to the enzyme activities of SOD and GR. In statistics, the enzyme activity levels of the antioxidant enzymes were inversely correlated to
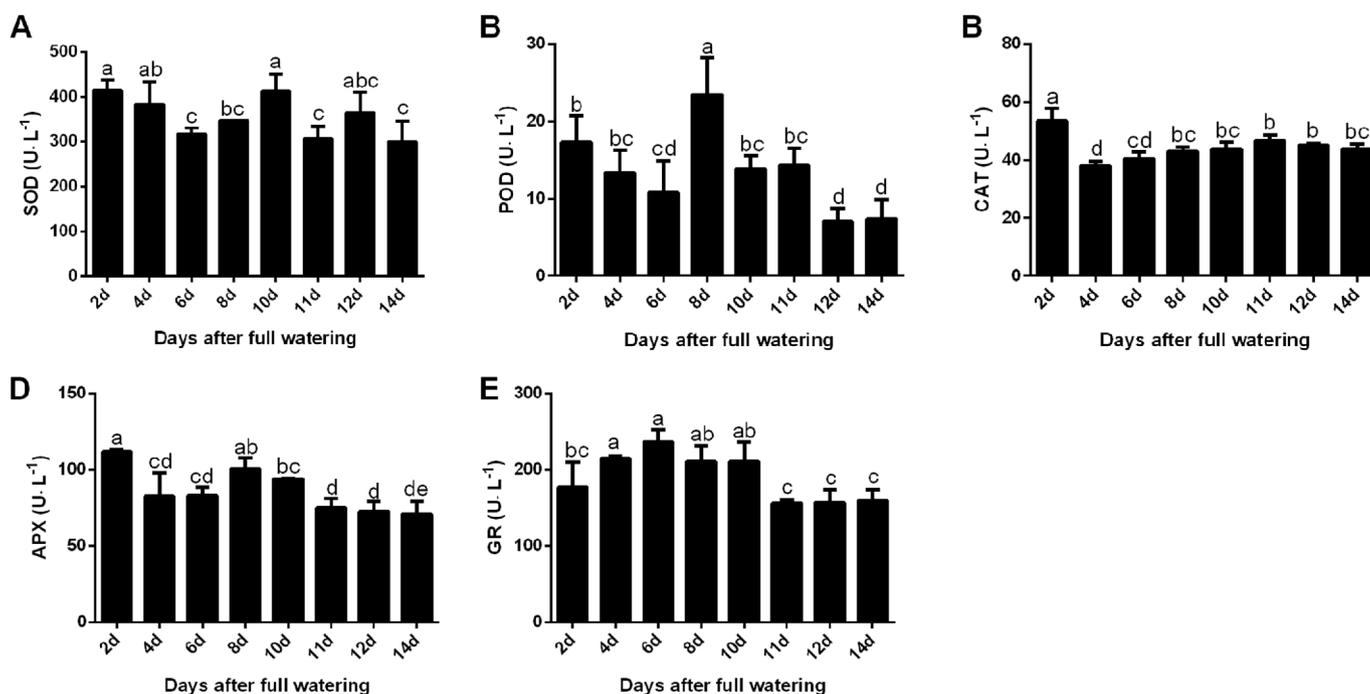
Fig. 3. Effects of antioxidant enzyme activity in peach leaf under drought stress. A, Superoxide dismutase (SOD). B, Peroxidase (POD). C, Catalase (CAT). D, Ascorbate peroxidase (APX). E, Glutathione peroxidase (GR). The data are from three replicated experiments, and represent means ± SE. Different lower-case letters in each analyzed indicate significant differences at p < 0.05.
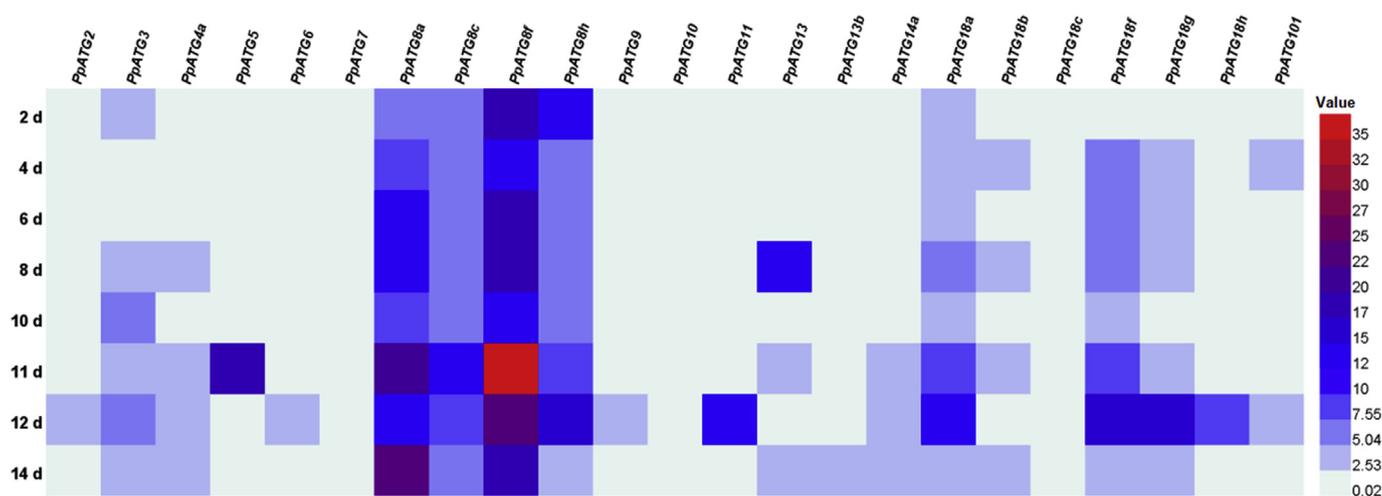
Fig. 4. Heatmaps of significantly expressed *PpATGs* at different developmental stages of peach response to natural drought stresses.

the expression levels of most *PpATGs*.

3.3. Physiological mechanism and autophagy monitoring in peach leaves under drought stress

As an indicator of the degree of plant stress, the MDA content was investigated. The MDA concentration was more than twofold greater in leaves 10 d after full watering than in leaves of plants during suitable soil-moisture conditions (Fig. 5A). The positive correlation between MDA and autophagosomes number found at the $p > 0.05$ level was 0.700^{**}. The proline concentration reached its highest level in leaves at 12 d after full watering (Fig. 5B). An MDC-microscope analysis was performed to monitor the autophagic processes in peach leaves under natural drought-stress conditions. Autophagy was involved in the peach response to natural drought stress in a time-dependent manner. Under drought stress, the number of autophagosomes was 3 times greater than under suitable soil-moisture conditions (Fig. 5C). A qualitative analysis of the autophagosomes in the peach leaves showed that their formation

decreased substantially within the first 6 d after full watering and returned to a significantly increased level at 10–12 d after full watering (Fig. 5D). There were no significant changes in the MDA content or the autophagy activity after rehydration, but the proline content decreased significantly.

3.4. A 1-μM EBR treatment alleviated the damage to peach leaves caused by drought stress

After rehydration, the damage cause by drought stress to peach leaves did not recover. Therefore, EBR was used to enhance the drought-stress tolerance of peach leaves. When compared with the other four treatments (untreated, 1 μM voriconazole, 100 nM EBR and 10 μM EBR), the MDA concentration decreased significantly after a 1-μM EBR treatment in peach leaves (Fig. 6A). The proline content of 1-μM EBR-treated leaves was lower than that of 10-μM EBR-treated leaves during the severe drought (Fig. S2). Moreover, the chlorophyll content, C_i, G_s, P_n and T_r values of 1-μM EBR–treated samples from the severe

Table 1
Correlation between antioxidant enzyme activity levels and autophagosome numbers as well as *ATG* gene expression levels in peach leaves.

| Pearson correlation	Superoxide dismutase (SOD)	Peroxidase (POD)	Catalase (CAT)	Ascorbate peroxidase (APX)	Glutathione peroxidase (GR)
Autophagosomes	− 0.455**	0.143	0.292	− 0.317	− 0.718**
PpATG2	0.011	− 0.313	0.242	− 0.246	− 0.513*
PpATG3	− 0.039	− 0.422	− 0.245	− 0.518*	− 0.330
PpATG4a	− 0.265	− 0.041	0.058	− 0.146	− 0.156
PpATG5	− 0.495*	− 0.019	0.214	− 0.381	− 0.481*
PpATG6	− 0.177	− 0.642**	0.079	− 0.594**	− 0.529*
PpATG7	− 0.03	0.330	− 0.095	− 0.087	− 0.076
PpATG8a	− 0.774**	− 0.185	− 0.070	− 0.525**	− 0.434*
PpATG8c	0.016	− 0.013	0.146	− 0.395*	− 0.347*
PpATG8f	− 0.045	− 0.100	0.266	− 0.425*	− 0.539**
PpATG8h	− 0.351*	− 0.202	0.344	− 0.016	− 0.244
PpATG9	− 0.248	− 0.605**	0.111	− 0.628**	− 0.620**
PpATG10	− 0.099	− 0.467*	0.069	− 0.523**	− 0.497**
PpATG11	− 0.462*	− 0.443*	0.092	− 0.533*	− 0.441*
PpATG13	− 0.436*	0.361	− 0.104	− 0.142	− 0.208
PpATG13b	− 0.895**	− 0.452*	0.059	− 0.541**	− 0.546**
PpATG14a	− 0.240	− 0.429*	− 0.123	− 0.749**	− 0.453*
PpATG18a	0.016	− 0.235	0.061	− 0.494**	− 0.447*
PpATG18b	− 0.483**	− 0.117	− 0.382*	− 0.640**	− 0.317
PpATG18c	− 0.193	− 0.158	− 0.308	− 0.505**	− 0.409*
PpATG18f	− 0.007	− 0.374*	− 0.108	− 0.569**	− 0.410*
PpATG18g	0.099	− 0.440*	− 0.055	− 0.451*	− 0.387*
PpATG18h	0.147	− 0.318	− 0.018	− 0.370*	− 0.395*
PpATG101	0.288	− 0.145	− 0.418*	− 0.284	− 0.059

Significant correlation between antioxidant enzyme activity and autophagosome numbers as well as gene expression levels are indicated by (*) $p < 0.05$ and (**) $p < 0.01$.
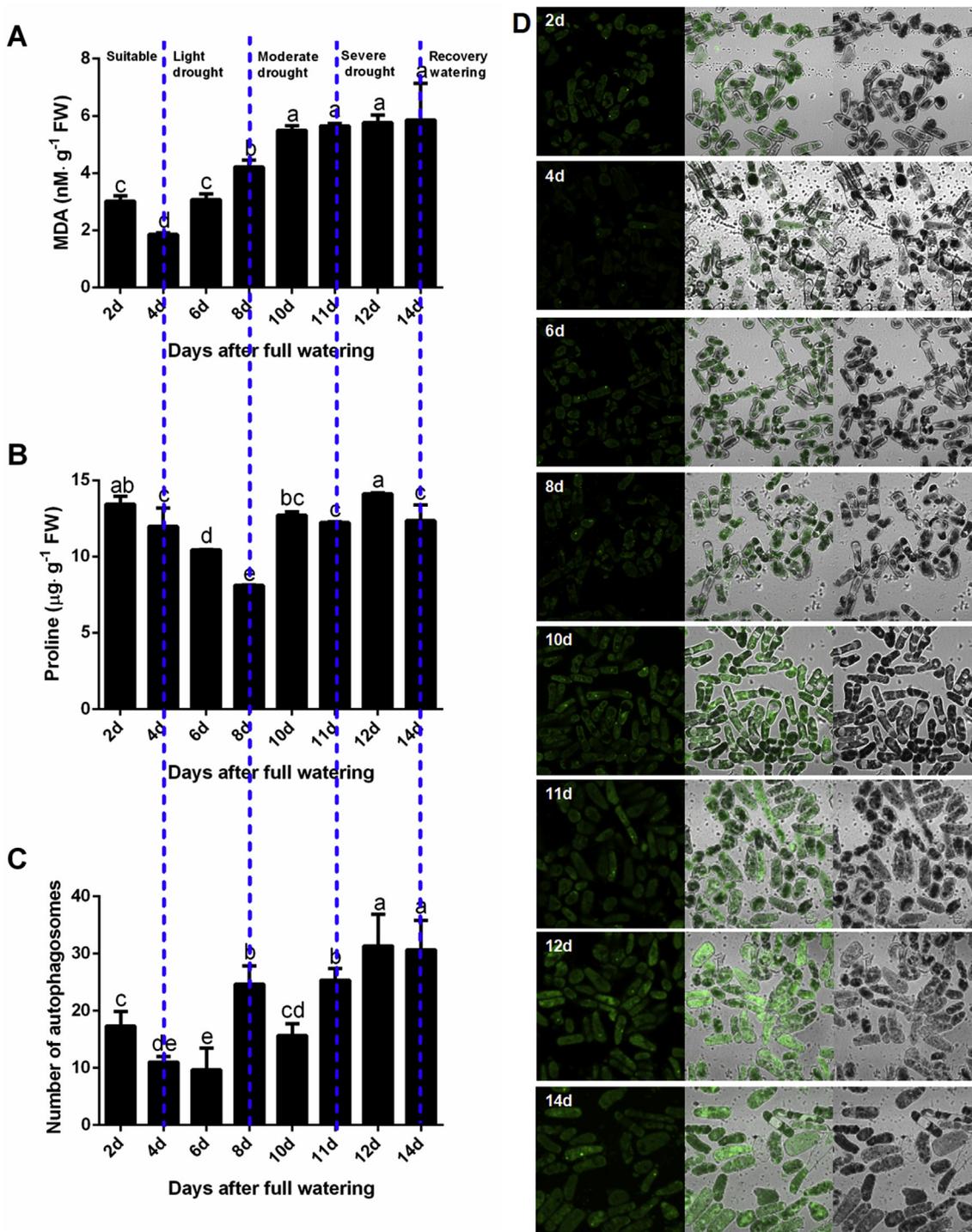
Fig. 5. MDA and proline contents, number of autophagosome, and autophagosome analysis in peach leaves under natural drought stress. A, Malondialdehyde (MDA). B, Proline concentration. C, Number of autophagosomes. D, Autophagosome monitoring. The data are from three replicated experiments, and represent means ± SE. Different lower-case letters in each analyzed indicate significant differences at $p < 0.05$.

drought were greater than those of the control (Fig. S3). Based on the phenotype, the 1-μM EBR-treated peach leaves had the highest growth vigor after 12 d of natural drought (Fig. 6B and C), and the capillary roots grew well under this treatment (Fig. 6D). Thus, the 1-μM EBR treatment appears to relieve drought-induced damage to peach leaves.

3.5. Peach autophagy monitoring after EBR relieves stress during severe drought

After the 1-μM EBR treatment, the activity levels of SOD, POD, CAT,

APX and GR significantly increased compared with the untreated and 1-μM voriconazole-treated peach leaves during the severe drought (Fig. 7A, S4). The total number of autophagosomes decreased during the 1-μM EBR treatment (Fig. 7B). The gene expression patterns of the 23 *PpATGs* were repressed by 1 μM EBR and 1 μM voriconazole under severe drought conditions (Fig. 7C). A MDC-microscopic analysis was performed to monitor the autophagic processes in peach leaves under drought conditions. The levels of autophagy in 1-μM EBR-treated and 1-μM voriconazole-treated leaves were lower than in the untreated leaves (Fig. 7D).
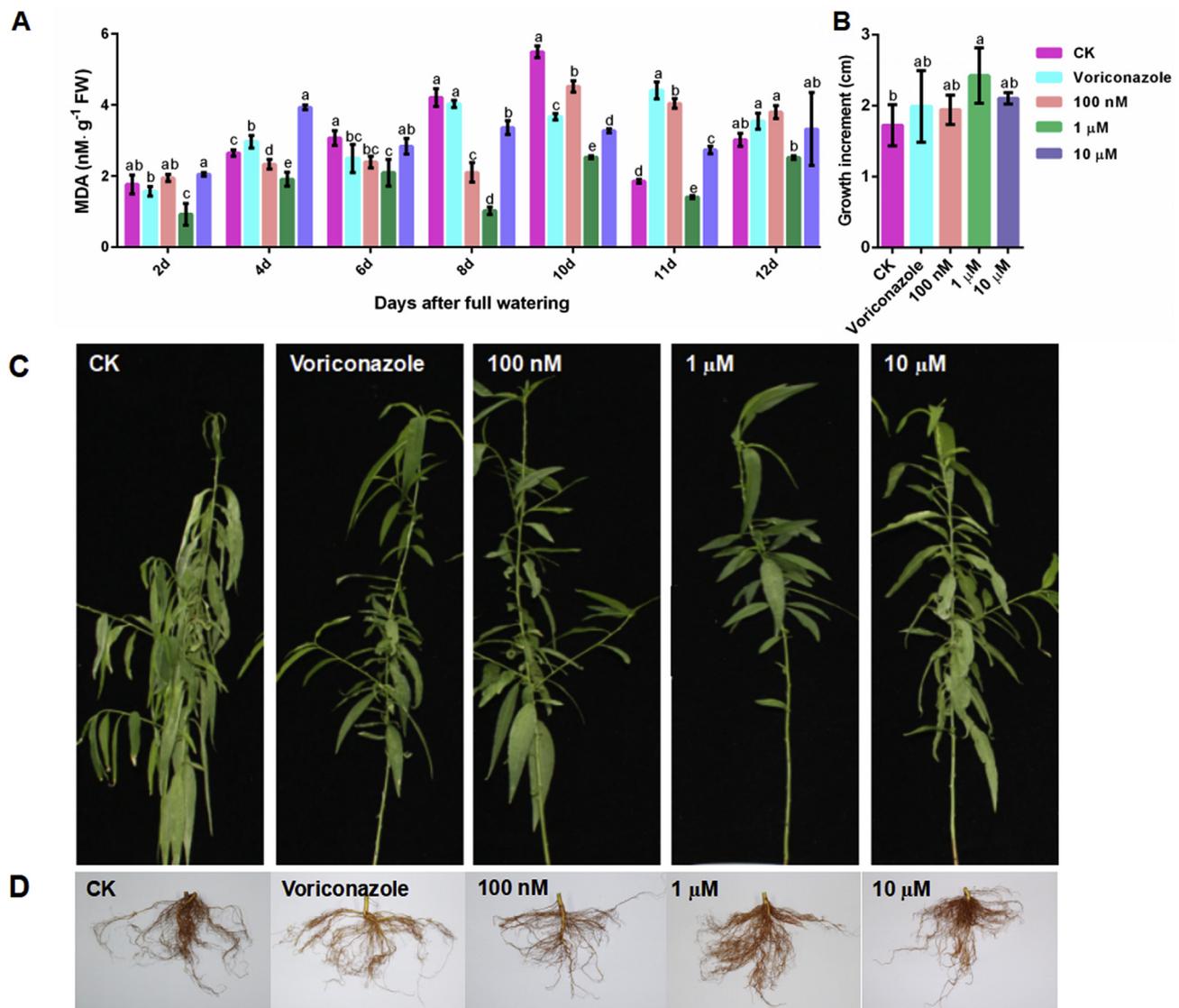
Fig. 6. Enhancing drought stress tolerance of peach by 24-Epibrassinolide. A, Malondialdehyde (MDA). B, Growth increment. C, Peach leaf phenotype. D, Peach root phenotype. The data are from three replicated experiments, and represent means ± SE. Different lower-case letters in each analyzed indicate significant differences at p < 0.05.

4. Discussion

4.1. Drought stress of peach

Under mild and severe drought-stress conditions, chlorophyll contents significantly decrease as do the Pn, Gs, Tr and Ci levels (Haider et al., 2018). In our findings, the chlorophyll, Ci, Gs, Pn, Tr and water utilization efficiency levels were significantly decreased in peach leaves under mild and severe drought conditions (Fig. 2). Like the results of Cao et al. (2017), the up-regulated antioxidant enzymes' activity levels removed ROS in peach leaves under moderate drought-stress conditions (Fig. 3). Unlike the results of Gilgen and Feller (2014) using the weed *Rumex obtusifolius*, the physiological mechanisms in peach leaves did not improve after rehydration. Thus, the damage to peach plants caused by drought stress was irreversible.

4.2. Drought and autophagy monitoring in peach leaves

The autophagy genes' transcriptional up-regulation is a prerequisite for increasing autophagic activity (Bernard et al., 2015). *ATG*s were involved in the drought-stress responses of peach (Fig. 4). The

autophagic vesicles form and are trafficked to their respective targets by associated conjugation cascades that couple the ATG8 and ATG12 proteins with phosphatidylethanolamine and the ATG5 protein (Chung et al., 2009). The expression levels of the *PpATG5, 8s, 11, 18a, 18f, 18g and 18h* genes were up-regulated during drought stress, especially at moderate and severe drought stages, and their expression levels positively correlated with the number of autophagosomes. These 10 ATGs are involved in the formation of autophagosomes and responses to drought stress. Therefore, we believe that peach autophagosomes were regulated by multiple *ATG*s in response to drought stress. The overexpression *AtAtg8* renders *A. thaliana* more sensitive to a mild salt stress and, to a lesser extent, to a mild osmotic stress (Slavikova et al., 2008). In wheat the *TdATG8*s were identified as drought stress-responsive genes (Kuzuoglu-Ozturk et al., 2012). In this study, the gene expression levels of *ATG8*s in peach leaves were greater than those of the other *ATG* genes under both non-drought and drought conditions. In apple plants, *ATG18a*'s overexpression enhances tolerance to drought stress owing to the greater autophagosome production and the higher autophagy frequency (Sun et al., 2018). *ATG18* genes in peach were active during drought. Therefore, we believe that *PpATG8*s and *PpATG18*s play vital roles in regulating peach leaf development and resisting
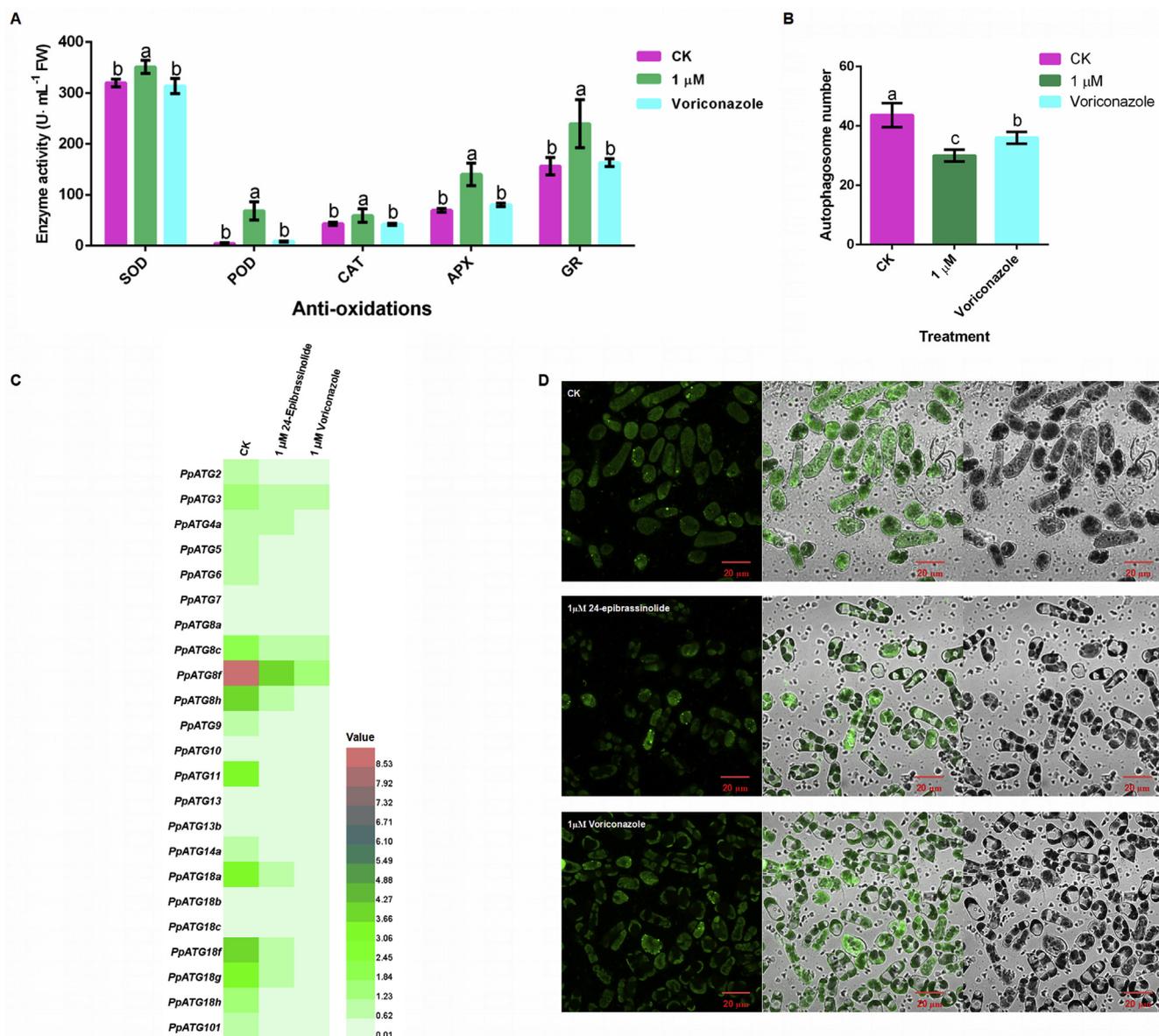
Fig. 7. Antioxidant enzyme activity and autophagy monitoring after 1 μM EBR treatment relieve severe drought injure of peach leaves. A, Antioxidant enzyme activity. B, Number of autophagosomes. C, Heatmaps of significantly expressed *PpATGs*. D, Autophagosome monitoring in peach leaves. The data are from three replicated experiments, and represent means ± SE. Different lower-case letters in each analyzed indicate significant differences at p < 0.05.

stress, but this requires further experimental proof.

4.3. Antioxidant enzymes and PpATGs in peach leaves

The *SlSOD* expression level decreases significantly under drought stress in tomato (Feng et al., 2016). Root and leaf SOD, POD, APX and GR activity levels increase the ROS scavenging capabilities during drought stress in *Robinia pseudoacacia* L (He et al., 2017). Here, the enzyme activities of SOD, POD, APX and GR increased during moderate drought, but they decreased during severe drought (Fig. 3). Under UV-C irradiation doses that do not damage cells, the apoptotic or autophagic cell death level remains unchanged, but the activity levels of CAT and SOD increase significantly in human melanoma cells (Ghosh et al., 2013). Peach leaves are slightly damaged under light and moderate drought conditions because antioxidant enzymes protect the leaves. We hypothesize that the production of ROS is first inhibited by antioxidant enzymes systems to alleviate membrane lipid peroxidation during light and moderate drought in peach (Li et al., 2016; Pérezpérez et al., 2012; Shangguan et al., 2018; Zhou et al., 2013). The SOD acticity of

Streptococcus suis serotype 2 scavenges ROS in infected macrophages (Fang et al., 2015). The Cu/Zn SOD 1 G93A mutant can upregulate autophagic activity in NSC34 cells (Wei, 2014). Reducing the CAT expression in TrkA-induced cells leads to autophagic cell death through ROS accumulation (Dadakhujaev et al., 2008). In this experiment, the number of autophagosomes was significantly greater during severe drought than during mid-drought (Fig. 5C and D). The MDA content did not increase during severe drought, and membrane lipid peroxide may be mainly cleared by autophagy. When drought stress aggravates membrane lipid peroxidation, leading to severe organelle damage in peach cells, autophagosomes are produced to degrade the damaged organelles, thereby reducing the level of plant injury caused by severe drought stress (Han et al., 2011; Zhou et al., 2014). Thus, autophagy may play an important role in protecting leaves of peach during severe droughts. However, more studies are needed to elucidate the mechanism.

In animals, the functions of *SOD2* can prevent the oxidative damage of mitochondrial components, and the activation of autophagy serves as an additional response that scavenges damaged mitochondria (Pi et al.,

2015). Mice with low autophagic capacities aggregate SOD1 earlier (Tokuda et al., 2016). CAT activity is required for immunity-triggered autophagic programmed cell death in *Arabidopsis*, and CAT activity inhibits basal autophagy in tobacco BY-2 cells (Tyutereva et al., 2018; Hackenberg et al., 2013). Here, the Pearson correlation showed that the *PpATGs*' expression levels were negatively correlated with antioxidant enzyme activity levels, and the SOD enzymes activity was negatively correlated with the number of autophagosome in peach leaves. Mason et al. (2013) found that, unlike many antioxidant treatments, GR activity cannot inhibit autophagy in humans with Huntington's disease. The phenomenon in plant species has not yet been reported, and here, we found that the number of autophagosomes was negatively correlated with GR activity. In animals, transglutaminase 2 acts as a multifunctional protein regulating autophagy and other cell processes (Gundemir et al., 2012). Knockouts of transglutaminase 2 in mice brain tissues significantly decrease the CAT and SOD2 protein expression levels, while, at the same time, Beclin1 (an autophagy protein) is downregulated (Barbara et al., 2013). This indicates that the antioxidant enzyme systems and autophagy may be two complementary and antagonistic systems that scavenge ROS in peaches' resistance to stress. They might be regulated by some of the same genes, but further experiments are required to confirm this hypothesis.

4.4. Autophagy monitoring during EBR-treatments mitigating drought stress in peach leaves

BRs have been proposed to increase the resistance of plants to drought stress for many years (Li et al., 2012). The MDA content of EBR-pretreated wheat seedlings decreases during drought-stress treatments compared with controls (Shakirova et al., 2016). In this study, the MDA concentration was lowest in leaves receiving the 1-μM EBR treatment; therefore, this treatment was considered to relieve drought damage to peach leaves (Fig. 6). EBR increased the CAT, POD and SOD activity levels in NaCl- and/or Cu-stressed *Cucumis sativus* (Fariduddin et al., 2013). After the 1-μM EBR treatment, the enzyme activity levels of SOD, POD, CAT, APX and GR significantly improved compared with the untreated and 1 μM voriconazole-treated plants (Fig. 7A, S4). Leaf senescence is accelerated by abiotic stresses (Wingler and Roitsch, 2010). ABA promotes senescence; however, its biosynthesis is inhibited by BRs. Therefore, BRs inhibit senescence in *Arabidopsis* (Hu and Yu, 2014). Increases in BR levels can increase the cytokinin levels to delay the senescence of rice (Ashikari et al., 2005). Here, the antioxidant enzymes still functioned to eliminate ROS during severe-drought stress (12 d) in 1-μM EBR-treated plants, which may delay senescence, resulting in the decreased drought damage to peach leaves.

An enhanced abiotic-stress tolerance can inhibit cell autophagy, and reducing the stress level in an environment can reduce autophagy (Shangguan et al., 2018). Transcription factors can directly regulate *ATG*s in plants during leaf ageing, which is senescence-related (Garapati et al., 2015a, 2015b). BES1 is targeted by selective autophagy through the ubiquitin receptor DSK2, and BIN2 phosphorylates DSK2, enhancing DSK2's interaction with ATG8 in *Arabidopsis* (Nolan et al., 2017). BER can promote BES1 transcription and inhibit BIN2 (Unterholzner et al., 2015; Hu and Yu, 2014). However, EBR may inhibit the expression of *BIN2* and *ATGs* in peach leaves. Accordingly, the 1-μM EBR treatment alleviated drought stress-related damage to peach leaves and the autophagy level also decreased. Confirming this hypothesis will require further experimentation.

5. Conclusions

We identified 23 ATG genes in peach. In total, 20 autophagy-related gene responded to drought stress. The *PpATG8s* and some *PpATG18s* appear to play vital functions in regulating peach leaf development and resistance to drought stress. SOD, POD, CAT, APX and GR activity levels were inversely correlated with the expression levels of different

PpATGs. The antioxidant enzyme systems alleviated membrane lipid peroxidation in peach under light and moderate drought conditions. When severe drought stress aggravates membrane lipid peroxidation, autophagosomes are produced to degrade the damaged organelles, thereby reducing the level of plant injury caused by severe drought stress. However, more evidence is needed to confirm this hypothesis. The 1-μM EBR treatment alleviated drought stress-related damage to peach leaves, and the number of autophagosomes also decreased. Additionally, the expression patterns of the 23 of *PpATG* genes were repressed by the 1-μM EBR treatment. More evidence is needed to confirm this mechanism.

Disclosure statement

No potential conflict of interest was reported by the authors.

Author contributions

XXW performed the experiments and wrote the manuscript. YGG, QJW, MC, XLY, DML and XDC provided technical and theoretical support. LL and DG supervised the project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.11.026.

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