Expression Patterns and Functional Analysis of E3 Ubiquitin Ligase Genes in Rice

huijuan zhang
Taizhou University https://orcid.org/0000-0003-4366-6833
Dewei Zheng
Taizhou University
Fengming Song
Zhejiang University
Ming Jiang (jiangming1973@139.com)
Taizhou University https://orcid.org/0000-0001-9556-2249

Original article

Keywords: rice, E3 ubiquitin ligases, biotic stress, abiotic stress, ROS, expression patterns

Posted Date: June 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-575739/v1

Abstract

Background: E3 ubiquitin ligases involve in many processes, containing the response to biotic and abiotic stresses. However, the functions of E3 ubiquitin ligases in rice were rarely studied.

Results: In this research, 11 E3 ubiquitin ligase genes were selected and the function analysis was done in rice. These 11 E3 ubiquitin ligase genes showed different expression patterns under different treatments. The BMV:Os06g13870- infiltrated seedlings showed decreased resistance to *Magnaporthe grisea* (*M. grisea*) when compared with BMV:00-infiltrated seedlings, while BMV:Os04g34030- and BMV:Os02g33590-infiltrated seedlings showed increased resistance. They involved in the resistance against *M. grisea* maybe by regulating the accumulation of reactive oxygen species (ROS) and expression levels of defense-related genes. The BMV:Os06g34390-infiltrated seedlings showed decreased tolerance to drought stress while BMV:Os02g33590-infiltraed seedlings showed increased tolerance, maybe through regulating proline content, sugar content and drought-responsive genes' expression. BMV:Os05g01940-infiltrated seedlings showed decreased tolerance to cold stress by regulating malondial dehyde (MDA) content and cold-responsive genes' expression.

Conclusion: These results showed that E3 ubiquitin ligases involved in the resistance to biotic and abiotic stresses in rice.

Introduction

Ubiquitin, a highly conserved 76-amino acid polypeptide, is widely distributed in eukaryotes. Ubiquitin is attached to target proteins by a cascade mediated by three sequential ubiquitin enzymes, E1 (the ubiquitin activating enzyme), E2 (the ubiquitin conjugating enzyme) and E3 (the ubiquitin ligase). The E3 ubiquitin ligase confers the specificity of the reaction and can be single-subunit (including HECT, RING finger and U-box domain family) or multi-subunit (such as SCF complex) (Morreale and Walden 2016).

E3 ubiquitin ligases have been reported to function in many processes, containing the responses to biotic and abiotic stresses (Ariain et al. 2017; Sun et al. 2019; Zhang et al. 2019). First, E3 ubiquitin ligases involved in response to biotic stresses. ATL subfamily containing the conserved RING-H2 domain was activated by elicitor, and played important roles in disease resistance may through regulating the elicitor signaling pathway including chitin (Berrocal-Lobo et al. 2010; Ni et al. 2010; Serrano and Guzman 2004; Deng et al. 2017; Chen et al. 2017). U-box domain family of E3 ubiquitin ligases also played important roles in defense response. The spl11 mutants increased resistance to multiple fungal and bacterial pathogens (Yin et al. 2000; Zeng et al. 2004). AtPUB22, 23 and 24 act as negative regulators of biotic stresses (Trujillo et al. 2008; Cho et al. 2008). In addition, E3 ubiquitin ligases involved in the basic resistance of plants. *HUB1* overexpression plants thickened cell wall to increase the resistance to *Botrytis cinerea* (*B. cinerea*), while the knock out mutants thinned cell wall to decrease the resistance to *B. cinerea* and *Alternaria brassicicola* (Dhawan et al. 2009). The overexpression of *OsBBI1* led to more

accumulation of H_2O_2 and phenolic compounds, thicker cell wall and increased resistance to *M. oryzae* (Li et al. 2011).

Second, E3 ubiquitin ligases involved in response to abiotic stresses, including drought and cold stresses. E3 ubiquitin ligases have functions on the response to drought stress, dependent on ABA pathway (Zhang et al. 2005, 2015; Stone et al. 2006; Ko et al. 2006; Joo et al. 2016; Yang et al. 2016; Lim et al. 2017; Chapagain et al. 2018; Qin et al. 2020), or independent on ABA pathway (Qin et al. 2008; Prasad et al. 2010; Suh and Kim 2015; Wu et al. 2015). In addition, E3 ubiquitin ligases have functions on the response to cold stress. In *Arabidopsis*, HOS1, AtATL78 and AtATL80 negatively regulated the tolerance to cold stress (Lee et al. 2001; Kim S and Kim W 2013; Suh and Kim 2015), while PUB25 and PUB26 positively regulated the tolerance to cold stress (Wang et al. 2019). OsDIRP1 positively regulated the tolerance to cold stress in rice (Cui et al. 2018).

E3 ubiquitin ligases comprise a huge protein family and are encoded by a large number of genes (Bhaskar and Joemar 2020). For example, more than 1200 in *Arabidopsis*. This large number indicates the importance of E3 ubiquitin ligase. In this study, the functional analysis of 11 E3 ubiquitin ligase genes in rice was done. The expression levels of some ubiquitin ligase genes were induced by one or several treatments we tested, although with different models. The silencing of *Os04g34030* (*OsPUB22*) or *Os02g33590* (*OsPUB23*) led to increased resistance to *M. oryzae* while the silencing of *Os06g13870* (*OsPUB21*) led to increased resistance. The silencing of *Os02g33590* led to increased tolerance to drought stress while the silencing of *Os06g34390* (*OsATL17*) led to decreased tolerance, maybe through regulating proline content, sugar content and expression levels of drought responsive genes. And the silencing of *Os05g01940* (*OsATL9*) led to decreased tolerance to cold stress, maybe through regulating MDA content and expression levels of cold responsive genes.

Methods

Characterization of E3 ubiquitin ligase genes

We selected 11 E3 ubiquitin ligase genes which are speculated to have function on response to biotic and abiotic stresses from *Arabidopsis*. Using these genes as queries to do search in rice genome database by BlastP program, the predicted nucleotide sequences and amino acid sequences for these genes were downloaded. Phylogenetic trees for rice, *Arabidopsis* and other MEDs were structured using the Neighbor-joining method by MEGA6 program with the *p*-distance, complete deletion, and 1000 bootstraps.

Plant growth condition and different treatments

Rice cv. Yuanfengzao, a pair of isogenic lines (H8R and H8S) and IR64 were used in this research for various purpose. The cv. Yuanfengzao was used for the gene expression analysis in response to treatments of hormone molecules and the abiotic stress. H8R and H8S were used for the analysis of gene expression with inoculation of *M. grisea*. IR64 was used for VIGS infiltration. In the treatment of hormone, the two weeks old cv. Yuanfengzao seedlings were treated with 1.5 mM salicylic acid (SA, pH 6.5), 100

μM jasmonic acid (JA), 100 μM 1-amino cyclopropane-1-carboxylic acid (ACC) and 100 μM abscisic acid (ABA) (Sigma-Aldrich, St. Lousi, USA). And the same volume of water or 0.1% ethanol was foliar sprayed as control.

M. grisea (strain 85-14B1, race ZB1) was cultivated on oatmeal medium at 25℃ for 10 days. The spores were collected and resuspended in water to final concentration 5×10^5 conidia/mL with 0.02% Tween-20. Then the spore solution was sprayed on the leaves of H8R and H8S (Luo *et al.*, 2005). Leaf samples were collected at indicated time points and stored at -80°C until use.

For the extreme temperature stress, three-week-old plants were suffered from 42°C and 4°C. For the drought stress, the hydroponic three-week-old plants were put on the floor of the frame in the greenhouse for growth after water on the surface of their roots were absorbed by filter paper. For salt stress, the hydroponic three-week-old plants were transferred to 200 mM NaCl solution. Then the samples were collected at indicated time points (Hong et al. 2016). All the seedlings mentioned above were grown in a room with 28°C, with a cycle of 14h light/10h dark. IR64 were used for VIGS assays and the infiltrated seedlings were put in a room with 24°C, with a cycle of 14h light/10h dark.

Vector construction and VIGS

200-400 bp fragments of target genes were constructed into BMV vector and confirmed by sequencing. The obtaining recombinant plasmids were transformed into *Agrobacterium tumefaciens* strain C58C1 by electroporation by a GENE PULSER II Electroporation System. The agrobacteria confirmed by colony PCR were cultivated in liquid YEP medium containing corresponding antibiotics at 28℃ overnight. The bacteria were collected and resuspended in induction buffer (10 mM $MgCl_2$, 10 mM MES, 200 μM acetosyringone, pH5.7) and kept at 28℃ for 5 h, stopped by centrifugation. Resuspended in an infiltration solution (10 mM MES, 10 mM $MgCl_2$, 0.4 g/L L-cysteine, 0.15 g/L DTT, 0.75 mg/L silver nitrate and 15 μl Silwet-77 in 10 % YEP) and incubated at 28°C until the OD600 value reached 2.0. Mixed with the same volume of agrobacteria harboring pC13/F1+2 before vacuum infiltration. 8-10 days old IR64 seedlings were submerged completely in the mixed Agrobacterium suspension with vacuum infiltration for 7 min with a pressure of 20 Kpa (model no. Rocker 410, Xiamen B&C Instrument Co. Ltd, China). Then these plants were put in a room with 24°C, with a cycle of 14h light/10h dark, which were recorded as BMV:target gene-infiltrated seedlings. The BMV:empty vector was transformed to seedlings as control, which were recorded as BMV:00-infiltrated seedlings.

qRT-PCR

RNA was extracted by Trizol as the instruction (Invitrogen, Shanghai, China). cDNA was got by AMV reverse transcriptase (TaKaRa, Dalian, China) following the instruction. The qRT-PCR was done by SYBR Premix Ex Taq™ followed the instruction (TaKaRa, Dalian, China) and performed in a CFX96 real-time PCR detection system (BioRad, Hercules, CA, USA).

Disease assay of *M. oryzae*

5 μL *M. oryzae* spore solution with appropriate concentration was dropped on the surface of leaves from four-week-old silencing plants which had already put on the wet cheese cloth. Then the inoculated leaves were kept in high humidity in the room for growth of silencing seedlings. After 7 days, the photos were taken and the lesion sizes were recorded. For the analysis of expression of defense-related genes and the measurement of *M. oryzae* in planta, we used the whole plants assay. It was carried out the same as done on the H8R and H8S seedlings.

Abiotic stress tolerance assay

In the analysis of drought stress, 4-week seedling BMV:Os06g34390-infiltrated plants and the BMV:00-infiltrated plants in the same pot were withholding water for 10 days before re-watering while 15 days for BMV:Os02g33590-infiltrated plants. After 12 days, the survival rate, water loss, proline content and sugar content were measured (Bates et al. 1973; Hong et al. 2016). In the analysis of cold stress, 4-week seedling of BMV:target gene-infiltrated plants and the BMV:00-infiltrated plants in the same pot were kept at 4 °C for 2 days before recovering to normal growth condition (Huang et al. 2016). The survival rate, MDA content, electrolyte leakage, chlorophyll content and expression levels of genes which were related to cold stress were done as reported before (Hong et al. 2016).

Results

Characterization of E3 ubiquitin ligase genes in rice

By Blastp searches against the rice genome database using the characterized 11 *Arabidopsis* genes as queries, corresponding genes were obtained. Phylogenetic tree analysis revealed that Os04g34030, Os02g33590 and Os01g64570 respectively showed similarity to *Arabidopsis* AtPUB22, AtPUB23 and AtPUB24 which were already reported to have functions in response to biotic and abiotic stresses. Os06g13870 showed similarity to AtPUB21, TdPUB21 and HvPUB21. Os06g34390 showed similarity to SbATL41 and ZmATL6, and Os05g01940 and AtATL9 were gathered in one cluster (Figure 1).

The expression levels of *Os06g13870*, *Os04g34030* and *Os02g33590* were strongly induced by the inoculation of *M. oryzae* and hormone molecules

To test whether these 11 genes have functions on responses to stress, the expression patterns of these genes with inoculation of *M. grisea* and treatment of hormone molecules were analyzed. As showed in Figure 2a, the expression of *Os06g13870*, *Os04g34030* and *Os02g33590* was strongly induced by the inoculation of *M. grisea* in incompatible interaction, while other genes not. The expression levels of these genes were analyzed with the treatment of hormone. As showed in Figure 2b, the expression levels of *Os06g13870*, *Os04g34030* and *Os02g33590* were strongly induced by SA, JA and ACC while the expression levels of other genes not. ABA is a well-known stress-related hormones in plants and involved in the responses to biotic and abiotic stress. We test the expression patterns of E3 ubiquitin ligase genes with ABA treatment. In the treatment of ABA, the expression levels showed no significant difference from control except *Os06g34390* and *Os05g01940* (Figure 2c).

The expression patterns of E3 ubiquitin ligase genes in response to abiotic stress

As reported, PUB genes were involved in the response to abiotic stresses containing drought, cold, heat and salt to different degrees (Lu et al. 2020). So drought, salt, cold and heat stresses were selected for the analysis of the expression patterns in abiotic stress. In the drought stress, almost all the genes had no changes except two genes, *Os06g34390* and *Os02g33590*. They increased dramatically 2 hours after treatment (Figure 3a). In the cold stress, the expression levels of all the genes showed no significant difference from control except *Os05g01940* which was strongly induced 12 hours after treatment (Figure 3b). In the heat and salt stresses, the expression levels of all genes showed no significant from control after treatment (Figure 3c-d).

BMV:Os06g13870-infiltrated plants showed decreased resistance to *M. grisea* when compared with BMV:00-infiltrated plants, while BMV:Os04g34030- and BMV:Os02g33590-infiltrated plants showed increased resistance

We explored the possible function of these genes in the resistance to *M. grisea* by comparing the phenotype of BMV:target genes- and BMV:00-infiltrated plants after the inoculation of *M. grisea*. The silencing efficiency was tested before inoculation and the really silencing plants were selected for disease assay (Figure 4a). After 7 days, the BMV:Os06g13870-infiltrated plants showed severer disease phenotype with larger lesion size and more fungi growth when compared with control while BMV:Os04g34030- and BMV:Os02g33590-infiltrated plants showed lighter disease phenotype with smaller lesion size and less fungi growth (Figure 4b-d).

In order to explore the mechanism of *Os06g13870*, *Os04g34030* and *Os02g33590*'s function in the resistance to *M. grisea*, we analyzed the condition of ROS accumulation and the expression levels of defense-related genes. First, we analyzed the condition of ROS accumulation. As showed in Figure 5a, there was no significant difference among BMV:Os06g13870-, BMV:Os04g34030-, BMV:Os02g33590- and BMV:00-infiltrated seedlings before *M. grisea* inoculation. While after *M. grisea* inoculation, the BMV:Os04g34030- and BMV:02g33590-infiltrated seedlings accumulated less ROS than BMV:00-infiltrated seedlings, while BMV:Os06g13870- infiltrated seedlings accumulated more. H_2O_2 content showed similar results. After *M. grisea* inoculation, H_2O_2 content in BMV:*Os04g34030*- and BMV:02g33590- infiltrated seedlings is lower than that in BMV:00-infiltrated seedlings while higher in BMV:06g13870-infiltrated seedlings (Figure 5b). SOD activity and CAT activity were analyzed to explore the reason for the changed H_2O_2 content in BMV:Os06g13870-, BMV:Os04g34030- and BMV:Os02g33590-infiltrated seedlings. As showed in Figure 5c-d, before *M. grisea* inoculation, SOD activity and CAT activity in BMV:target genes- and BMV:00-infiltrated seedlings showed no significant difference. After *M. grisea* inoculation, SOD activity in BMV:Os04g34030- and BMV:02g33590- infiltrated seedlings decreased while CAT activity increased when compared with BMV:00-infiltrated seedlings (Figure 5c-d). SOD activity in BMV:Os06g13870- infiltrated seedlings increased while CAT activity decreased when compared with BMV:00-infiltrated seedlings after *M. grisea* inoculation (Figure 5c-d).

Second, we analyzed the expression levels of defense-related genes. As showed in Figure 6, the expression levels of *OsLOX1*, *OsPR3*, *OsNH1*, *OsPR1a* and *OsWRKY45* decreased in BMV:*Os06g13870*-infiltrated plants when compared to control after *M. grisea* inoculation while increased in BMV:Os04g34030- and BMV:Os02g33590- infiltrated seedlings. These results indicated *Os06g13870*, *Os04g34030* and *Os02g33590* involved in the resistance to *M. grisea*, may through regulating the accumulation of ROS and the expression of defense-related genes.

The BMV:Os02g33590-infiltrated plants increased the tolerance to drought stress while BMV:Os06g34390-infiltrated plants decreased the tolerance to drought stress

To explore the possible function of these 11 genes in response to abiotic stress, we compared the phenotype of BMV:target gene- and BMV:00-infiltrated plants after suffered from abiotic stress. None had dramatic difference from control expect BMV:Os06g34390- and BMV:Os02g33590-infiltrated plants. The BMV:Os02g33590- infiltrated plants showed increased tolerance while BMV:*Os06g34390*-infiltrated plants showed decreased tolerance to drought when compared with control (Figure 7a and 8a). Water loss and survival rate further confirmed this conclusion. Water loss in BMV:Os06g34390-infiltrated plants is higher than control while lower in BMV:Os02g33590-infiltrated plants (Figure 7b, 8b). The survival rate, proline content, and sugar content decreased dramatically in BMV:Os06g34390-infiltrated plants (Figure 7c-e) while increased dramatically in BMV:Os02g33590-infiltrated plants (Figure 8c-e). We also tested the expression levels of drought-responsive genes. The expression levels of drought-responsive genes decreased in BMV:Os06g34390- infiltrated plants while increased dramatically in BMV:Os02g33590-infiltrated plants (Figure 9).

BMV:Os05g01940-infiltrated plants decreased the tolerance to cold stress

In the cold stress, BMV:Os05g01940-infiltrated plants showed decreased resistance when compared with control (Figure 10a). The survival rate of BMV:Os05g01940- infiltrated plants was 19.62% , while 83.65% in control (Figure 10b). The MDA content and electrolyte leakage in BMV:Os05g01940-infiltrated plants increased when compared with control (Figure 10c-d) while chlorophyll content in BMV:Os05g01940-infiltrated plants decreased (Figure 10e). The expression levels of cold-responsive genes were analyzed next. As showed in Figure 10f, the expression levels of cold-responsive genes decreased significantly in BMV:Os05g01940- infiltrated plants when compared with control.

Discussion

E3 ubiquitin ligases had important role in ubiquitin-proteasome pathway which is one of the most important protein degradation pathway in eukaryotic organism (Pickart and Eddins 2004; Wang and Deng 2011). There are many E3 ubiquitin ligases in plants, for example, at least 60 in *Arabidopsis*. The study of E3 ubiquitin ligases is a hot topic and the functions of E3 ubiquitin ligases are explored gradually. However, functions of E3 ubiquitin ligases in rice were rarely studied. In this research, 11 E3 ubiquitin ligase genes which were speculated to have function on the response to biotic or abiotic stresses from *Arabidopsis* were selected and the homologous genes in rice were found. Phylogenetic tree analysis

revealed that these genes in rice may have similar functions as the homologous genes in *Arabidopsis* (Figure 1).

Plants are unavoidably suffered from abiotic and biotic stresses. And plants have formed sophisticated mechanisms to adapt to such adverse conditions. Phytohormones have an important role in helping plants to suit for environmental situations (Verma et al. 2016). Many phytohormone signaling pathways dependent on the ubiquitin proteasome system, specifically E3 ubiquitin ligases which can perceive and initiate signaling transduction (Kelley 2018; Tal et al. 2020). So the expression of these 11 genes with hormone treatment such as JA, ACC and SA were done first. The expression levels of all genes were not induced by JA, ACC and SA treatment except Os06g13870, Os04g34030 and Os02g33590 (Figure 2b). This result indicates that these genes may have functions on the response to biotic stress. Next, the functions of these genes on the response to *M. grisea* were done. As Figure 2a and 4 showed, the expression levels of Os06g13870, Os04g34030 and Os02g33590 were induced by *M. grisea*, and the silencing of Os06g13870, Os04g34030 and Os02g33590 led to changed resistance to *M. grisea*. *Os04g34030* and *Os02g33590* negatively regulated the resistance to *M. grisea*. The functions of Os04g34030 (OsPUB22) and Os02g33590 (OsPUB23) in rice on response to biotic stress were similar with AtPUB22 and AtPUB23 in *Arabidopsis*, negative regulators of biotic stresses (Trujillo et al. 2008; Cho et al. 2008). However, Os01g64570 (OsPUB24) seemed to have no function on the resistance to *M. grisea*. CMPG1, highly related to *Arabidopsis* PUB20 and PUB21, positively regulated the response to disease resistance in tomato and tobacco (Gonzalez et al. 2006). Likely, Os06g13870 (OsPUB21) positively regulated the resistance to *M. grisea* (Figure 4). These results further confirmed previous reports that E3 ubiquitin ligases did regulate the resistance to biotic stress, positively or negatively (Chen et al. 2017; Ni et al. 2010; He et al. 2015; You et al. 2016; Wang et al. 2016).

ROS production is important for the activation of immune responses against pathogen infection and the expression levels of defense-related genes are closely related to disease resistance (Lehmann et al. 2015; Waszczak et al. 2018; Qi et al. 2018; Segal and Wilson 2018). E3 ubiquitin ligase can also regulated the resistance to biotic stress by these two ways (Zhou and Zeng 2018; Yaeno and Iba 2008). APIP6 silencing results in reduced resistance to *M. oryzae* in rice, by reducing flg22-induced ROS generation and suppressing defense-related gene expression (Park et al. 2012). In our study, ROS accumulation and the expression levels of defense-related genes were analyzed to explore the mechanism for the changed resistance by gene silencing. As showed in Figure 5a-b, the silencing of Os06g13870 led to more ROS accumulation and H_2O_2 content while the silencing of Os04g34030 and Os02g33590 led to less accumulation and H_2O_2 content. Plants have a highly efficient system for maintaining ROS homeostasis (Mittler et al. 2004). They have two ways to scavenge ROS, one is by small molecules (containing glutathione, ascorbic acid, flavo-noids, alkaloids and carotenoids), and the other is by detoxifying enzymes including superoxide dismutase (SOD), catalase (CAT), peroxidase and peroxiredoxins (Lehmann et al. 2014). SOD activity and CAT activity were done to explain the changed ROS accumulation. The changed SOD activity and CAT activity in BMV:target genes-infiltrated seedlings may explain the changed ROS accumulation (Figure 5c-d). The expression levels of defense related genes

were done next. *LeATL6* regulates elicitor-activated defense responses via a JA-dependent signaling pathway in tomato (Hondo et al. 2007). ASK1/ASK2 and cullin 1 formed SCF ubiquitin ligase complex, as the signaling receptor of JA, through degradation of the inhibitor JAZ of JA pathway to activate the expression of JA-response genes (Devoto et al. 2002; Xu et al. 2002; Ren et al. 2005; Thines et al. 2007; Chini et al. 2007). MdPUB29 increases the resistance to *Botryosphaeria dothidea* by SA pathway (Han et al. 2019). So SA-responsive genes, JA-responsive genes and *OsWRKY45* (a positive regulator in response to fungal pathogen) were selected. As showed in Figure 6, the expression level of *OsWRKY45* decreased in BMV:Os06g13870-infiltrated plants while increased in BMV:Os04g34030- and BMV:Os02g33590-infiltrated plants. The expression levels of JA-responsive genes (*OsLOX1* and *OsPR3*) and SA-responsive genes (*OsNH1* and *OsPR1a*) all decreased in BMV:Os06g13870-infiltrated plants when compared with control while increased in BMV:Os04g34030- and BMV:Os02g33590-infiltrated plants. These results indicate that Os06g13870, Os04g34030 and Os02g33590 involved in the response to *M. grisea* maybe through SA and JA/ET pathways.

Drought and cold are major abiotic stresses which seriously affect plant growth and productivity. E3 ubiquitin ligases have been reported to involve in the response to drought stress, positively or negatively. OsiSAP7 negatively regulated ABA stress signaling and imparted sensitivity to drought stress in *Arabidopsis* (Sharma et al. 2015). AIRP1 positively regulated the response to drought. Its overexpression plants increased stomatal closure, ROS accumulation, the expression of drought-responsive genes and ABA-responsive bZIP transcript factor (Ryu et al. 2010). *SDIR1* positively regulated the ABA pathway. The loss-function mutants were less sensitive to ABA and the overexpression plants increased the stomatal closure and the resistance to drought in *Arabidopsis* (Zhang et al. 2007). Similarly, the overexpression *RHA2a/RHA2b* plants were highly sensitive to ABA, increased the stomatal closure, decreased the loss of water, and increased the resistance to drought (Bu et al. 2009, Li et al. 2011). Wheat TaPUB1 positively regulated the tolerance to drought stress by improving antioxidant capability (Zhang et al. 2017). Our study showed that the expression levels of *Os06g34390* and *Os02g3359*0 were induced by drought stress (Figure 3a). Os06g34390 (OsATL17) positively regulated tolerance to drought stress (Figure 7), consistent with previous report that AtATL78 act as positive regulators of drought stress (Trujillo et al. 2008; Cho et al. 2008). While Os02g33590 negatively regulated tolerance to drought stress (Figure 8), consistent with previous report that AtPUB22, 23 and 24 act as negative regulators of drought stress (Trujillo et al. 2008; Cho et al. 2008). In order to explore the reason for the changed tolerance to drought stress caused by the silencing of *Os06g34390* or *Os02g3359*0, proline content, sugar content and the expression levels of drought-responsive genes were analyzed. Proline content and sugar content in BMV:Os06g34390-infiltrated plants decreased after drought stress when compared with control (Figure 7d-e) while increased in BMV: Os02g33590-infiltrated plants (Figure 8d-e). The expression levels of drought-responsive genes increased in BMV:Os02g33590-infiltrated plants while decreased in BMV:Os06g34390- infiltrated plants when compared with control (Figure 9). These results showed that *Os06g34390* or *Os02g3359*0 have functions on tolerance to drought stress maybe through regulating proline content, sugar content and the expression levels of drought-responsive genes.

E3 ubiquitin ligases also have known to involve in the tolerance to cold stress. AtATL78 and AtATL80 negatively regulated the tolerance to cold stress in *Arabidopsis* (Lee et al. 2001; Kim S and Kim W 2013; Suh and Kim 2015). OsDIRP1 positively regulated the tolerance to cold stress in rice (Cui et al. 2018). In our study, we found the expression level of *Os05g01940* was induced by cold stress (Figure 3b). And the BMV:Os05g01940-infiltrated seedlings showed decreased resistance to cold resistance when compared with control (Figure 10a), with lower survival rate and chlorophyll content (Figure 10b and 10e), but higher MDA content and electrolyte leakage (Figure 10c-d). And the expression levels of cold-responsive genes in BMV:Os05g01940-infiltrated seedlings were all downregulated when compared with control. These results indicate that Os05g01940 regulates the tolerance to cold stress maybe through MDA content and the expression levels of cold responsive genes.

ABA is a critical signaling mediator which regulated diverse biological processes in various organisms (Kumar et al. 2019). The pathways of plants regulating response to abiotic stress have two, one is ABA-dependent, and the other is ABA-independent. E3 ubiquitin ligases regulate the tolerance to abiotic stressed dependent or independent on ABA. AtARRE negatively regulates ABA signaling in *Arabidopsis thaliana* (Wang et al. 2018). *PeCHYR1* elevates the tolerance to drought stress by ABA-induced stomatal closure via ROS production in *Populus euphratica* (He et al. 2018). Rma1H1 responded to drought by mediating the ubiquitination of water channel protein isoenzyme PIP2;1 to downregulate the expression of water channel protein, independent on ABA (Lee et al. 2009, Son et al. 2009, Bae et al. 2011). Our results showed that, the expression levels of *Os06g34390* and *Os05g01940* were induced by ABA while the expression level of *Os02g33590* not (Figure 2c). This may indicated that *Os06g34390* and *Os05g01940* regulated the response to abiotic stress dependent on ABA, while *Os02g33590* regulated the response to abiotic stress independent on ABA.

E3 ubiquitin ligases have been reported to have function on the response to heat stress. AtSAP5 had function in heat stress tolerance (Kim et al. 2015). *AtPPRT1* increased the tolerance to heat stress in *Arabidopsis* (Liu et al. 2020). SlSIZ1 positively regulated the tolerance to heat stress in tomato (Zhang et al. 2018). HTD1 negatively regulated thermotolerance in *Arabidopsis* (Kim et al. 2014). However, in our study, we found the expression level of no E3 ubiquitin ligase gene was induced by heat stress (Figure 3c). And because of the abolishment of VIGS by high temperature, we didn't do research on function on response to heat stress. Whether these 11 E3 ubiquitin ligase genes have functions on the resistance to heat stress can be explored through transgenic lines.

Conclusion

Os06g13870 positively regulated the resistance to *M. grisea*, while Os04g34030 and Os02g33590 negatively regulated, maybe by regulating the accumulation of ROS and expression levels of defense-related genes. Os06g34390 positively regulated tolerance to drought stress while Os02g33590 negatively, maybe through regulating proline content, sugar content and drought-responsive genes' expression. Os05g01940 negatively regulated the tolerance to cold stress by regulating MDA content and cold-responsive genes' expression.

Abbreviations

M. grisea: *Magnaporthe grisea*

ROS: reactive oxygen species

MDA: malondial dehyde

B. cinerea: *Botrytis cinerea*

ABA: abscisic acid

SA: salicylic acid;

JA: jasmonic acid

ACC: 1-amino cyclopropane-1-carboxylic acid

Declarations

Acknowledgements

We thank Dr. Rongyao Cai (Zhejiang Academy of Agricultural Sciences) for providing *M. grisea*. Dayong Li (Zhejiang University) are gratefully acknowledged for technical assistance.

Author Contributions

F.S. conceived the study. H.Z. and M.J. designed the experiments. H.Z., D.Z. and L.Y. performed the experiments. H.Z. and F.S. analyzed the data. M.J. drafted the manuscript, and all authors read and approved the final manuscript.

Funding

This work was supported by Taizhou Municipal Science and Technology Project (20ny18), Science Foundation for Distinguished Young Scholars of Taizhou University (2019JQ001) and Zhejiang Provincial Natural Science Foundation of China (LY19C150004).

Availability of Data and Materials

All relevant data are provided within the article and its supplementary information files.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

All authors are consent for publication.

Competing Interests

The authors declare that they have no competing interests.

Author details

[1] College of Life Science, Taizhou University, Taizhou, Zhejiang 318000, P. R. China; [2]National Key Laboratory for Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, Zhejiang 310058, China

References

1. Ariani P, Vandelle E, Wong D, Giorgetti A, Porceddu A, Camiolo S, Polverari A (2017) Comprehensive workflow for the genome-wide identification and expression meta-analysis of the ATL E3 ubiquitin ligase gene family in grapevine. J Vis Exp 130:56626. doi: 10.3791/56626.
2. Bae H, Kim SK, Cho SK, Kang BG, Kim WT (2011) Overexpression of OsRDCP1, a rice RING domain-containing E3 ubiquitin ligase, increased tolerance to drought stress in rice (*Oryza sativa* L.). Plant Sci 180:775-782.
3. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207.
4. Berrocal-Lobo M, Stone S, Yang X, Antico J, Callis J, Ramonell KM, Somerville S (2010) ATL9, a RING zinc finger protein with E3 ubiquitin ligase activity implicated in chitin- and NADPH oxidase-mediated defense responses. PLoS One 5:e14426.
5. Bhaskar S, Joemar T (2020) Genome-wide analysis of the U-box E3 ubiquitin ligase enzyme gene family in tomato. Sci Rep 10(1):9581.
6. Bu Q, Li H, Zhao Q, Jiang H, Zhai Q, Zhang J, Wu X, Sun J, Xie Q, Wang D, Li C (2009) The *Arabidopsis* RING finger E3 ligase RHA2a is a novel positive regulator of abscisic acid signaling during seed germination and early seedling development. Plant Physiol 150:463-481. doi: 10.1104/pp.109.135269.
7. Chapagain S, Park YC, Kim JH, Jang CS (2018) *Oryza sativa* salt-induced RING E3 ligase 2 (OsSIRP2) acts as a positive regulator of transketolase in plant response to salinity and osmotic stress. Planta 247(4):925-939.
8. Chen SC, Zhao HJ, Wang MM, Li JD, Wang ZH, Wang FH, Liu AR, Ahammed G (2017) Overexpression of E3 ubiquitin ligase gene *AdBiL* contributes to resistance against chilling stress and leaf mold disease in tomato. Front Plant Sci 8:1109. doi: 10.3389/fpls.2017.01109. eCollection 2017.
9. Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano F, Ponce M, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signaling. *Nature*, **448**, 666-671. doi: 10.1038/nature06006. Epub 2007 Jul 18.

10. Cho SK, Ryu MY, Song C, Kwak JM, Kim WT (2008) *Arabidopsis* PUB22 and PUB23 are homologous U-box E3 ubiquitin ligases that play combinatory roles in response to drought stress. The Plant Cell **20**:1899-1914.
11. Cui LH, Min HJ, Byun MY, Oh HG, Kim WT (2018) OsDIRP1, a putative RING E3 ligase, plays an opposite role in drought and cold stress responses as a negative and positive factor, respectively, in rice (*Oryza sativa* L.) Front Plant Sci 9:1797.
12. Deng F, Guo T, Lefebvre M, Scaglione S, Antico CJ, Jing T, Yang X, Shan W, Ramonell K (2017) Expression and regulation of ATL9, an E3 ubiquitin ligase involved in plant defense. PLoS One 12(11): e0188458. doi: 10.1371/journal.pone.0188458. eCollection 2017.
13. Devoto A, Nieto-Rostro M, Xie D, Ellis C, Harmston R, Patrick E, Davis J, Sherratt L, Coleman M, Turner J (2002) COI1 links jasmonate signaling and fertility to the SCF ubiquitin-ligase complex in *Arabidopsis*. Plant J 32:457-466. doi: 10.1046/j.1365-313x.2002.01432.x.
14. Dhawan R, Luo H, Foerster AM, Abuqamar S, Du HN, Briggs SD, Scheid O, Mengiste T (2009) HISTONE MONOUBIQUITINATION1 interacts with a subunit of the mediator complex and regulates defense against necrotrophic fungal pathogens in *Arabidopsis*. Plant Cell 21:1000-1019. doi: 10.1105/tpc.108.062364.
15. Dong CH, Agarwal M, Zhang Y, Xie Q, Zhu JK (2006) The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. Proc Natl Acad Sci USA 103:8281-8286.
16. Gonzalez LR, Tsitsigiannis DI, Ludwig AA, Panicot M, Shirasu K, Jones JD (2006) The U-box protein CMPG1 is required for efficient activation of defense mechanisms triggered by multiple resistance genes in tobacco and tomato. Plant Cell 18(4): 1067-1083.
17. Han PL, Dong YH, Gu KD, Yu JQ, Hu DG, Hao YL (2019) The apple U-box E3 ubiquitin ligase MdPUB29 contributes to activate plant immune response to the fungal pathogen *Botryosphaeria dothidea*. Planta 249(4):1177-1188.
18. He F, Wang HL, Li HG, Su Y, Li S, Yang Y, Feng CH, Yin W, Xia X (2018) PeCHYR1, a ubiquitin E3 ligase from *Populus euphratica*, enhances drought tolerance via ABA-induced stomatal closure by ROS production in *Populus*. Plant Biotechnol J 16(8):1514-1528.
19. He Q, McLellan H, Boevink PC, Sadanandom A, Xie C, Birch PR, Tian Z (2015) U-box E3 ubiquitin ligase PUB17 acts in the nucleus to promote specific immune pathways triggered by *Phytophthora infestans*. J Exp Bot 66:3189-3199.
20. Hondo D, Hase S, Kanayama Y, Yoshikawa N, Takenaka S, Takahashi H (2007) The LeATL6-associated ubiquitin/proteasome system may contribute to fungal elicitor-activated defense response via the jasmonic acid-dependent signaling pathway in tomato. Mol Plant-Microbe Interact 20:72-81.
21. Hong Y, Zhang, H, Huang L, Li D, Song F (2016) Overexpression of a stress-responsive NAC transcription factor gene ONAC022 improves drought and salt tolerance in rice. Front Plant Sci 7:4-22.

22. Huang L, Hong Y, Zhang H, Li D, Song F (2016) Rice NAC transcription factor ONAC095 plays opposite roles in drought and cold stress tolerance. BMC Plant Biol 16:203.
23. Joo H, Lim CW, Lee SC (2016) Identification and functional expression of the pepper RING type E3 ligase, CaDTR1, involved in drought stress tolerance via ABA-mediated signaling. Sci Rep 6:30097.
24. Kelley DR (2018) E3 ubiquitin ligases: key regulators of hormone signaling in plants. Mol Cell Proteomics 17(6):1047-1054.
25. Kim GD, Cho YH, Yoo SD (2015) Regulatory functions of evolutionarily conserved AN1/A20-like Zinc finger family proteins in *Arabidopsis* stress responses under high temperature. Biochem Biophys Res Commun 457:213-220.
26. Kim SH, Lee JH, Seo KI, Ryu B, Sung Y,Chung T, Deng XW, Lee J (2014) Characterization of a novel DWD protein that participates in heat stress response in *Arabidopsis*. Mol Cells 37:833-840. doi: 10.14348/molcells.2014.0224.
27. Kim SJ, Kim WT (2013) Suppression of *Arabidopsis* RING E3 ubiquitin ligase AtATL78 increases tolerance to cold stress and decreases tolerance to drought stress. FEBS Lett 587(16):2584-2590.
28. Ko JH, Yang SH, Han KH (2006) Upregulation of an *Arabidopsis* RING-H2 gene, *XERICO*, confers drought tolerance through increased abscisic acid biosynthesis. Plant J 47:343-355.
29. Kumar M, Kesawat MS, Ali A, Lee SC, Gill SS, Kim AH (2019) Integration of abscisic acid signaling with other signaling pathways in plant stress responses and development. Plants (Basel) 8(12):592.
30. Lee H, Xiong L, Gong Z, Ishitani M, Stevenson B, Zhu JK (2001) The *Arabidopsis HOS1* gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleo-cytoplasmic partitioning. Genes Dev 15:912-924.
31. Lee HK, Cho SK, Son O, Xu Z, Hwang I, Kim WT (2009) Drought stress-induced Rma1H1, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic *Arabidopsis* plants. Plant Cell 21: 622-641.
32. Lehmann M, Schwarzlander M, Obata T, Sirikantaramas S, Burow M, Olsen CE, Tohge T, Fricker M, Lindberg B, Fernie A, Sweetlove L, Laxa M (2009) The metabolic response of *Arabidopsis* roots to oxidative stress is distinct from that of heterotrophic cells in culture and highlights a complex relationship between the levels of transcripts, metabolites, and flux. Mol Plant 2: 390-406. doi: 10.1093/mp/ssn080.
33. Lehmann S, Serrano M, Haridon FL, Tjamos SE, Metraux JP (2015) Reactive oxygen species and plant resistance to fungal pathogens. Phytochemistry 112:54-62.
34. Li H, Jiang H, Bu Q, Zhao Q, Sun J, Xie Q, Li C. (2011) The *Arabidopsis* RING finger E3 ligase RHA2b acts additively with RHA2a in regulating abscisic acid signaling and drought response. Plant Physiol 156:550-563.
35. Lim CW, Park C, Kim JH, Joo H, Hong E, Lee SC (2017) Pepper CaREL1, a ubiquitin E3 ligase, regulates drought tolerance via the ABA-signaling pathway. Sci Rep 7(1): 477.
36. Liu Y, Xiao SY, Sun HR, Pei LS, Liu YY, Peng L, Gao XM, Liu Y, Wang J. (2020) AtPPRT1, an E3 ubiquitin ligase, enhances the thermotolerance in *Arabidopsis*. Plants (Basel) 9(9):1074. doi:

10.3390/plants9091074.

37. Lu XK, Shu N, Wang DL, Wang JJ, Chen XG, Zhang BL, Wang S, Guo L, Chen C, Ye W (2020) Genome-wide identification and expression analysis of PUB genes in cotton. BMC Genomics 21(1):213.
38. Luo H, Song F, Zheng Z (2005) Overexpression in transgenic tobacco reveals different roles for the rice homeodomain gene OsBIHD1 in biotic and abiotic stress responses. J Exp Bot 56:2673-2682.
39. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490-498.
40. Morreale FE, Walden H (2016). Types of ubiquitin ligases. *Cell* 165(1):248-248.
41. Ni X, Tian Z, Liu J, Song B, Xie C (2010) Cloning and molecular characterization of the potato RING finger protein gene *StRFP1*and its function in potato broad-spectrum resistance against *Phytophthorainfestans*. J Plant Physiol 167:488-496.
42. Park CH, Chen SB, Shirsekar G, Zhou B, Khang CH, Songkumarn P, Afzal AJ, Ning Y, Wang R, Bellizzi M, Valent B, Wang G (2012) The *Magnaporthe oryzae* effector AvrPiz-t targets the RING E3 ubiquitin ligase APIP6 to suppress pathogen-associated molecular pattern-triggered immunity in rice. Plant Cell 24(11):4748-4762. doi: 10.1105/tpc.112.105429.
43. Pickart CM, Eddins MJ (2004) Ubiquitin: structures, functions, mechanisms. Biochim Biophys Acta 1695:55-72.
44. Prasad ME, Schofield A, Lyzenga, W, Liu H, Stone SL (2010) *Arabidopsis* RING E3 ligase XBAT32 regulates lateral root production through its role in ethylene biosynthesis. Plant Physiol 153:1587-1596.
45. Qi J, Song CP, Wang BS, Zhou JM, Kangasjärvi J, Zhu JK, Gong ZZ (2018) Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. J Integr Plant Biol 60(9):805-826.
46. Qin F, Sakuma Y, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Fujita M, Umezawa T, Sawano Y, Miyazono K, Tanokura M, Shinozaki K, Yamaguchi-Shinozaki K (2008) *Arabidopsis* DREB2A-interacting proteins function as RING E3 ligases and negatively regulate plant drought stress-responsive gene expression. Plant Cell 20:1693-1707. doi: 10.1105/tpc.107.057380.
47. Qin Q, Wang YX, Huang LY, Du FP, Zhao XQ, Li ZK, Wang WS, Fu YB (2020) A U-box E3 ubiquitin ligase OsPUB67 is positively involved in drought tolerance in rice. Plant Mol Biol 102(1-2):89-107. doi: 10.1007/s11103-019-00933-8.
48. Ren C, Pan J, Peng W, Genschik P, Hobbie L, Hellmann H, Estelle M, Gao B, Peng J, Sun C, Xie D (2005) Point mutations in *Arabidopsis* Cullin1 reveal its essential role in jasmonate response. Plant J 42:514-524. doi: 10.1111/j.1365-313X.2005.02394.x.
49. Ryu MY, Cho SK, Kim WT (2010) The *Arabidopsis* C3H2C3-type RING E3 ubiquitin ligase AtAIRP1 is a positive regulator of an abscisic acid-dependent response to drought stress. Plant Physiol 154:1983-1997.
50. Segal LM, Wilson RA (2018) Reactive oxygen species metabolism and plant-fungal interactions. Fungal Genet Biol 110:1-9.

51. Serrano M, Guzman P (2004) Isolation and gene expression analysis of *Arabidopsis thaliana* mutants with constitutive expression of *ATL2*, an early elicitor-response RING-H2 zinc-finger gene. Genetics 167:919-929.
52. Sharma G, Giri J, Tyagi AK (2015) Rice OsiSAP7 negatively regulates ABA stress signaling and imparts sensitivity to water-deficit stress in *Arabidopsis*. Plant Sci 237:80-92.
53. Son O, Cho SK, Kim EY, Kim WT (2009) Characterization of three *Arabidopsis* homologs of human RING membrane anchor E3 ubiquitin ligase. Plant Cell Rep 28:561-569.
54. Stone SL, Williams LA, Farmer LM, Vierstra RD, Callis J (2006) KEEP ON GOING, a RING E3 ligase essential for *Arabidopsis* growth and development, is involved in abscisic acid signaling. Plant Cell 18:3415-3428.
55. Suh JY, Kim WT (2015) *Arabidopsis* RING E3 ubiquitin ligase AtATL80 is negatively involved in phosphate mobilization and cold stress response in sufficient phosphate growth conditions. Biochem Biophys Res Commun 463:793-799.
56. Sun JH, Sun YH, Ahmed RI, Ren A, Xie MM (2019) Research progress on plant RING-finger proteins. Genes (Basel) 10(12):973.
57. Tal L, Gil M, Guercio A, Shabek N (2020) Structural aspects of plant hormone signal perception and regulation by ubiquitin ligases. Plant Physiol 182(4):1537-1544.
58. Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He S, Howe G, Browse J (2007) JAZ repressor proteins are targets of the SCF (COI1) complex during jasmonate signaling. Nature 448:661-665. doi: 10.1038/nature05960.
59. Trujillo M, Ichimura K, Casais C, Shirasu K (2008) Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in *Arabidopsis*. Current Biology 18:1396-1401.
60. Verma V, Ravindran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. BMC Plant Biol 16:86.
61. Wang B, Li C, Kong X, Li Y, Liu Z, Wang J, Li X, Yang Y (2018) AtARRE, an E3 ubiquitin ligase, negatively regulates ABA signaling in *Arabidopsis thaliana*. Plant Cell Rep 37(9):1269-1278. doi: 10.1007/s00299-018-2311-8.
62. Wang F, Deng XW (2011) Plant ubiquitin-proteasome pathway and its role in gibberellin signaling. Cell Res 21:1286-1294.
63. Wang T, Chang C, Gu C, Tang S, Xie Q, Shen Q (2016) An E3 ligase affects the NLR receptor stability and immunity to *Powdery Mildew*. Plant Physiol 172(4):2504-2515.
64. Wang X, Ding YL, Li ZY, Shi YT, Wang JL, Hua J, Gong ZZ, Zhou JM, Yang SH (2019) PUB25 and PUB26 promote plant freezing tolerance by degrading the cold signaling negative regulator MYB15. Dev Cell 51(2):222-235. doi: 10.1016/j.devcel. 2019.08.008.
65. Waszczak C, Carmody M, Kangasjärvi J (2018) Reactive oxygen species in plant signaling. Annu Rev Plant Biol 69:209-236.

66. Wu H, Ye H, Yao R, Zhang T, Xiong L (2015) OsJAZ9 acts as a transcriptional regulator in jasmonate signaling and modulates salt stress tolerance in rice. Plant Sci 232:1-12.
67. Xu L, Liu F, Lechner E, Genschik P, Crosby W, Ma H, Peng W, Huang D, Xie D (2002) The SCF (COI1) ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. Plant Cell 14:1919-1935. doi: 10.1105/tpc.003368.
68. Yaeno, T., and Iba, K. (2008) BAH1/NLA, a RING-type ubiquitin E3 ligase, regulates the accumulation of salicylic acid and immune responses to *Pseudomonas syringae* DC3000. *Plant Physiol.* **148**, 1032-1041.
69. Yang L, Liu Q, Liu Z, Yang H, Wang J, Li X, Yang Y (2016) *Arabidopsis* C3HC4-RING finger E3 ubiquitin ligase AtAIRP4 positively regulates stress-responsive abscisic acid signaling. J Integr Plant Biol 58:67-80.
70. Yin ZC, Chen J, Zeng LR, Goh ML, Leung H, Khush GS, Wang GL (2000) Characterizing rice lesion mimic mutants and identifying a mutant with broad-spectrum resistance to rice blast and bacterial blight. Molecular Plant-Microbe Interactions 13:869-876.
71. You Q, Zhai K, Yang D, Yang W, Wu J, Liu J, Pan W, Wang J, Zhu X, Jian Y, Liu J, Zhang Y, Deng Y, Li Qun, Lou Y, Xie Q, He Z (2016) An E3 ubiquitin ligase-BAG protein module controls plant innate immunity and broad-spectrum disease resistance. Cell Host Microbe 20:758-769. doi: 10.1016/j.chom.2016.10.023.
72. Zeng LR, Qu S, Bordeos A, Yang C, Baraoidan M, Yan H, Xie Q, Nahm B, Leung H, Wang G (2004) Spotted leaf 11, a negative regulator of plant cell death and defence, encodes a U-box/armadillo repeat protein endowed with E3 ubiquitin ligase activity. The Plant Cell 16:2795-2808. doi: 10.1105/tpc.104.025171.
73. Zhang S, Wang SJ, Lv JL, Liu ZB, Wang Y, Ma N, Meng QW (2018) SUMO E3 ligase SlSIZ1 facilitates heat tolerance in tomato. Plant Cell Physiol 59(1):58-71.
74. Zhang CY, Hao ZY, Ning YS, Wang GL (2019) SINA E3 ubiquitin ligases: versatile moderators of plant growth and stress response. Mol Plant 12(5):610-612.
75. Zhang GQ, Zhang M, Zhao ZX, Ren YQ, Li QX, Wang W (2017) Wheat TaPUB1 modulates plant drought stress resistance by improving antioxidant capability. Sci Rep 7(1):7549.
76. Zhang H, Liu J, He F, Wang Z, Ning Y, Wang GL (2015) OsHUB1 and OsHUB2 interact with SPIN6 and form homo- and hetero-dimers in rice. Plant Signal Behav 10:e1039212.
77. Zhang X, Garreton V, Chua NH (2005) The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation. Genes Dev 19:1532-1543.
78. Zhang Y, Yang C, Li Y, Zheng N, Chen H, Zhao Q, Gao T, Guo H, Xie Q (2007) SDIR1 is a RING finger E3 ligase that positively regulates stress-responsive abscisic acid signaling in *Arabidopsis*. Plant Cell 19:1912-1929. doi: 10.1105/tpc.106.048488.
79. Zhou B, Zeng L (2018) The tomato U-Box type E3 ligase PUB13 acts with group III ubiquitin E2 enzymes to modulate FLS2-mediated immune signaling. Front Plant Sci 9:615.

Tables

Table 1 The list of primer sequences used in this study

Primers	Sequences (5’-3’)
Os06g13870-qRT-F	CCAGAGATATCGTTGCTGAGAC
Os06g13870-qRT-R	GATCGGGCACACGAAGTT
Os06g34390-qRT-F	AAGCGGCGATCATCAACTAC
Os06g34390-qRT-R	CGACCGCACAACCACAA
Os04g34030-qRT-F	CGAGGGAGATGCTCAAGATG
Os04g34030-qRT-R	GAGGGTAGGAAGCATTCAAGT
Os02g33590-qRT-F	CCTCGGCAAGGACAATGG
Os02g33590-qRT-R	TTCAGGAGGAGGATGGCATA
Os04g34140-qRT-F	CGATGCCTCTGTCACTTCTT
Os04g34140-qRT-R	GCGTTCTTCTCGAACTCGT
Os09g32690-qRT-F	GGCAGTAGTTGATCTGGAAGTAG
Os09g32690-qRT-R	TCTGGAGAGAGGCAGTGTATAA
Os09g29310-qRT-F	CTACTACGCGACCAACTTCAG
Os09g29310-qRT-R	GAAGAAGCCGAGGAAGAAGAA
Os02g46100-qRT-F	CGAACAAGGGCGTCAAGA
Os02g46100-qRT-R	GAACTCCACGAGGCAGATG
Os05g01940-qRT-F	GCATCTTCCGCAATGTGTTC
Os05g01940-qRT-R	TCAGACGCATCGTTCAACTC
Os06g09310-qRT-F	CCGTTGGTGGTGAGCAA
Os06g09310-qRT-R	TCTCTTGGCGTAGAGGTAGAG
Os01g64570-qRT-F	GGGTGAAGACCAAGGAGAAG
Os01g64570-qRT-R	TGGGTAAAGCGCCAAGAA
Os06g13870-vigs-F	ATACCTAGG GCGCTCACGGTGTTCTTCCC
Os06g13870-vigs-R	TATCCATGG TCGCTCGTCCCCTTGTCGG
Os06g34390-vigs-F	TATCCATGG GACGACGACGACCACCACCA
Os06g34390-vigs-R	ATACCTAGGCCACCACCTTCCCTTGACAGC
Os04g34030-vigs-F	ATACCTAGG GCGGGAGGAGCTGATGGCT
Os04g34030-vigs-R	TATCCATGG TTCCCTGCATCCGCGAGA

Os02g33590-vigs-F	ATACCTAGG GCTCATCCAGGCGTGGTGC
Os02g33590-vigs-R	TATCCATGGCGGACGGCTTGAGGGAGTAGA
Os04g34140-vigs-F	ATACCTAGG CTCCGCAGCCTCATCTCCCA
Os04g34140-vigs-R	TATCCATGG CGCCTCTTGTTCGCGTCCTC
Os09g32690-vigs-F	ATACCTAGG GCCTGTGGCAGTAGTTGA
Os09g32690-vigs-R	TATCCATGG AAGGTAAATACGGTGGAAAT
Os09g29310-vigs-F	ATACCTAGGCCTCATGCTTCTCCTCCTGCTC
Os09g29310-vigs-R	TATCCATGG CGCCCTTGACGGACTTGTGC
Os02g46100-vigs-F	ATACCTAGG AACAAGGGCGTCAAGAAGGA
Os02g46100-vigs-R	TATCCATGG ACGAGCACGCGGCGGCACGA
Os05g01940-vigs-F	ATACCTAGGGCAGCCACATCTACCACCAGG
Os05g01940-vigs-R	TATCCATGGGCGACCAAAGCACGAGAACAC
Os06g09310-vigs-F	ATACCTAGG AGGAGGCGCTCGAGTGCGCG
Os06g09310-vigs-R	TATCCATGG TTGGCGACGTCGTCGTGGGC
Os01g64570-vigs-F	ATACCTAGG TGCCGTCCTACTTCGTCTGCC
Os01g64570-vigs-R	TATCCATGG TGCCCTGCTATCCTCGCACTCC
OsActin-qRT-F	A AGCTGCGGGTATCCATGAGA
OsActin-qRT-R	GCAATGCCAGGGAACATAGTG
eEF1-qRT-F	CAACCCTGACAAGATTCCCT
eEF1-qRT-R	AGTCAAGGTTGGTGGACCTC
28s rDNA-qRT-F	TACGAGAGGAACCGCTCATTCAGATAATTA
28s rDNA-qRT-R	TCAGCAGATCGTAACGATAAAGCTACTC
OsLOX1-qRT-F	AAACGCTCGCTGGCATCAAC
OsLOX1-qRT-R	ATCGCCTCCTCCACCGTCAT
OsNH1-qRT-F	GCGGCGTCTCCTTGATGTCCTT
OsNH1-qRT-R	CGAGTTGTGGGTCCCTTCTTTC
OsPR1a-qRT-F	TCGTATGCTATGCTACGTGTTT
OsPR1a-qRT-R	CACTAAGCAAATACGGCTGACA
OsPR3-qRT-F	CACATACTGCGAGCCCAA

OsPR3-qRT-R	TTGTAGGTGATCTGGATGGG
OsWRKY45-qRT-F	CGGGCAGAAGGAGATCCAAAACT
OsWRKY45-qRT-R	GCCGATGTAGGTGACCCTGTAGC
OsAP37-qRT-F	AAGTGACTCCGACTCCTCGTC
OsAP37-qRT-R	GTTCAGATCCAGATCGAAAGCT
OsbZIP23-qRT-F	GGAGCAGCAAAAGAATGAGG
OsbZIP23-qRT-F	GGTCTTCAGCTTCACCATCC
OsPP2C68-qRT-F	CGCAGCTCCGACAACATCT
OsPP2C68-qRT-R	GCTGGGTGACACTCTCTCTACAAG
OsRAB21-qRT-F	CCACGGCACCGGGATGACC
OsRAB21-qRT-R	AGCTTCTCCTTGATCTTGTCCA
OsERD1-qRT-F	ACTGTAGTATTACTTGATGAGATA
OsERD1-qRT-R	CAATATTTGATGTCATGACAAT
Myb-qRT-F	ACGGCGGTGGGATTTCTTA
Myb-qRT-R	GCGATGCGAGACCACCTGTT
CDPK7-qRT-F	AACATGCCCGATGCTTTTCTT
CDPK-qRT-R	ATTGTTCTTCGTCCGACTCCC
Fer1-qRT-F	GGGAAAGGGAAGGAGGTGCT
Fer1-qRT-R	GTAGGCGAAAAGGGAGTGGT
Trx23-qRT-F	GTTCCCTGGTGCTGTCTTCC
Trx23-qRT-R	GCTTCACGATGGTGTTCTGG
Lti6a-qRT-F	CGGCGTCTTCTTCAAGTTCG
Lti6a-qRT-R	TGAGCAGCAAGCAGATCCAG

Figures

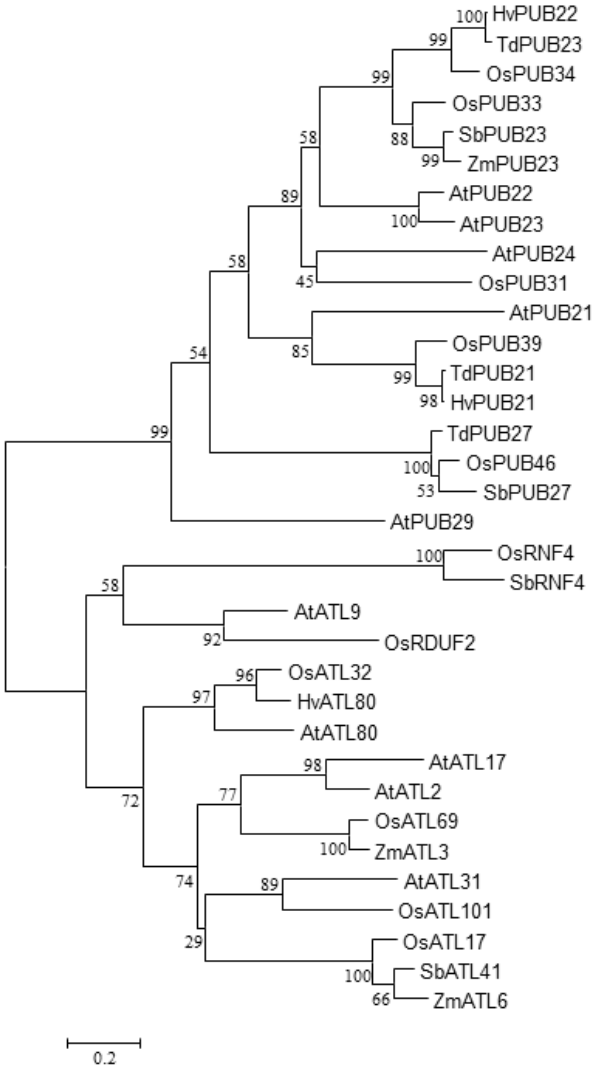

Figure 1

Phylogentic tree of 11 E3 ubiquitin ligase genes studied in this research. Phylogenetic tree was drawn by neighbor-joining method.

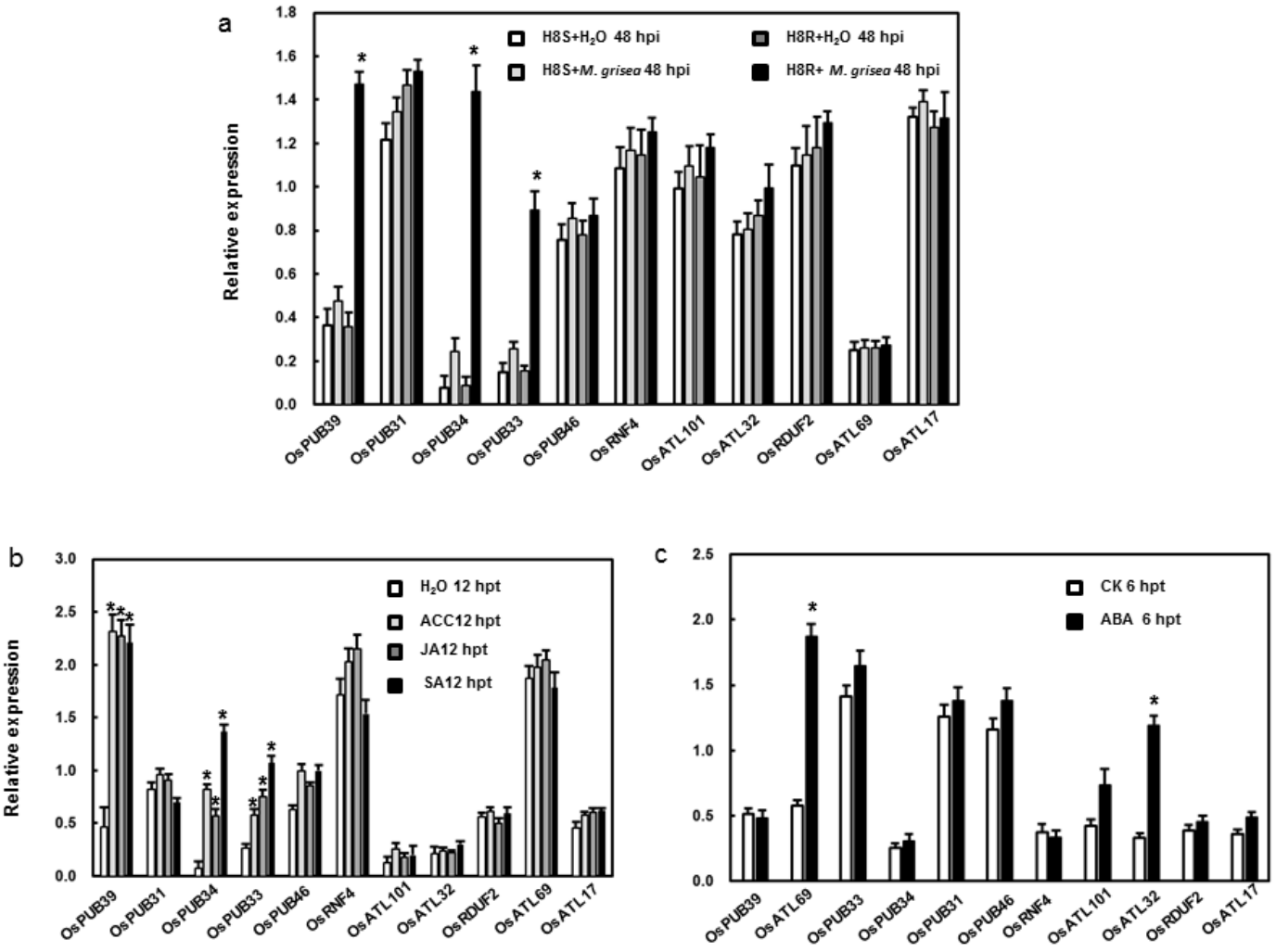

Figure 2

Expression patterns of E3 ubiquitin ligase genes in response to infection with M. grisea and hormone treatment. a Expression of E3 ubiquitin ligase genes in response to M. grisea. Leaves of H8R and H8S seedlings was sprayed with solution of M. grisea spores. b Expression of E3 ubiquitin ligase genes in response to signaling hormones. Leaves of 4-week-old cv. Yuanfengzao seedlings were treated with 1.5 mM SA, 100 μM JA and 100 μM ACC solutions by spraying, respectively. JA and ACC solution were made in 0.1% ethanol while SA in water. The same volume of 0.1% ethanol or distilled sterilized water was used as control. c Expression patterns of E3 ubiquitin ligase genes in response to ABA. Leaves of 4-week cv. Yuanfengzao seedlings were sprayed with 100 μM ABA solutions. 0.1% ethanol was used as control. The samples were harvested at specific time points for analysis of gene expression. Expression levels were shown as folds of the OsActin expression level which was uesd as a standardization. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between the plants with treatments and control.

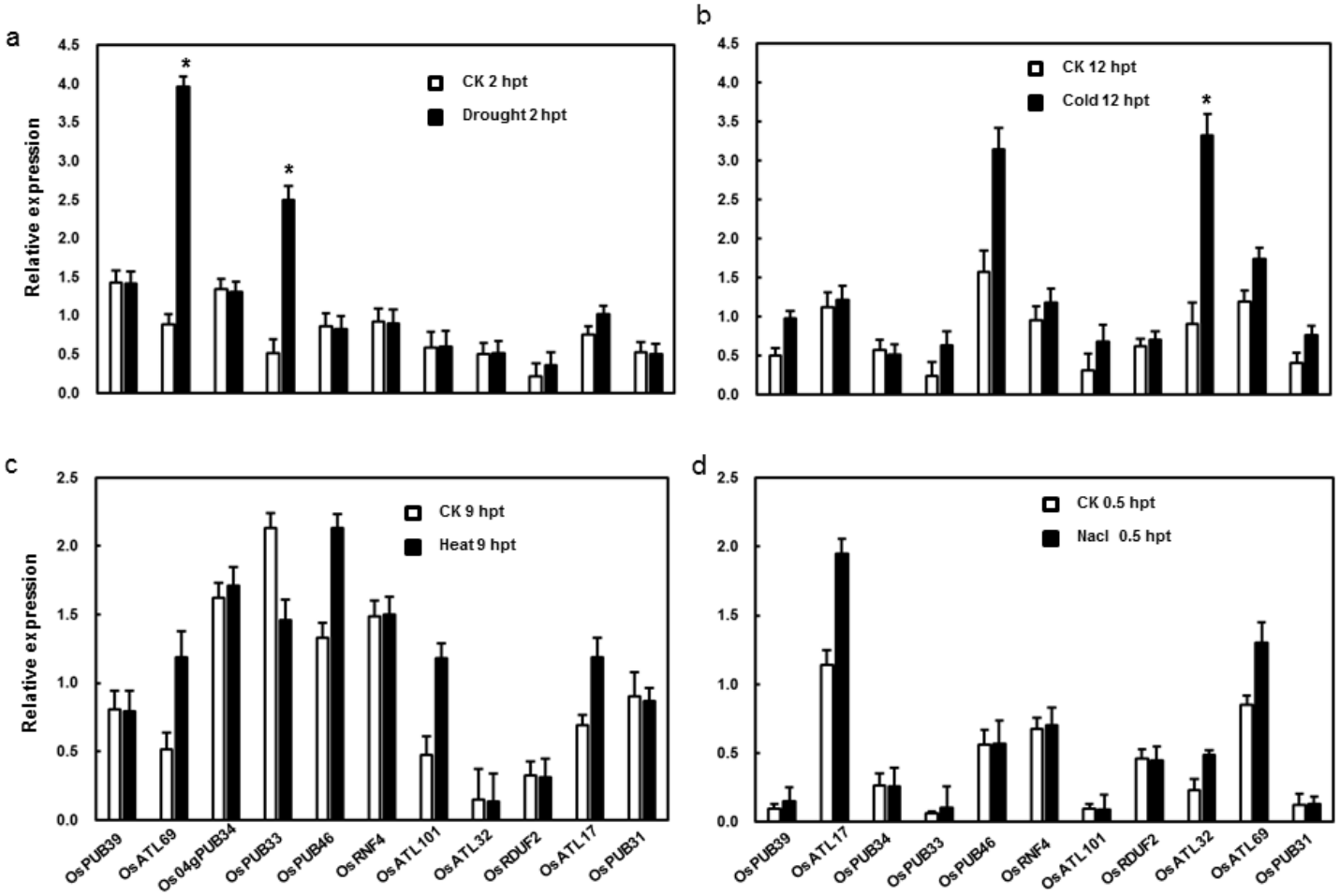

Figure 3

Expression patterns of E3 ubiquitin ligase genes in response to abiotic stress. a For drought stress, the hydroponic three-week plants were put on the frame floor in the green house after the water on the surface of root was dried by filter paper. For cold (b) and heat stress (c), three-week plants were put in climatic cabinet 4□C and 42□C. d For salt stress, the hydroponic three-week plants were put in 200mM NaCl solution. The samples were harvested at specific time points for analysis of gene expression. Expression levels were shown as folds of the OsActin expression level which was uesd as a standardization. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between the plant with and without treatments.

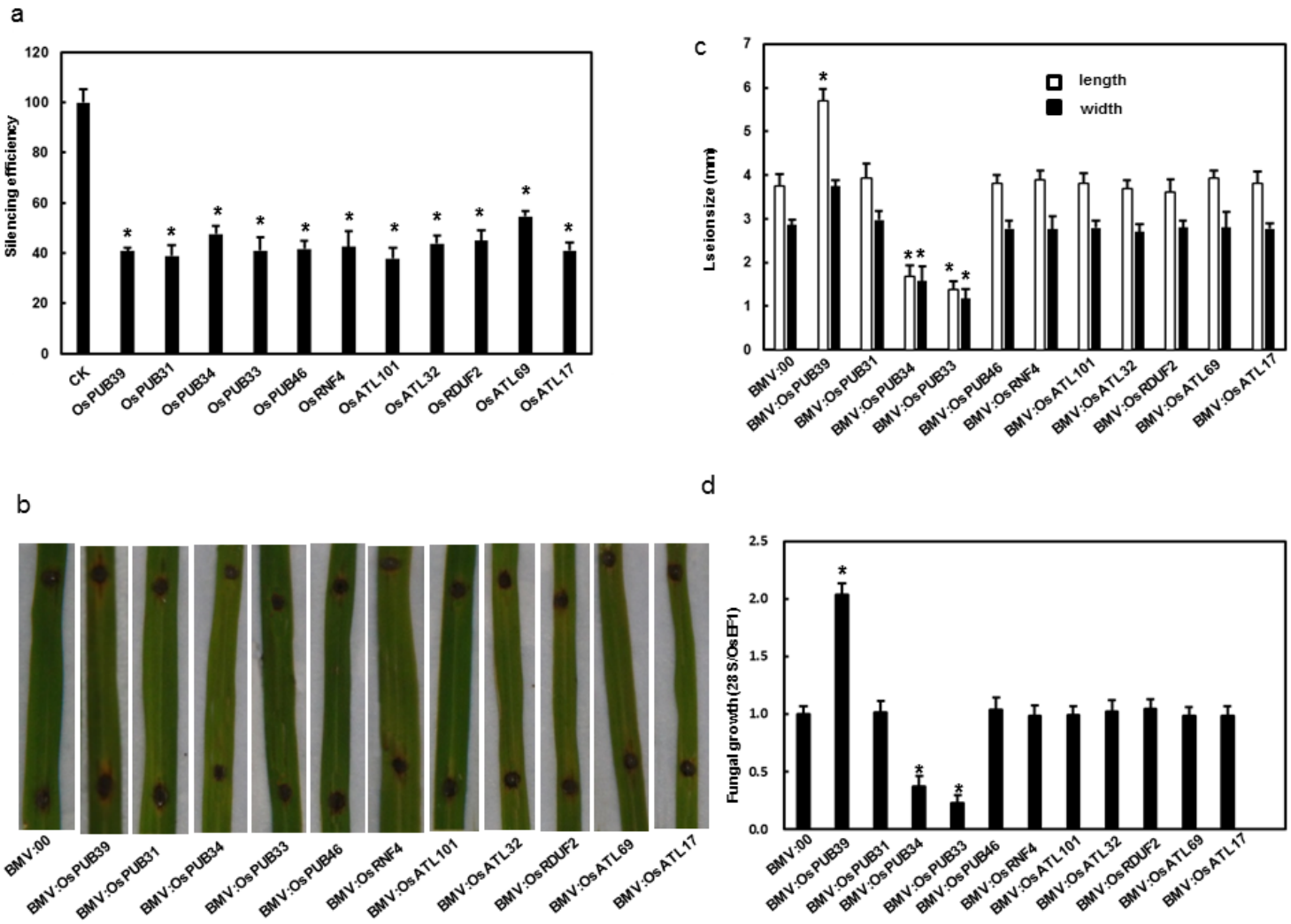

Figure 4

BMV:Os04g34030- and BMV:Os02g33590-infiltrated seedlings increased resistance to M. oryzae dramatically when compared with the control while BMV:Os06g13870-infiltrated seedlings decreased the resistance to M. oryzae. a The silencing efficiency of the BMV:target gene-infiltrated plants. b The lesions on the leaves of rice 7 days after inoculation. c The length and width of the lesions on BMV:target gene- and BMV:00-infiltrated seedlings. d The quantities of bacterial in leaves of BMV:target gene- and BMV:00-infiltrated seedlings.

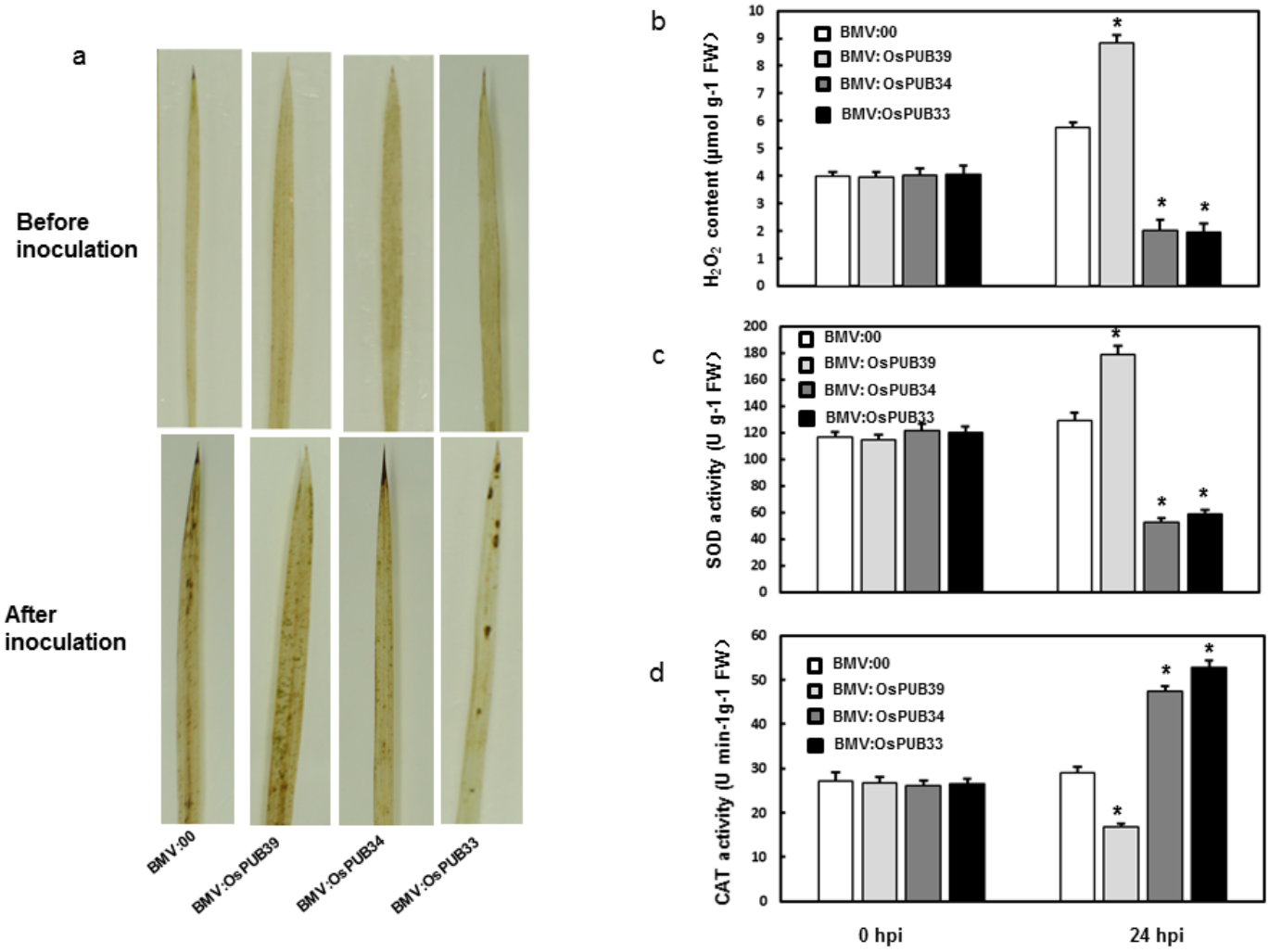

Figure 5

The ROS accumulation in BMV:target gene- and BMV:00-infiltrated seedlings before and after M. oryzae inoculation. a The DAB staining of leaves from BMV:target gene- and BMV:00-infiltrated seedlings before and after M. oryzae inoculation. b H2O2 content in BMV:target gene- and BMV:00-infiltrated seedlings before and after M. oryzae inoculation. The SOD activity (c) and CAT activity (d) in BMV:target gene- and BMV:00-infiltrated seedlings before and after M. oryzae inoculation.

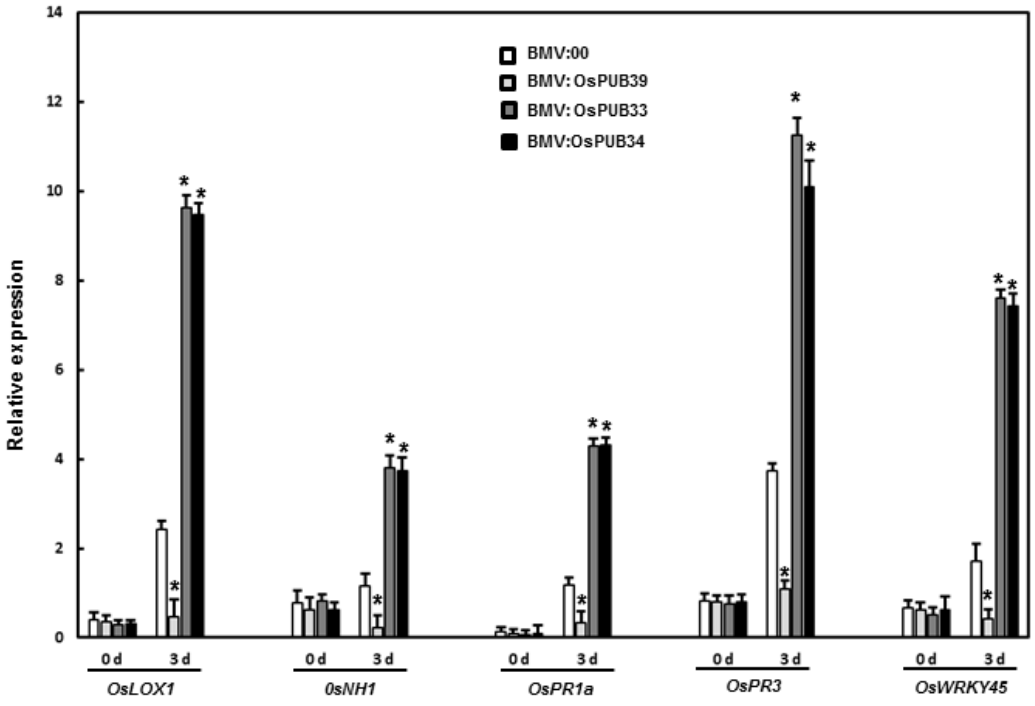

Figure 6

The expression levels of defense-related genes changed after the inoculation of M. oryzae. Expression levels of defense-related genes were shown as folds of the OsActin expression level which was uesd as a standardization. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between BMV:target gene- and BMV:00-infiltrated seedlings.

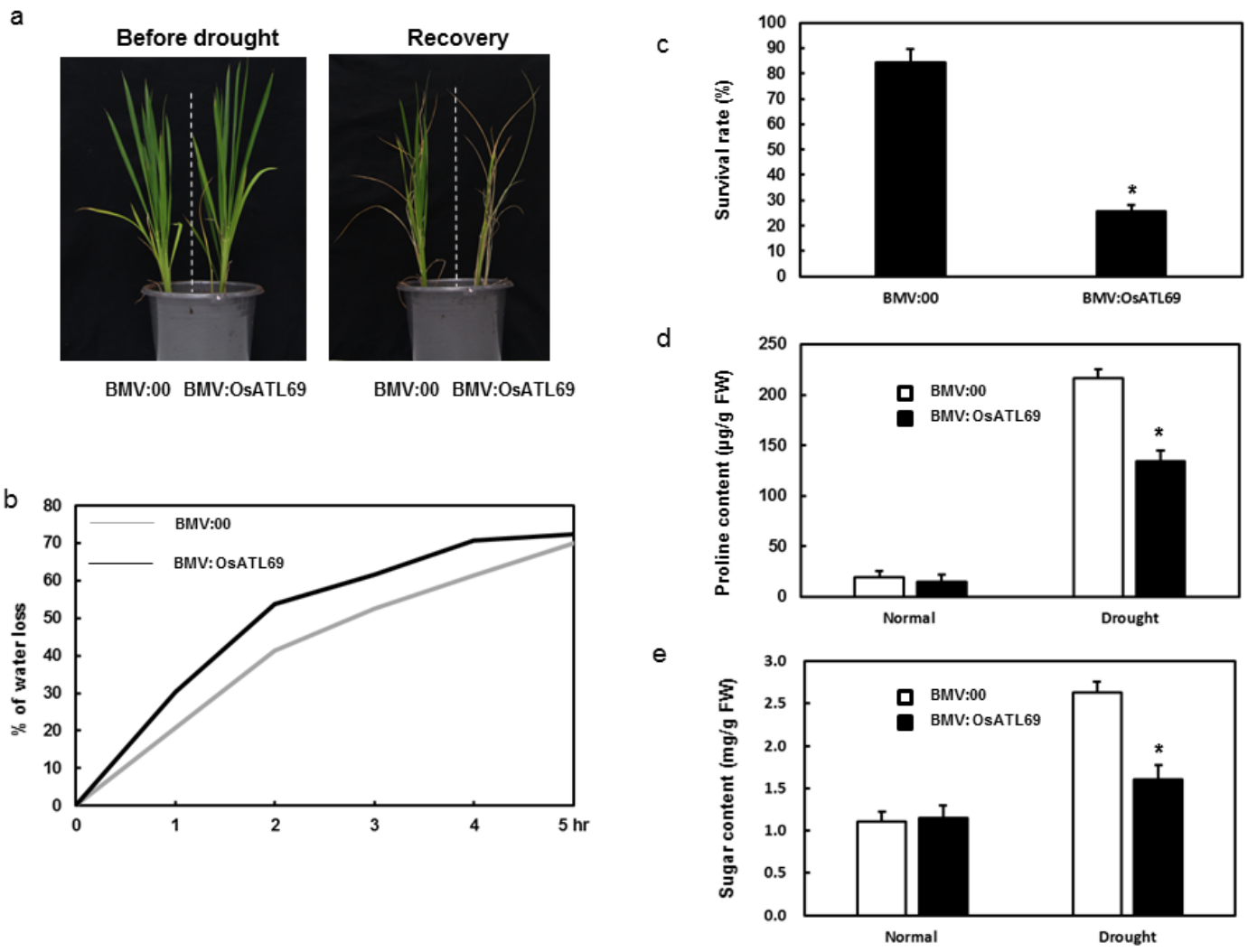

Figure 7

BMV:Os06g34390-infiltrated seedlings decreased tolerance to drought stress. The BMV:Os06g34390- and BMV:00-infiltrated seedlings in the same pot were withholded watering for 10 days and recovered with normal watering for another 12 days. a The phenotype of BMV:Os06g34390- and BMV:00-infiltrated seedlings showed when suffered from drought stress. Water loss (b) and survival rate (c) of BMV:Os06g34390- and BMV:00-infiltrated seedlings after suffered from drought stress. Proline content (d) and sugar content (e) of BMV:Os06g34390- and BMV:00-infiltrated seedlings after suffered from drought stress. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between the BMV:Os06g34390- and BMV:00-infiltrated seedlings.

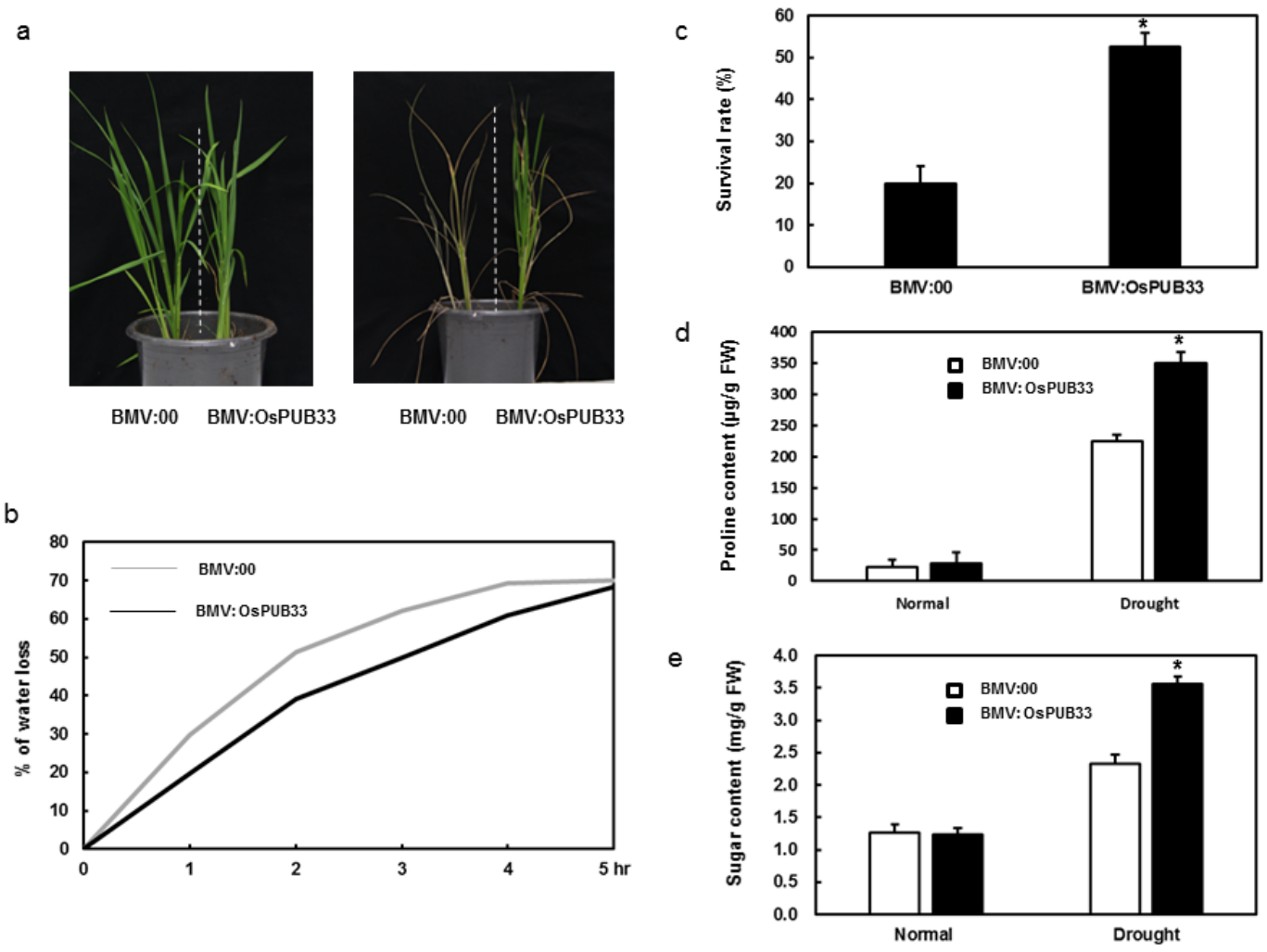

Figure 8

BMV:Os02g33590-infiltrated seedlings increased tolerance to drought stress. The BMV:Os02g33590- and BMV:00-infiltrated seedlings in the same pot were withholded watering for 15 days and recovered with normal watering for another 12 days. a The phenotype of BMV:Os02g33590- and BMV:00-infiltrated seedlings showed when suffered from drought stress. Water loss (b) and survival rate (c) of BMV:Os02g33590- and BMV:00-infiltrated seedlings after suffered from drought stress. Proline content (d) and sugar content (e) of BMV:Os02g33590- and BMV:00-infiltrated seedlings after suffered from drought stress. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between the BMV:Os02g33590- and BMV:00-infiltrated seedlings.

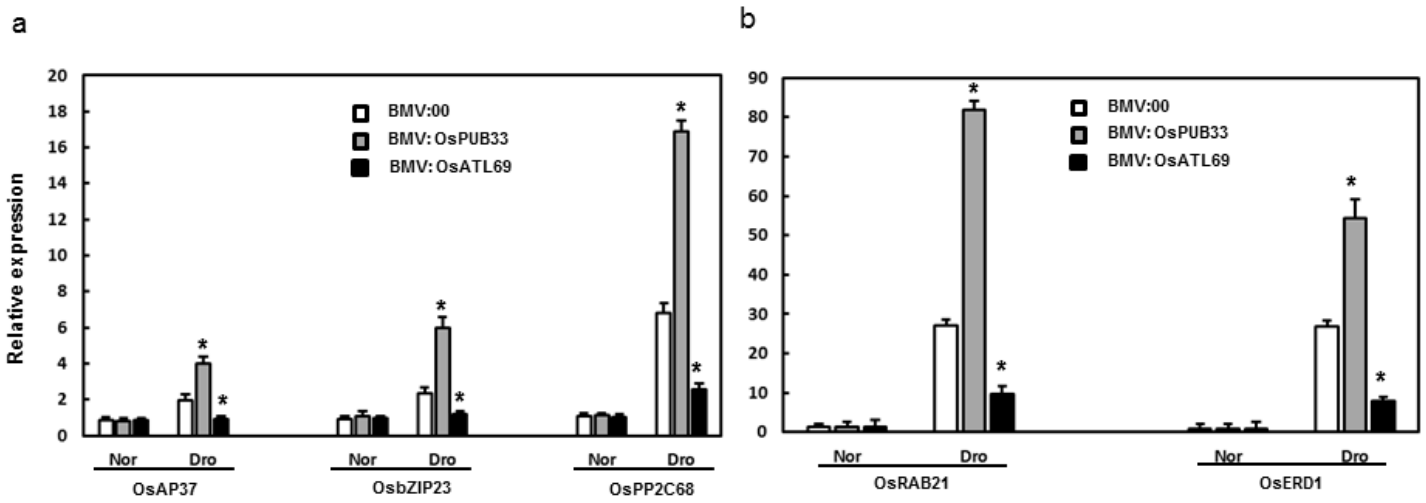

Figure 9

The expression levels of drought-responsive genes upregulated in BMV:Os02g33590-infiltrated plants while downregulated in BMV:Os06g34390- infiltrated plants when compared with control after drought stress. Expression levels were shown as folds of the OsActin expression level which was uesd as a standardization. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between BMV:target genes- and BMV:00-infiltrated plants.

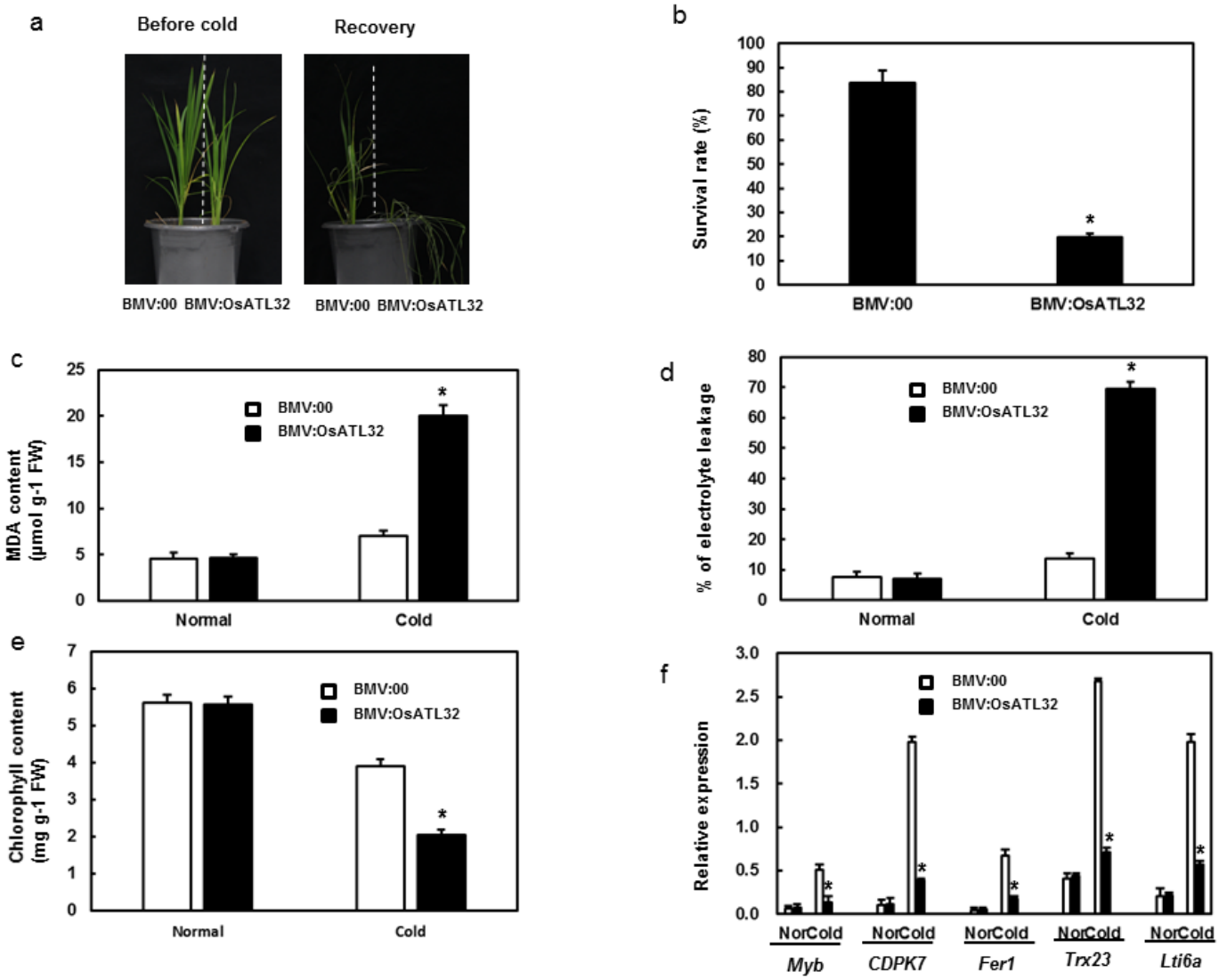

Figure 10

BMV:Os05g01940-infiltrated seedlings led to decreased tolerance to cold stress when compared with control. 4-week-old BMV:Os05g01940- and BMV:00- infiltrated seedlings in the same pot were put in a growth chamber with 4 □C for 2 days, then transferred to normal growth condition. 7 days later, the survival rate, MDA content, chlorophyll content, electrolyte leakage and expression levels of cold-responsive genes were analyzed. A The phenotype of BMV:Os05g01940- infiltrated plants when suffered from cold stress. b The survival rate were recorded. MDA content (c), electrolyte leakage (d) and chlorophyll content (e) were analyzed in BMV:Os05g01940- and BMV:00-infiltrated seedlings with and without cold stress. f Cold-responsive genes' expression in BMV:Os05g01940- and BMV:00-infiltrated seedlings with and without cold stress. Expression levels of cold-responsive genes were analyzed using qRT-PCR with gene-specific primers. Data presented are the means ± SD from three independent experiments and * above the columns indicate significant differences at $p<0.05$ level between BMV:Os05g01940- and BMV:00- infiltrated plants.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementdata.tif