Transcriptomic Profiling of Rice Reveals Positive Role of OsNCED3 in Rice Resistance Against Brown Planthopper

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Research Article

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Abstract

Background: Exogenous abscisic acid (ABA) could improve rice resistance to brown planthopper (BPH) *Nilaparvata lugens*. The 9-*cis*-epoxycarotenoid dioxygenase (NCED) is the rate-limiting enzyme for ABA biosynthesis in higher plants. In rice, *OsNCED3* gene promoted ABA synthesis and improved abiotic stress tolerance, but the function of *OsNCED3* in regulating rice defense against BPH remains unclear.

Results: In this study, the average injury level, functional plant loss index and EPG results of rice conferred that insect-resistance of OE rice lines was significantly higher than that of WT. Then transcriptome analysis of overexpression (OE), RNA interference (RNAi) and wild type (WT) of *OsNCED3* in Zhonghua11 rice lines after BPH infestation were performed. Seventeen RNA libraries were obtained from OE, RNAi and WT rice lines at 12 h post BPH infestation. The number of all differential expression genes (DEGs) between OE and WT or RNAi and WT were mostly up-regulated. These accounted for more than 75% of the total number of DEGs for each other. The number of DEGs between RNAi and WT rice lines fed by BPH increased significantly, higher than that between OE and WT, and most of these DEGs were related to the adversity stress and the biosynthesis of JA (jasmonic acid).

Conclusions: overexpression of *OsNCED3* gene could improve rice resistance to BPH.

Introduction

Due to incremented population and urbanization, food security has become one of the most important issues across the globe (Wang et al., 2018). The brown planthopper (BPH), *Nilaparvata lugens* Stål is one of the most devastating pests of rice (*Oryza sativa* L.) in Asia (Xue et al., 2010). Since 2005, BPH attacks result in millions of tons of rice yield loss every year in China (Cheng and Zhu, 2006). In rice growing areas, BPH was being controlled by application of chemical pesticides, however, the abuse of pesticides could cause serious environmental problems and the development resistance of BPH. Therefore, it is necessary to devise new control strategies and develop new and efficient green pesticides to reduce the use of chemical pesticides.

Abscisic acid (ABA) is one of the five major phytohormone which affects the growth and developmental processes of seeds such as maturity, dormancy and germination. In addition, ABA is considered as a key player in plant development and regulation of abiotic stress tor including cold, drought, salinity, waterlogging and other environmental stresses (Huang et al., 2015; Rodrigues et al., 2016). However, compared with other plant hormones, such as jasmonic acid (JA), salicylic acid (SA) and ethylene (ET), only limited research showed the pivotal role of ABA played in plant defense against insect pests (Zhou et al., 2019; Li et al., 2017). It is reported that ABA deficiency could increase the susceptibility of plants to herbivores (Thaler and Bostock, 2004; Bodenhausen and Reymond, 2007; Dinh et al., 2013). Previous studies showed that exogenous ABA enhanced the resistance of rice to BPH by promoting callose formation (Liu et al., 2014) (Liu et al., 2017). However, the molecular mechanism underlying ABA-mediated rice defense is still poorly understood.

9-*cis*-epoxycarotenoid dioxygenase (NCED) is a key enzyme in the rate-limiting step of biosynthesis of ABA in higher plants (Hussain et al., 2019). The *NCED* gene was first found in *Zea mays vp14* mutant and characterized by lower endogenous ABA contents, higher transpiration rate and reduced seeds germination rate than wild-type (WT) plants (Tan et al., 1997). Subsequently, *NCED* genes were isolated from other plants such as *Solanum lycopersicum* (Burbidge et al., 1999), *Persea americana* (Chernys and Zeevaart, 2000), *Arabidopsis thaliana* (Rock and Zeevaart, 1991; Tan et al., 2003), *Malus domestica* (Xia et al., 2014) and *Brassica napus* (Xu and Cai, 2017). In previous research reports, the *AtNCED3* of *Arabidopsis thaliana* is induced to express under drought stress, and can improve the level of endogenous ABA in *Arabidopsis thaliana* mutant, thus improving drought resistance (Jia et al., 2008). Moreover, the expression of *MeNCED3* in cassava can be significantly induced by salt stress, and ABA is synthesized in large quantities, thus positively regulating the salt tolerance of plants (Ding et al., 2016). There are five genes in rice *NCED* gene family (Ni et al., 2015). In these genes, studies have confirmed expression of *OsNCED3* in various tissues of rice under normal as well as stress conditions (Yuan et al., 2018). Salinity, PEG and H_2O_2 stress could induce its expression and indicated important role of *OsNCED3* in rice resistance to abiotic stress (Huang et al., 2018). Hwang et al (2010) reported that heterologous expression of *NCED3* in *Arabidopsis thaliana* increased ABA levels. The study also found that the expression of *OsNCED3* in rice was induced by drought stress, and was rapidly down-regulated after rehydration. Overexpression *OsNCED3* rice transgenic lines (OE) under drought stress had higher ABA levels (Xu et al., 2008). ABA improves plant resistance to drought stress, but there are relatively few reports on resistance to biotic stress. In many studies, it had been found that overexpression of *OsNCED3* significantly improved rice resistance to abiotic stress. Therefore, whether *OsNCED3* could improve rice resistance to BPH had aroused our interests.

In this study, transcriptome analysis of leaf sheaths after BPH feeding in rice seedlings was performed to find out whether *OsNCED3* was related to rice BPH-resistance, and the average injury level, functional plant loss index (FPLI) and BPH feeding behavior using EPG method were further evaluated.

Methods

Rice and insect materials

The wide type rice cultivar Zhonghua 11 (ZH11) was used in the experiment and *OsNCED3* overexpression (OE) and gene interference (RNAi) lines were provided by the College of Life Sciences of South China Agricultural University (Xu et al., 2018). Seeds were selected, soaked and germinated, and then sown in plastic boxes filled with soil (length 38 cm, width 22 cm, height 8 cm). 4-leaf seedlings were cultivated in plastic cups (diameter 5 cm, height 12 cm) and supplied with fertilizer and water. At 4 leaf stage, rice lines were used as experimental materials. BPH were collected from China Rice Research Institute (Hangzhou, China) and reared in a greenhouse at Yangzhou University.

Measurement of *OsNCED3* expression level in different rice lines

Single-plant rice seedlings was shifted into a plastic cup (8 cm diameter, 14 cm height), At 4 leaf stage, WT, OE and RNAi rice transgenic lines were used for experiments. 0.5 g of rice lines leaves were wrapped in tin foil and placed in liquid nitrogen. After sampling, they were stored in an ultra-low temperature refrigerator at -80 ^{0}C. The expression level of *OsNCED3 gene* was then determined.

Determination of the average injury level of rice after BPH feeding on OE rice lines

15 rice seedlings of WT and OE transgenic rice lines at 4 leaf stage with the same growth status were selected. We wrapped a cylinder with a flexible transparent PVC (Polyvinyl chloride) sheet and inserted that into the soil along the rim of the cup. Thirty BPH nymphs of 3rd instar were starved for 1 hour, then all the nymphs were poured into a self-made transparent PVC cover and sealed with gauze. After 7 days, the injury level of rice in each plastic cup was checked, and the identification standard of Wu et al (1986) was used to calculate the average injury level (Tab. 1).

Measurement of plant functional plant loss index (FPLI) after BPH feeding on OE rice lines

After the determination of the average injury level was finished, rice plants were cut off and washed, and then dried at 110 ^{0}C for 20 min, and then dried to constant weight at 60 ^{0}C. The dry weight of each plant was measured with an electronic balance of one ten thousandth of a gram, and the functional plant loss index (FPLI) was calculated. The method of Chen et al (2009) was used for the determination of FPLI.

$$FPLI = 100 - \frac{\text{Dry weight of injured plant}}{\text{Dry weight of uninjured plants}} \times \left(1 - \frac{\text{Injured level}}{9}\right) \times 100$$

EPG analysis of feeding behavior of *OsNCED3* transgenic rice to BPH

The main EPG wave-forms of BPH on rice were non-penetration (NP), stylet penetrating into rice epidermis (N1), salivation and movement of stylet (N2), extracellular movement of stylet near the phloem region (N3), sap ingestion in phloem (N4) and water ingestion in xylem (N5). EPG waveform characterization and quantification were performed according to Liu et al (2017) method.

Single rice seedling (4-leaf stage) with good growth was transplanted into a plastic cup. After being starved for 1 hour, each third instar BPH nymph was placed on a leaf sheath of the rice to be tested and a gold wire (3-5 cm long, 20 μm diameter) was connected from its dorsum to EPG instrument. A set of EPG instrument was placed in a climate-controlled room (25 ± 2 ^{0}C) and was linked to a computer running PROBE software. BPH with a gold wire attached to the dorsum by conductive silver glue was then allowed to probe the rice sheath through the parafilm. The gold wire from each insect and a copper wire (0.1 mm dia.) immersed in the food were linked to a model CR-8 DC-EPG amplifier (Wuhan Pusaisi Electronic Technology Co., Ltd.). Data were analyzed using ANA version 3.0 software (Wageningen University). EPG recordings were carried out for 6 h per insect per plant, with 10 replicates for each treatment.

Rice RNA library construction and transcriptome sequencing

At tillering stage, six seedlings of OE, RNAi and WT transgenic rice lines were selected. Each plant was used for BPH feeding covered with a self-made plastic cover, sampling was carried out at 12 h after BPH feeding, and the other was used for control (without BPH feeding). Each treatment and control was replicated three times. Every sample was weighed 0.2 g leaf sheath, wrapped in tin foil and frozen in liquid nitrogen, and then stored in an ultra-low temperature refrigerator at -80 ^{0}C.

RNA library construction and quality control refer to method of Wang et al (2018). Total RNA was extracted from these samples using the Total RNA Kit (Tiangen, Beijing, China), then the samples were concentrated using oligo (dT) magnetic adsorption and served as a template for the synthesis of first-strand cDNA using random hexamers and reverse transcriptase Second-strand cDNA was synthesized and purified by the AMPure XP beads and resolved in EB buffer for poly(A) addition and adapter addition. The cDNA fragments with suitable lengths and insert sizes XP were selected by AMPure XP beads to construct the final cDNA libraries. The cDNA libraries were checked using Qubit 2.0 and Agilent 2100 before they were sequenced using the Illumina HiSeq. 2500 High-throughput Sequencing machine at Genepioneer Biotechnologies Co, Ltd. (Nanjing, China). Differential expression analysis was performed according to the expression level of genes in the sample group, and GO functional annotation and KEGG pathway enrichment analysis were performed on the differential expression genes (DEGs).

Statistical analysis

Library construction was performed with a NEBNext Multiplex RNA Library Prep Set for Illumina (NEB) and sequenced on an Illumina Hiseq 2000 Platform (Gao et al., 2015). The raw data after quality control, namely clean data (reads), was compared with the reference genome to obtain mapped data (reads) for subsequent transcript assembly and expression calculation. Meanwhile, the comparison results of this transcriptome sequencing were evaluated, including sequencing saturation, gene coverage, distribution of reads in different regions of the reference genome and distribution analysis of reads in different chromosomes. Comparing with the reference genome by Cufflinks (http://cole-trapnell-lab.github.io/cufflinks/), identify PE fragments from different shearers, search for overlapping fragments, and get the difference information between this sequencing and the original annotation. For gene expression analysis, the number of fragments per kilobase of exon per million fragments (FPKM) was calculated. The DEGs were defined as that fulfilling the following requirement: p-adjust $\leq$ 0.05 and default difference multiple = 2. DAVID24 was used for the analysis of over-representation of gene classes. The statistical significance of any difference between treatments was analyzed by analysis of variance (ANOVA; Systat Inc.) followed by a Fisher's Protected Least Significant Difference (PLSD) test for multiple comparisons. For the ANOVA, the data were analyzed directly if normally distributed, if not, then all the data were transformed to ensure homogeneity of variances among different groups. Data were denoted as means±SE, and analyzed using SPSS 11.0 software (SPSS).

Results

Measurement of *OsNCED3* expression level in different rice lines

The expression of *OsNCED3* gene in OE and RNAi rice transgenic lines was measured. The results showed that the expression of OE rice lines increased by 106.3% compared with WT, and RNAi rice lines was significantly down-regulated by 66.6% (Fig. 1).

Determination of average injury level, FPLI of rice lines and EPG analysis of BPH feeding behaviour

After BPH infestation, the average damage level and FPLI in OE rice lines showed a significant lower damage level than that of WT, and the decreasing rate was 14.64% and 9.41%, significantly (Fig. 2). The EPG behavior measurement results were shown in Fig. 3. There was no significant difference in the duration of the NP wave between the three rice lines in the non-probing stage. The N1 wave represents the times of penetrations. During the feeding process of BPH, the frequency of the N1 wave in RNAi rice lines was significantly higher than that of the other two lines, which was 39.27% and 29.21% higher than that of WT and OE, significantly. N2 wave indicates the duration of saliva secretion, N3 wave indicates the duration of the extra-cellular movement of BPH needle near phloem. There was no significant difference in the duration of N2 and N3 waves among the three rice lines. OE, RNAi and WT had significant differences in the duration of the N4 wave, N4 wave is the wave that appears when BPH sucks juice in the phloem. The duration of the N4 wave of OE was significantly reduced by 20.13%, while RNAi was increased by 16.49%, compared with WT. The process of BPH feeding on xylem sap is represented by wave N5. It was found that there was no significant difference in the duration of wave N5 between WT and RNAi rice lines, but there was a significant difference between OE and WT rice lines, and OE rice lines was 53.61% lower than that of WT, and RNAi rice lines was 118.89% higher than that of OE rice lines (Fig. 3).

Transcriptome data statistics

The transcriptome sequencing data of 17 rice samples are shown in Tab. 2. After quality control, 128.00 Gb Clean Data was obtained, and the Clean Data of each sample reached 6.19 Gb or above. The clean reads of each sample were compared with the designated reference genome, and the comparison efficiency was 91.36% or above. The percentages of Q30 bases in the 17 rice samples were all greater than 89.29%, and the base quality was qualified. In addition, the percentage of GC content of the four bases in clean reads was between 48.81% and 52.25% (Tab. 2). It is evident that the quantity and quality of the sequencing results were qualified and could be further analyzed.

General situation of DEGs of OE and RNAi transgenic rice lines after BPH feeding

Statistical analysis of DEGs in different comparative groups was carried out, and the number of up-regulated and down-regulated genes in each comparative group was obtained (Fig. 4). The DEGs between RNAi and OE (R vs OE) was the lowest, and that between RNAi and RNAi +BPH (R vs RB) was the highest. Comparing rice lines fed by BPH with non-BPH infestation (ZH vs ZHB, OE vs OEB, R vs RB), the total number of DEGs exceeded 500, among which the up-regulated genes exceeded 50%. After RNAi rice lines

fed by BPH, the number of up-regulated genes was 592.57%, which was higher than that of down-regulated genes, accounting for 87.38% of the total number of DEGs in this group. It could also be seen that the number of DEGs between RNAi and WT fed by BPH (ZHB vs RB) was significantly higher than that in non-BPH-infestation group (ZH vs R) (Fig. 4).

Under the treatment of 2 ~7 times difference multiple analysis, there were still a large number of up-regulated and down-regulated DEGs in the four comparative groups (Tab. 3), and the DEGs were mostly up-regulated. At the same time, the number of up-regulated genes in RNAi rice lines fed by BPH was higher than that of other groups in every difference multiple. When the difference multiple was 2, there were 1724 DEGs, which was significantly more than other control groups in the table (Tab. 3).

Venn analysis of DEGs

Without BPH infestation, the number of overlapping DEGs between OE and WT rice lines, and that between RNAi and WT was 96, accounting for 29.63% and 24.74% of the total number of DEGs, respectively (Fig. 5A). After BPH infestation, there were 117 DEGs overlapped, respectively, accounting for 48.15% and 5.82% of the DEGs of WT respectively (Fig. 5B). The above four DEGs set in the case of BPH infestation and non-BPH infestation were collected together for Venn diagram analysis, the results showed 51 DEGs overlapped, accounting for 2.54%, 20.99%, 15.75% and 13.14% of the total number of DEGs in each control groups (Fig. 5C).

GO function annotation analysis of DEGs

The Gene Ontology (GO) database could classify the genes in the selected gene set, such as the biological process, the cellular component, the molecular function, etc. Without BPH infestation, the DEGs between OE and WT, and that between RNAi and WT were annotated into GO molecular function "binding" (GO :0005488) which had the largest number of DEGs (Fig. 6A). The number of DEGs in annotate "catalytic activity" (GO:0003824), "cell part" (GO:0044464), "membrane part" (GO:0016020), "metabolic process" (GO:0008152) and "cellular process" (GO:0009987) all exceeded 100(Fig. 6A). After BPH infestation, the number of DEGs annotated to each GO function in RNAi rice lines increased significantly, among which, the most DEGs that were annotated to "binding" in GO molecular function were 845, followed by "catalytic activity", "cell part", "metabolic process" and so on. The number of DEGs in the first six GO pathways was greater than 600 (Fig. 6B). After BPH infestation, the number of DEGs annotated to "binding" in OE rice lines was also the largest, but the number was only 114, followed by "metabolic process", "cell process", "cell part" and so on. However, there were less than 100 DEGs in each GO pathway, and the number of DEGs in each GO pathway was significantly less than that of RNAi rice lines fed by BPH (Fig. 6B).

Comment analysis of KEGG function

Without BPH infestation, the DEGs between OE and WT were annotated into KEGG classification map, and the results of analysis pathways showed that the DEGs were mainly concentrated in 15 pathways.

They were amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, energy metabolism, lipid metabolism, metabolism of cofactors and vitamins, metabolism of other amino acids, metabolism of terpenoids and polyketides, signal transduction, transport and catabolism and environmental adaptation pathways (Fig. 7A), among them, 12 genes were involved in energy metabolism pathway which had the largest number of DEGs, followed by 8 genes in carbohydrate metabolism pathway and signal transduction pathway. The DEGs between RNAi rice lines and WT were obtained and annotated on KEGG classification map (Fig. 7B). The results showed that the DEGs were mainly concentrated in 14 pathways. They were amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, energy metabolism, lipid metabolism, metabolism of cofactors and vitamins, metabolism of other amino acids, metabolism of terpenoids and polyketides, signal transduction, transport and catabolism, and environmental adaptation pathways (Fig. 7B). There were 19 DEGs annotated to the carbohydrate metabolism pathway, which is the pathway containing the most DEGs, followed by 12 DEGs in biosynthetic pathways for other secondary metabolites.

After BPH infestation, the classification results of KEGG pathway of DEGs between OE and WT rice lines were consistent with those of non-BPH-infestation. But the highest number of DEGs was 9 in carbohydrate metabolism pathway, followed by 7 in terpenoids and polyketones metabolism pathway (Fig. 8A). After RNAi rice lines fed by BPH, the DEGs were distributed in 19 KEGG pathways, and there were 4 new pathways more than those of non-BPH-infestation. They were glycan biosynthesis and metabolism, nucleotide metabolism, environmental adaptation and endocrine and metabolic disease, among which carbohydrate metabolism pathway contains the most DEGs, with 67 DEGs, followed by signal transduction pathway, with 50 DEGs (Fig. 8B). A large number of DEGs after RNAi rice lines fed by BPH were evenly distributed in many different KEGG pathways, but not aggregated in one or several pathways.

Quantitative qRT-PCR Verification

Six DEGs related to hormone pathway and rice resistance were selected for qRT-PCR verification. *OsABA8ox2* (Os08g36860) and *OsPYL9* (Os06g36670) were ABA decomposition genes and ABA receptor genes, respectively. The results showed that the expression of the two genes in OE rice lines increased significantly after BPH infestation (Fig. 9a-b). *OsAOS1* (Os03g55800) is an important gene for JA biosynthesis, and *OsJAZ1* (Os04g32480) is a transcriptional inhibitor of JA, and the expression of *OsJAZ1* increased while that of *OsAOS1* decreased after RNAi rice lines fed by BPH (Fig. 9c-d). The relative expression of rice stress-tolerance gene *OsbZIP23* (Os02g52780) and BPH-resistance gene *Osbph6* (Os04g35210) in OE rice lines was always higher than that of WT (Fig. 9e-f). The results of qRT-PCR were consistent with those of transcriptome analysis.

Discussion

EPG analysis, the average injury level and functional plant loss index showed that overexpression of *OsNCED3* gene might enhance rice lines resistance against BPH.

EPG can accurately record the probing behavior and position of the insect's mouth needle in the host tissue (Miao and Han 2008). Therefore, EPG technology can also be used as a bio-assay method for rapid screening of resistant plants (Lei and Xu 1998). According to our observations by means of EPG e.g.N3 wave, overexpression of *OsNCED3* gene could significantly shorten the duration of extra-cellular movement of mouth needle of BPH near phloem. Similarly, N4 wave appears during sucking of in phloem juice by BPH. In this experiment, N4 wave indicated that BPH did not like to eat in rice lines with the overexpression of *OsNCED3* gene, and on the other hand, it further proved that the function of *OsNCED3* gene could improve plant resistance to BPH. The decrease in N4 wave duration in our study can be considered as presence of resistance factors in phloem for a short time because it is an important indicator to measure plant resistance (Luo et al., 2005).

The function of *OsNCED3* gene was confirmed with transcriptomics analysis. The number of DEGs in WT, OE and RNAi rice lines were considerably impacted by BPH Feeding. It has already been concluded that transcriptomic profiling of BPH resistant and susceptible plants are distinct.. BPH attack incremented the DEGs between OE and WT rice lines, as well as between RNAi and WT rice lines. The number of DEGs in RNAi rice lines was significantly higher than that of OE particularly after BPH infestation.. Our findings are in line with earlier report stating that Susceptible plants i.e. rice show more DEGs in response to BPH attack (Lv et al., 2014). Our results suggest that the loss of *OsNCED3* function might affect the regulation of genes actions in the plant body after BPH infestation, and most of the genes were up-regulated.

The DEGs expressed in OE plants were related to the synthesis of lignin, chitin and serotonin. *OsCCR17* (Cinnamoyl-CoA reductase 17) was involved in the biosynthesis of secondary metabolites in OE transgenic rice lines such as lignin. This expression of *OsCCR17* has already been reported in rice lines facing abiotic and biotic stresses e.g. high temperature, salinity, *Magnaporthe grisea* and *Xanthomonas oryzea attack* (Park et al. 2017), etc. Our results have again confirmed this report. Up-regulation of *OsCCR17* gene in OE rice lines revealed its function in promoting the synthesis of lignin and this up-regulation was independent of BPH infestation. The role of lignin in plant defense is crystal clear. For example, *CmMYB15*, a homologous gene of *AtMYB15*, commonly existed in the promoter of lignin biosynthesis genes and enhanced the resistance of chrysanthemum against aphids (An et al. 2019). Similarly, silencing *OsSLR1* gene enhanced constitutive levels of defence-related compounds, such as phenolic acids, lignin and cellulose, and improved rice resistance to BPH (Jin et al., 2017). The direct link between expression of recorded genes and biosynthesis of defense associated compounds is adequate evidence of rice defense in OE lines. This also clarifies the functional connection between expression of *OsNCED3* and metabolic adjustments to confer resistance against BPH infestation. In the carbohydrate metabolism pathway, we found three chitinase genes *Oscht1*, *Oscht4* and *Oscht5*, among which *Oscht1* increased significantly in OE rice lines compared with WT in spite of BPH feeding or not. *Oscht4* and *Oscht5* increased significantly in OE rice lines compared with WT only after BPH feeding. Chitinase has been involved in plant defense and overexpression of pepper chitinase genes in heterogeneous transgenic plants enhanced disease-resistance as well as stress-tolerance (Asrorov et al., 2017; Hong et al., 2005). We have an opinion that involvement of different chitinase in rice defense against BPH specifies their function as disease related proteins. Our opinion is advocated by the findings of Rajendran

et al. (2011) describing increased plant chitinase activity as a key player in reducing the number of aphids, They have also suggested that chitinase could be induced by both pathogens as well as pests. Similarly, presence of chitinase has been detected in different plant tissues such as leaves, fruits, seeds and roots and its expression was recorded as a protein against fungi and pests (Silva et al., 2017). *OsTDC1* encodes tryptophan decarboxylase which catalyzed the conversion of tryptophan to tryptamine. Tryptamine was further catalyzed by tryptamine 5-hydroxylase to form 5-hydroxytryptamine/serotonin. We found that *OsTDC1* existed both in amino acid and other secondary metabolite pathways after BPH infestation. It was speculated that 5-hydroxytryptamine was likely to participate in the process of resisting adversity stress. Our research confirmed this point. *OsTDC1* can be significantly induced by stress, and the increasing of 5-hydroxytryptamine in resistant-rice-mutants led to the disappearance of plant resistance to pests (Kang et al., 2009; Lu et al., 2018), The expression of *OsTDC1* decreased after OE rice lines were fed by BPH ans main reason for this decline is change in 5-hydroxytryptamine levels as explained in above stated evidence. According to the KEGG analysis, many DEGs were related to plants resistance to adversity stress, and the overexpression of *OsNCED3* would improve rice tolerance to pathogens and pests.

ABA and JA may interact each other against BPH. JA is considered as one of the key hormones for plant insect-resistance. Our study found that three JA synthesis genes (*OsHI-LOX*, *OsLOX7* and *OsOPR10*) were expressed in large quantities after OE plants were fed by BPH. *OsHI-LOX* (13-lipoxygenase) and *OsLOX7* (lipoxygenase 7) were two lipoxygenase (LOX) family genes found in the lipid metabolism pathway of OE rice lines. *OsHI-LOX* was involved in the synthesis of JA induced by pests.Loss of function of *OsHI-LOX* made rice more vulnerable to chewing insects but at the same time enhanced its resistance to phloem feeding insects (Zhou et al., 2009). The *OsOPR10* (12-oxo-phytodienoic acid reductase 10) gene also belonged to lipid metabolism pathway, and it was a key enzyme in JA biosynthesis. *OsOPR10* was up-regulated in OE rice lines that reveals its significance in rice defense. OPRs have been identified for their involvement in JAs biosynthesis in rice, tomato and *Arabidopsis*. *OsOPRs* responds to various biological and abiotic stresses such as mechanical damage, salt ions, plant signal molecules and pathogen infection (Jang et al 2009),

The results of fluorescence quantification showed that the expression of ABA decomposition related gene *OsABA8ox2* in OE rice lines was significantly increased. On the other hand, *OsPYL9* was an ABA receptor, *OsPYLs* positively regulate ABA response during rice seed germination, and overexpression of *OsPYL9* could significantly improve rice drought-tolerance and cold-tolerance (Tian et al., 2015). The expression of *OsPYL9* in OE rice line was always significantly higher than that of RNAi rice lines. Combined with the determination results of these two ABA pathway genes, we speculate that the ABA content in OE rice increased after BPH feeding, and the excess ABA was hydrolyzed at the same time. *OsAOS1* was related to JA biosynthesis. The expression of *OsAOS1* in RNAi rice lines was inhibited at 12 h post BPH infestation, which represented the decrease of JA synthesis. JAZ protein, a transcription inhibitor of JA signal, inhibited JA activation by inhibiting MYC2 positive TF, and JAZ protein relies on JA signal pathway to negatively regulate plant defense against biotic and abiotic stress (Browse, 2009; Fu et al., 2017). Combined with the determination results of two genes of JA pathway, it can be inferred that JA

content in RNAi rice lines were significantly lower than that in WT and OE rice. The results of qPCR verification showed that *OsNCED3* and *OsBZIP23* had synergistic relationship. *OsbZIP23* has already been reported for its function in abiotic stress tolerance in rice (Park et al., 2015). The expression of *Osbph6* in OE rice lines was significantly higher than that in WT in absence of BPH, which indicated that OE rice lines had higher resistance against BPH. The expression of *Osbph6* gene in three rice lines was down-regulated after BPH infestation, but he expression of *Osbph6* in OE rice lines was still higher than that in WT and RNAi rice lines after BPH infestation. It could be inferred that OE rice lines was resistant to BPH compared with WT under the same treatment. *Osbph6* was an important resistance gene in rice, which endowed rice with resistance to BPH (Guo et al., 2018).

Conclusions

Based on the above results, we speculated that the function of *OsNCED3* would not only improve rice resistance to abiotic stress, but also induce rice to develop resistance to BPH to a certain extent, which might include the synthesis of lignin, chitin and other substances, and the interaction between ABA and JA, etc, which still needs further research and verification.

Abbreviations

ABA: Abscisic acid; JA: Jasmonic acid; ET: Ethylene; BPH: Brown planthopper; NCED: 9-*cis*-epoxycarotenoid dioxygenase; OE: Overexpression; RNAi: RNA interference; WT: wild type; EPG: Electrical penetration graph; FPLI: Functional plant loss index; DEGs: Differential expression genes; ZH11: Zhonghua 11; PVC: Polyvinyl chloride; FPKM: Fragments per kilobase of transcript per million mapped reads; qRT-PCR: Quantitative real time polymerase chain reaction; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

All authors have provided consent for publication.

Availability of supporting data

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Author'Contributions

LS, YL, LC and JL designed the research. LS and YL performed the experiments. LS, JL and NA analyzed the data. LS and JL wrote the manuscript. All authors approved the manuscript.

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Tables

Due to technical limitations, table 1-4 is only available as a download in the Supplemental Files section.

Figures

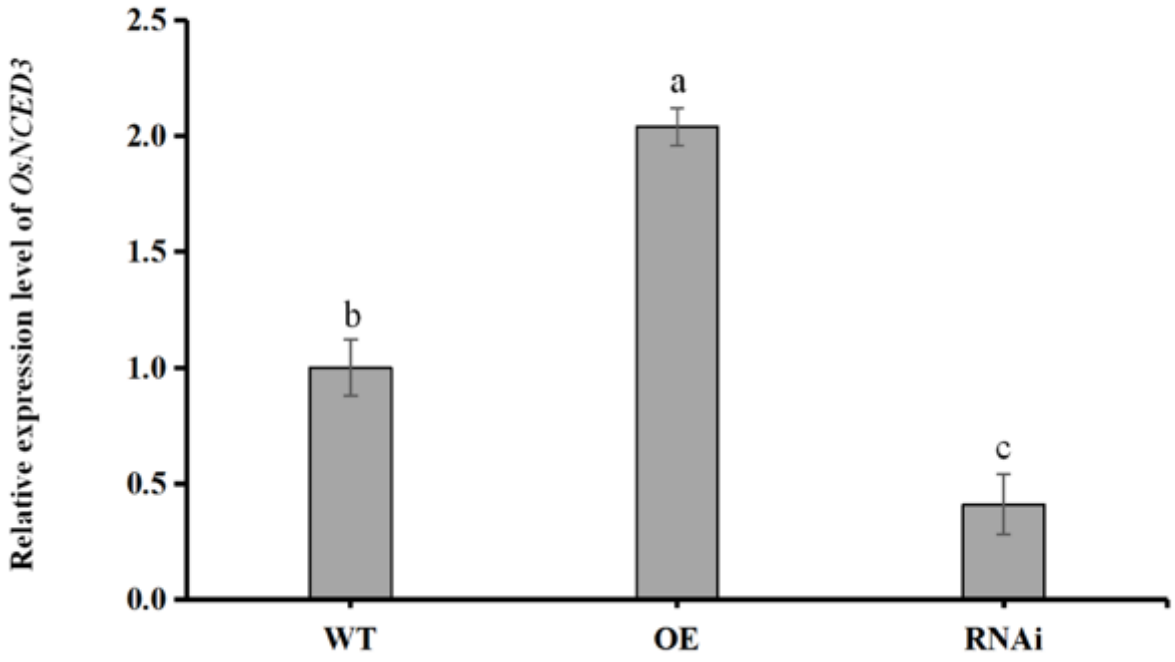

Figure 1

Measurement of OsNCED3 expression level in different rice lines. The relative expression level of OsNCED3 was determined by qPCR. Error bars represent the means ± SE, n = 3 biological independent

experiments with at least three replicates for each. Bars with different letters show significant different at P < 0.05 by Duncank´s multiple range test.

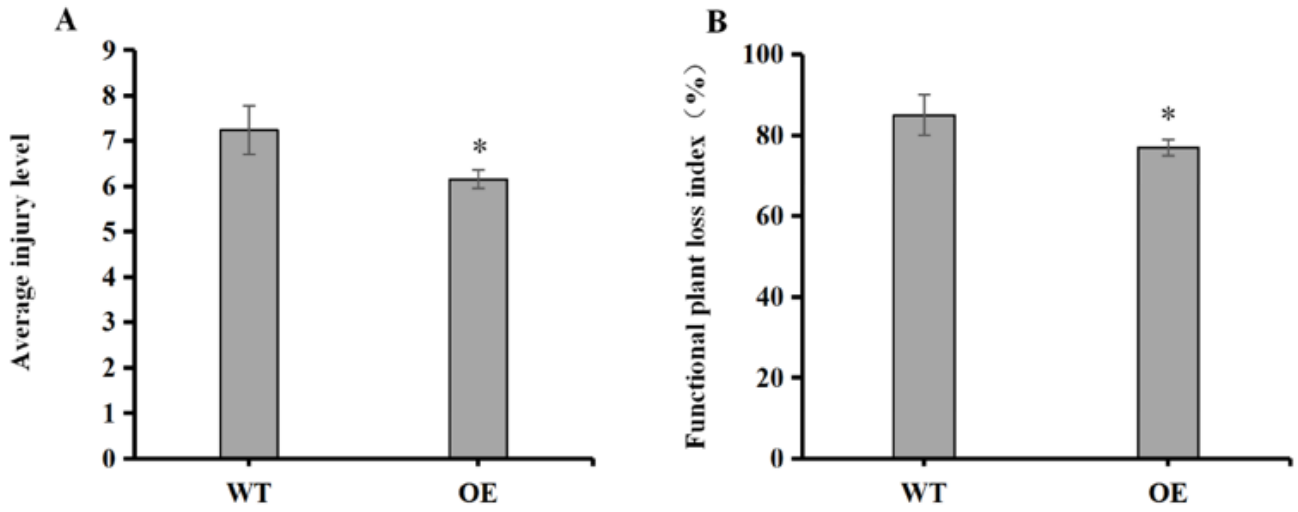

Figure 2

Average injury level and functional plant loss index after BPH feeding on WT and OE rice plants. A total of 30 BPH were taken to feed on WT and OE rice plants for 7 days, and the corresponding determination results were obtained. a. Average injury level result of WT and OE rice plants. b. Functional plant loss index result of WT and OE rice plants. Error bars represent the means ± SE, n = 3 biological independent experiments with at least three replicates for each. Bars with different letters show significant different at P < 0.05 by Duncank´s multiple range test.

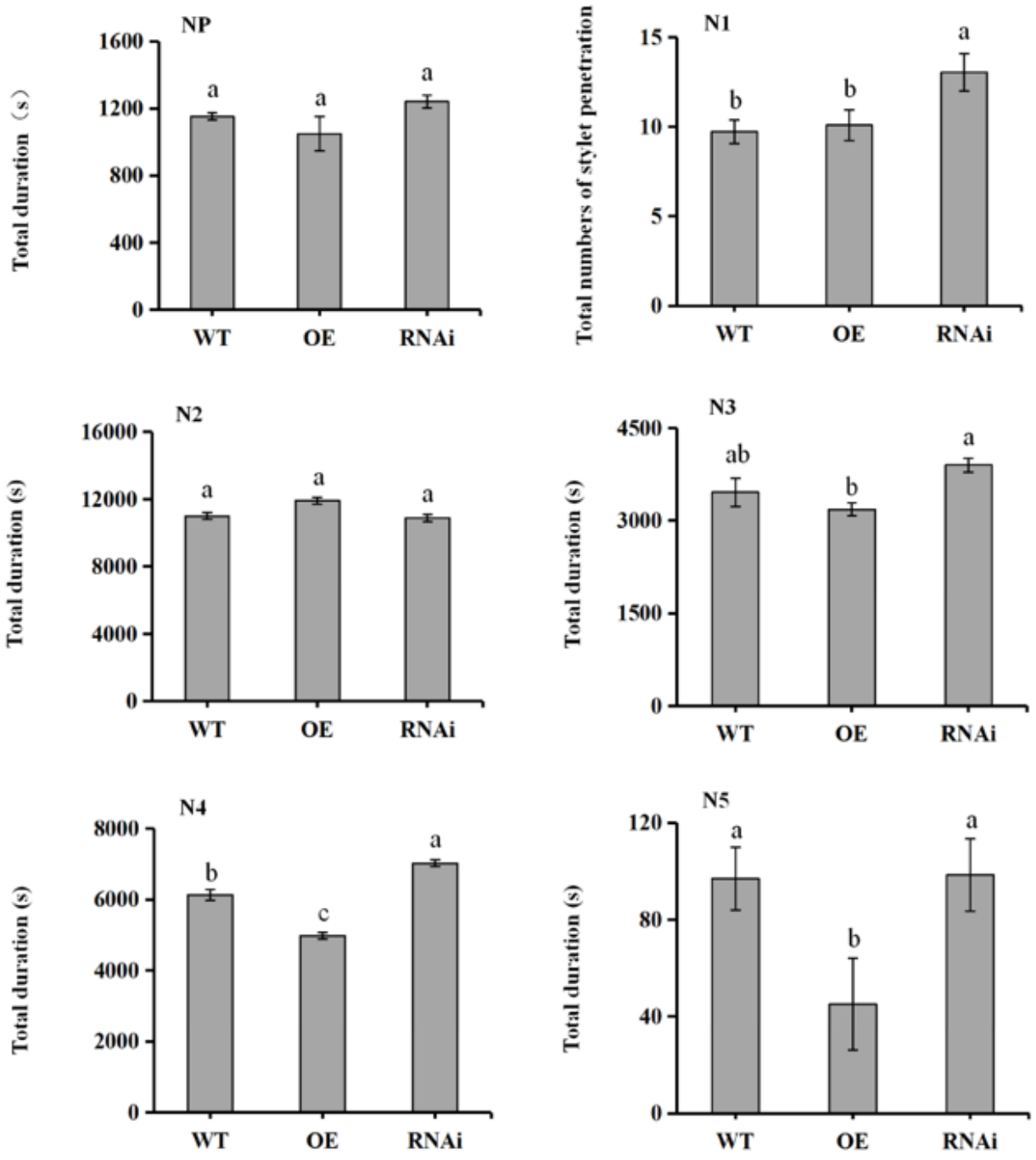

Figure 3

EPG behavior analysis of BPH feeding on WT, OE and RNAi rice plants. A single BPH fed on a single rice plant, and the effective feeding time was 6 hours. NP: The duration of non probing stage. N1: The times of probing. N2: The duration of the mouth needle movement in vascular bundle. N3: The duration of the mouth needle movement outside phloem cells. N4: The time the mouth needle sucks the juice in the phloem. N5:The duration of the mouth needle sucking xylem juice. Error bars represent the means ± SE, n = 3 biological independent experiments with at least three replicates for each. Bars with different letters show significant different at $P < 0.05$ by Duncank´s multiple range test.

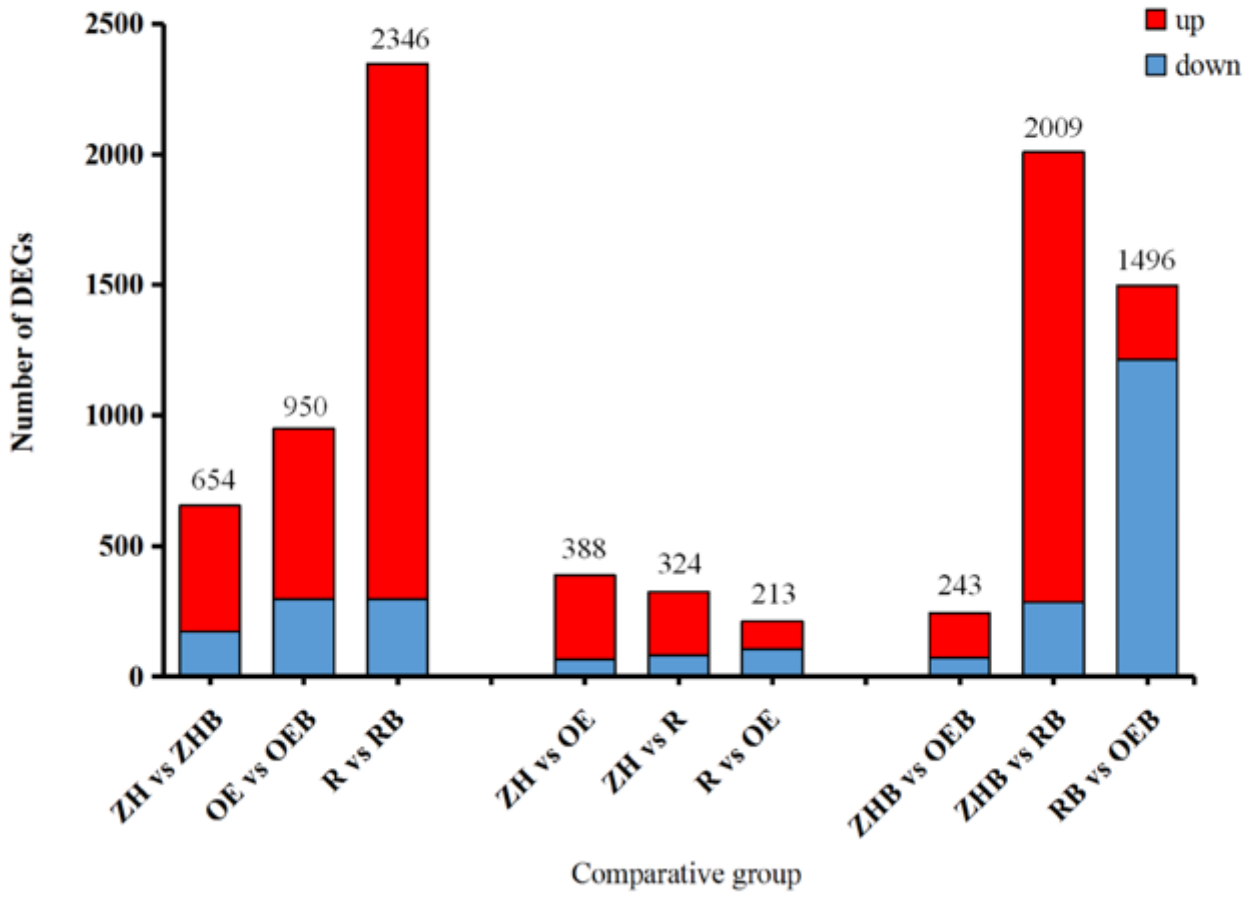

Figure 4

Up and down-regulated DEGs in different comparative groups. Red columns represent up-regulated DEGs, blue columns represent down-regulated DEGs, and column lengths reflect the number of DEGs. ZH: WT without BPH feeding. ZHB: WT with BPH feeding. OE: OE rice plants without BPH feeding. OEB: OE rice plants with BPH feeding. R: RNAi rice plants without BPH feeding. RB: RNAi rice plants with BPH feeding.

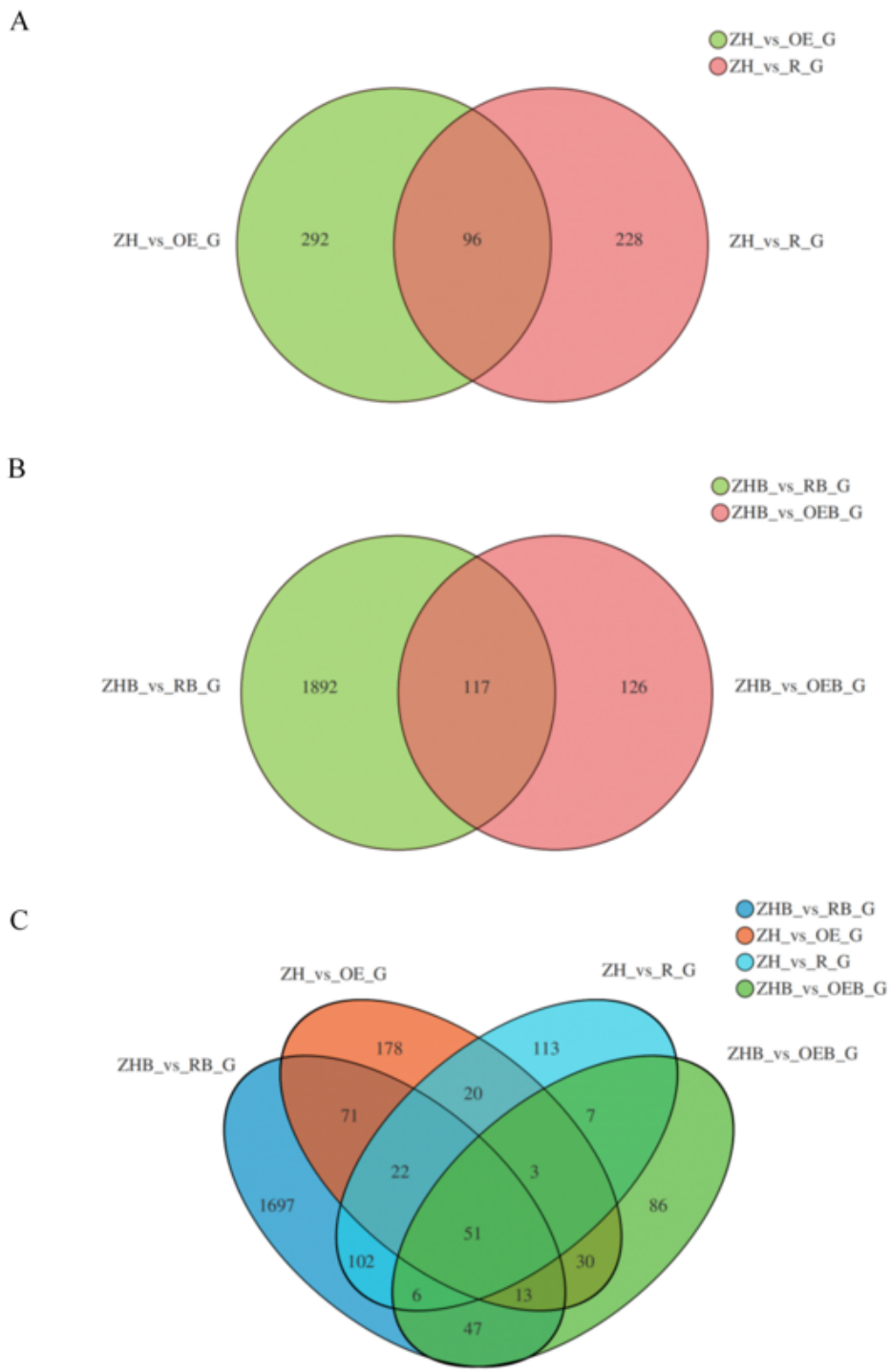

Figure 5

Venn diagram analysis of DEGs in different comparative groups. a. ZH_vs_R_G: The group of DEGs between ZH11 and RNAi rice plants; ZH_vs_OE_G: The group of DEGs between ZH11 and OE rice plants. b. ZHB_vs_OEB_G: The group of DEGs between ZH11 and OE rice plants after BPH feeding; ZHB_vs_RB_G: The group of DEGs between ZH11 and RNAi rice plants after BPH feeding. c. The group of DEGs between four comparative groups.

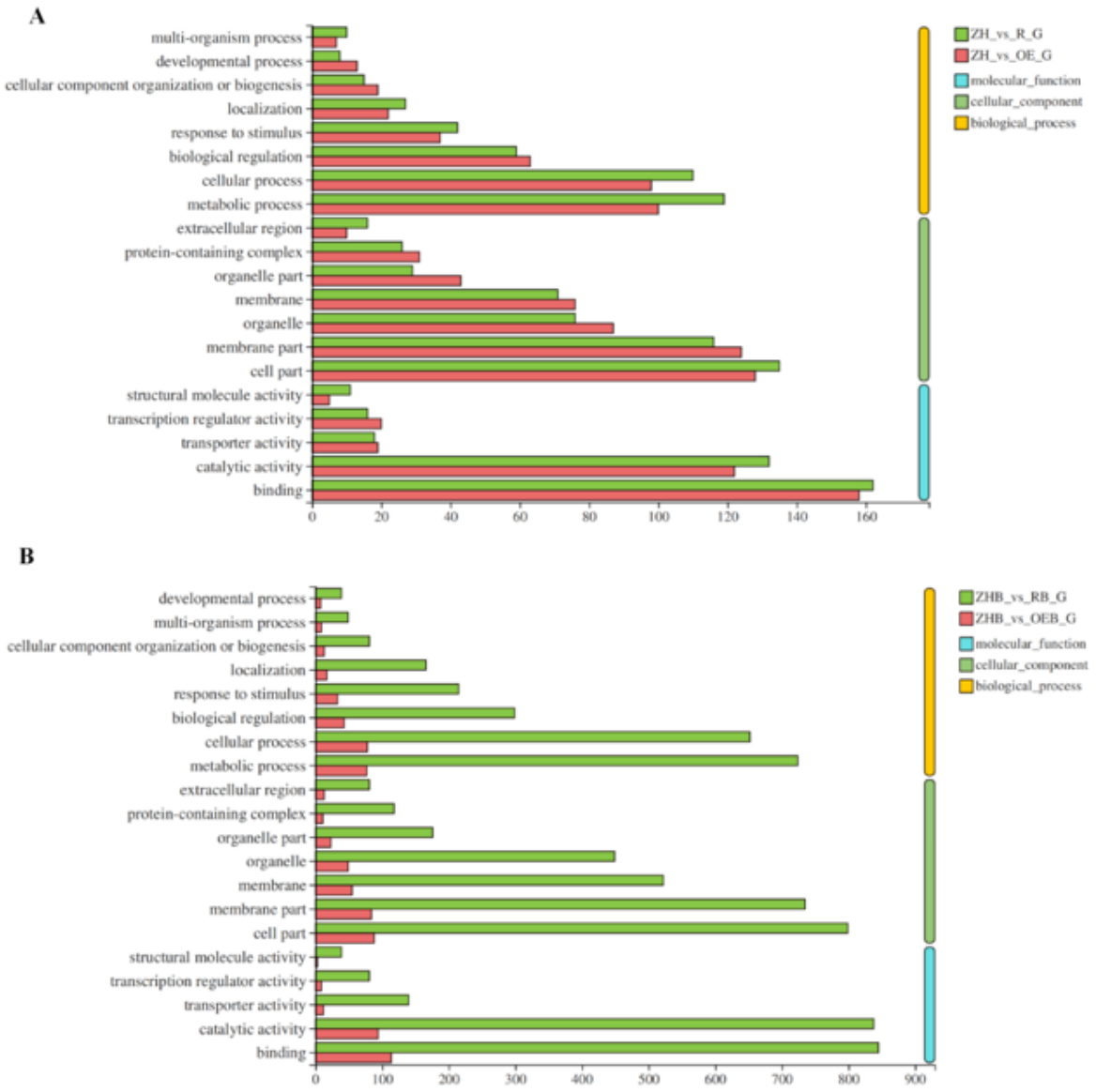

Figure 6

Go annotation classification chart of DEGs in different comparative groups. a. ZH_vs_R_G: The group of DEGs between ZH11 and RNAi rice plants; ZH_vs_OE_G: The group of DEGs between ZH11 and OE rice plants. b. ZHB_vs_OEB_G: The group of DEGs between ZH11 and OE rice plants after BPH feeding; ZHB_vs_RB_G: The group of DEGs between ZH11 and RNAi rice plants after BPH feeding.

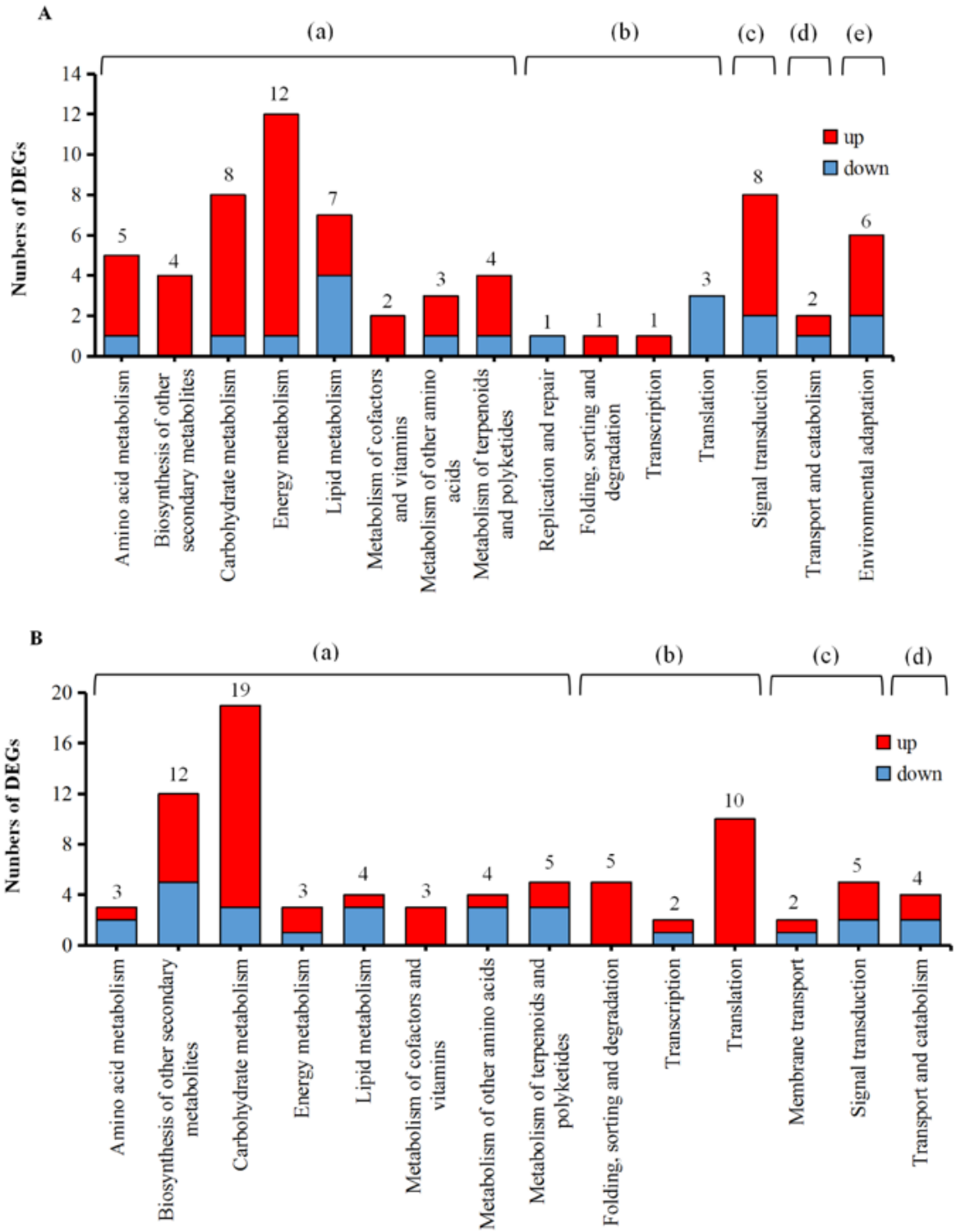

Figure 7

KEGG annotation classification of DEGs in different comparative groups without BPH feeding. a. Annotation results of up-regulated and down-regulated DEGs in OE rice plants on KEGG pathways without BPH feeding. b. Annotation results of up-regulated and down-regulated DEGs in RNAi rice plants on KEGG pathways without BPH feeding. (a). Metabolism. (b). Genetic Information Processing. (c). Environmental Information Processing. (d). Cellular Processes. (e). Organismal Systems.

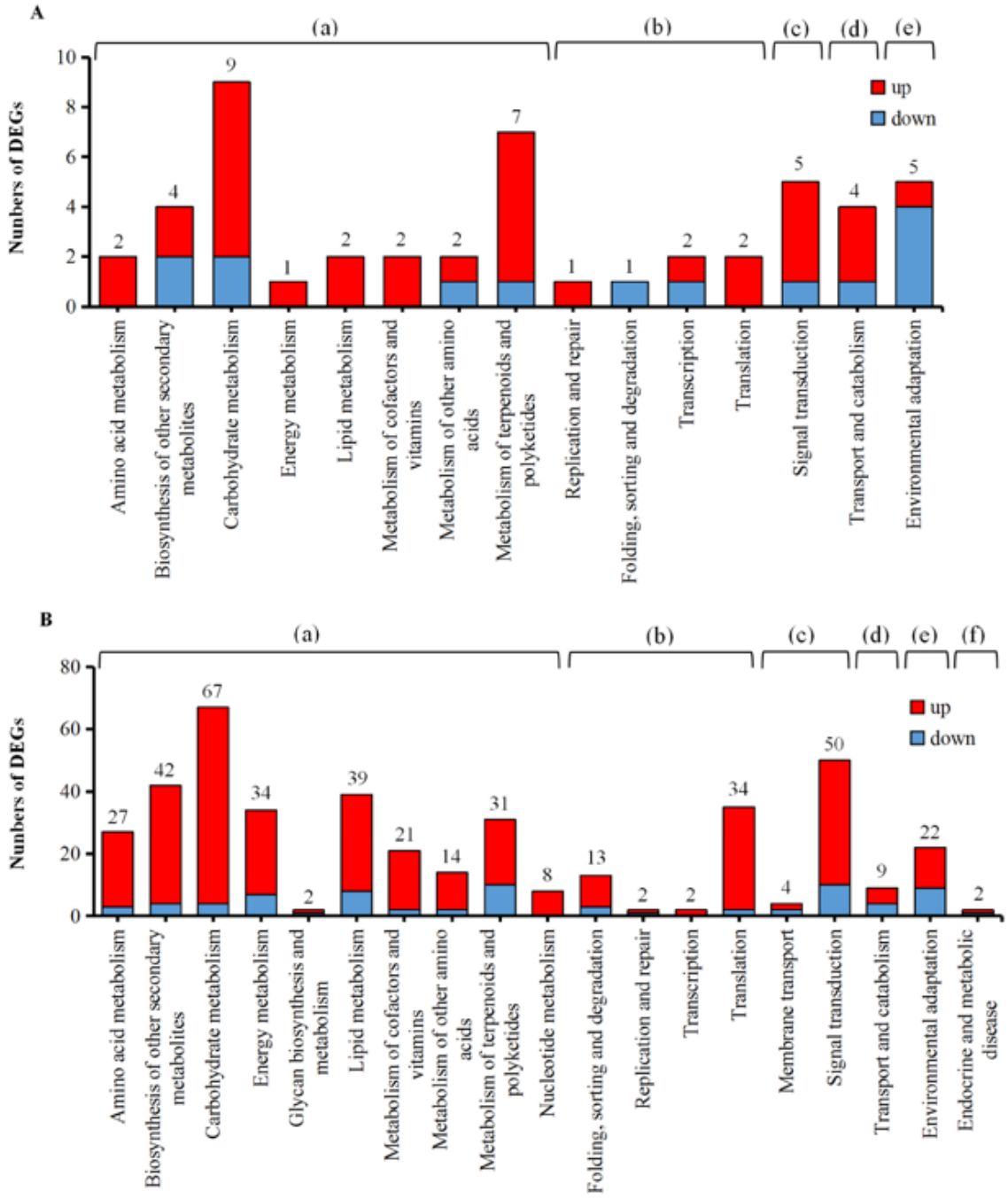

Figure 8

KEGG annotation classification of DEGs in different comparative groups with BPH feeding. a. Annotation results of up-regulated and down-regulated DEGs in OE rice plants on KEGG pathways with BPH feeding. b. Annotation results of up-regulated and down-regulated genes in RNAi rice plants on KEGG pathways with BPH feeding. (a). Metabolism. (b). Genetic Information Processing. (c). Environmental Information Processing. (d). Cellular Processes. (e). Organismal Systems. (f). Human Diseases.

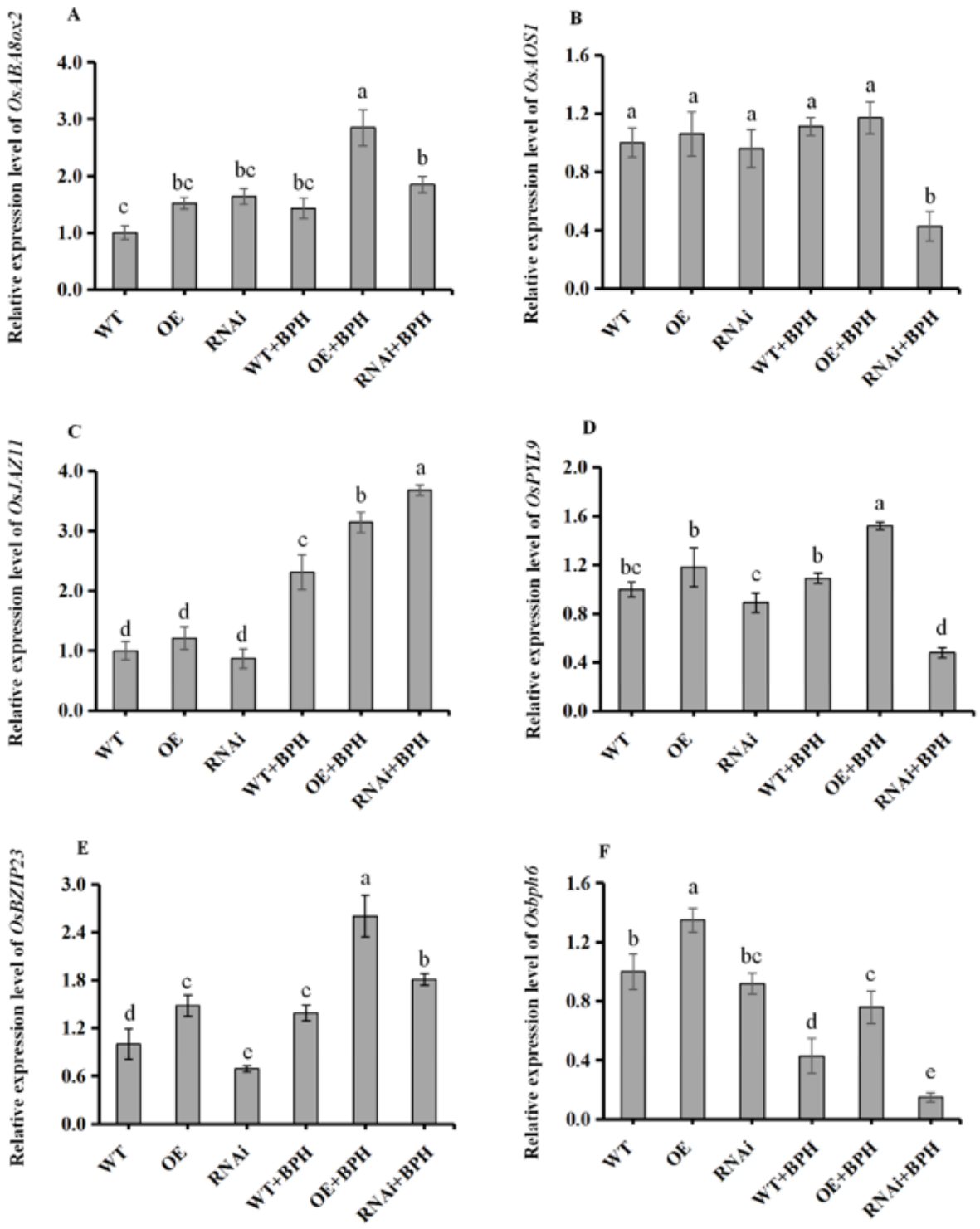

Figure 9

qRT-PCR verification of key genes. Six genes related to ABA, JA and rice defense were selected, and the relative expression level was determined by qRT-PCR and compared with the transcriptome results. a. OsABA8ox2, ABA 8'-Hydroxylase (LOC_Os08g36860). b. OsAOS1, allene oxide synthase (LOC_Os03g55800). c. OsJAZ11, jasmonate ZIM-domain protein (LOC_Os04g32480). d. OsPYL9, pyrabactin resistance-like abscisic acid receptor (LOC_Os06g36670). e. OsBZIP23, bZIP transcription

factor (LOC_Os02g52780). f. Osbph6, leucine rich repeat family protein (LOC_Os04g35210). Error bars represent the means ± SE, n = 3 biological independent experiments with at least three replicates for each. Bars with different letters show significant different at P < 0.05 by Duncank´s multiple range test.

Supplementary Files

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