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Comparative transcriptomic and physiological analyses reveal the key role of abscisic acid in *hydrangea macrophylla* responding to *Corynespora cassiicola*

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Abstract

Background Bigleaf hydrangea (*Hydrangea macrophylla*) is a widely cultivated ornamental plant species. Leaf spot disease, caused by *Corynespora cassiicola*, poses a significant threat to the ornamental quality and economic value of hydrangeas. However, the disease resistance breeding of hydrangea is limited due to the lacking of resistant varieties and genes.

Results This study evaluated ten hydrangea varieties for their resistance to leaf spot disease. Among them, 'White Angel' and 'Ocean Heart' were screened out as representative varieties for resistance and susceptibility, respectively, on the basis of evaluation. Physiological and biochemical indices, phytohormones, and transcriptomic changes were measured in the leaves of both varieties at 0 and 24 h post inoculation with *C. cassiicola*. The results showed that *C. cassiicola* infection significantly increased abscisic acid (ABA) contents in both varieties; however, the increase was significantly higher in the susceptible variety 'Ocean Heart' compared to the resistant variety 'White Angel' ($p < 0.05$). Moreover, exogenous ABA (100 μM) decreased the leaves' resistance to *C. cassiicola* of both varieties, underscoring its key role in reduced disease resistance. Transcriptome profiling revealed 17,087 differentially expressed genes (DEGs) responding to *C. cassiicola* between the two varieties. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis indicated significant enrichment of DEGs in "Plant hormone signal transduction", particularly related to ABA signaling (*HmPP2C* and *HmABFs*). In addition, the expression of ABA biosynthesis genes (*HmZEP3*, *HmABA2*, *and HmAAO3*) was upregulated in both varieties. Meanwhile, the ABA catabolism gene (*HmCYP707A4*) exhibited significantly upregulated expression in the resistant variety 'White Angel' and downregulated expression in the susceptible variety 'Ocean Heart'. Intriguingly, the expression of *HmCYP707A4* was 15-fold higher in 'White Angel' than in 'Ocean Heart'.

Conclusion In summary, these findings highlight the crucial role of ABA in the resistance of bigleaf hydrangea to leaf spot disease and provide valuable genetic resources for breeding programs to enhance the disease resistance in hydrangeas.

Keywords *Hydrangea macrophylla*, Leaf spot disease, Disease resistance evaluation, Transcriptome, ABA, *CYP707A4*

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Introduction

Bigleaf hydrangea (*Hydrangea macrophylla* (Thunb.) Ser.) is a cherished flowering shrub in the Hydrangeaceae family [1]. It is widely favored as a garden plant, a potted flower, an addition to bouquets, and a landscape feature globally [2, 3]. However, *H. macrophylla* is prone to foliar pathogen infections, which often result in leaf spot disease. Previous studies have identified *Corynespora cassiicola* as the primary pathogen responsible for leaf spot disease of hydrangea [3]. As a necrotrophic pathogen, *C. cassiicola* has a broad host range, infecting over 500 plant species worldwide [4]. The primary symptoms of this disease include mottling, wilting, and defoliation of leaves [3, 4]. Consequently, the occurrence of this disease in hydrangeas leads to reduced blooming, smaller flowers, and diminished growth vigor, causing substantial economic losses for producers.

Plants have evolved a complex defense system to combat pathogen invasions over long periods of evolution [5]. Constitutive defense serves as the initial protective barrier following pathogen infection, typically comprising physical and chemical defenses. When constitutive defenses are compromised, induced defense mechanisms are activated. These mechanisms primarily include pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI), which work in concert to resist pathogen invasion [6]. Pattern recognition receptors (PRRs) located on the surface and within plant cells initiate PTI by detecting molecular pattern signals associated with pathogen infection, leading to physiological responses such as the change of enzymatic activities, the production of signaling compounds, and the release of plant hormones [7].

The activity of antioxidant enzymes, soluble sugar (SS) and proline (Pro) contents indicate the ability of plant disease resistance [8]. Former studies have demonstrated that plants have developed antioxidant systems, including malondialdehyde (MDA), Pro, catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and various other compounds, to combat superoxide radicals, their derivative free radicals, and other organic radicals, thereby protecting themselves against pathogens [9, 10]. For instance, infection by *Fusarium oxysporum* f. sp. *phaseoli* has varying effects on oxidative metabolism in resistant versus susceptible soybean varieties. In the roots of resistant soybean variety, the levels of H_2O_2 and SOD activity significantly increased, whereas CAT activity decreased [11]. In kiwifruit, the activities of SOD, POD, CAT, and phenylalanine ammonia-lyase (PAL) were enhanced in response to *Botrytis cinerea* infection [12]. The carbohydrates produced by plants through photosynthesis not only serve as the primary energy source and substrates for the synthesis of other organic compounds, but also act as damage-associated molecular patterns (DAMPs) to detect pathogens and induce certain pathogenesis-related (PR) proteins [13].

Plants' responses to environmental cues are primarily regulated by phytohormones [14]. Numerous studies suggest that abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), along with their interactions, play crucial roles in plant defense against biotic stresses [15–18]. SA is central to both local and systemic resistance against biotrophic and hemi-biotrophic pathogens [14]. Upon activation by signaling, SA facilitates the reduction of disulfide bonds in the oligomeric protein nonexpresser of pathogen resistance gene 1 (NPR1) via thioredoxins, permitting its constituent monomers to translocate from the cytosol to the nucleus, where they bind to specific sites and upregulate resistance-associated genes [16]. Both JA and ET are essential in the plant's response to necrotrophic pathogens [19]. ET inactivates receptors and the constitutive triple response 1 (CTR1) protein, thus halting the repression of EIN2 and EIN3, which allows for the upregulation of ET signaling and the expression of defense genes, resulting in enhanced necrotrophic resistance [20]. Recent studies have indicated that ABA was also involved in plant defense signaling pathways; however, its role remains poorly understood.

In recent years, transcriptomic analyses have been utilized to identify key genes associated with resistance traits in plants, providing molecular references for the study of plant-pathogen interactions and resistance breeding [21]. In various species, significant resistance and susceptibility genes have been identified from differentially expressed genes (DEGs) associated with disease resistance through comparisons of resistant and susceptible varieties, such as tobacco [22], mulberry [23], and bluegrass [21]. However, further research is still required to identify resistant genes related to leaf spot disease in hydrangea.

This study aimed to investigate mechanism underlying the response of *H. macrophylla* varieties with different resistances to *C. cassiicola*. Transcriptome sequencing, along with physiological and biochemical analyses, were conducted on the resistant variety 'White Angel' and the susceptible variety 'Ocean Heart' under *C. cassiicola* infection. The results will serve as a valuable reference for breeding resistant varieties of *H. macrophylla* and provide insights into effective strategies for preventing leaf spot disease.

Materials and methods

Plants and pathogen materials

To evaluate the resistance/sensitivity of various *H. macrophylla* varieties to *C. cassiicola*, ten varieties with different performance to leaf spot disease on the basis of

former field investigation, including 'Sweet Fantasy', 'Ocean Heart', 'Cocktail', 'You and Me Emotion', 'Blue Danube', 'Wish You Peace', 'Girl in Red', 'Spring Bird', 'White Angel', and 'ShuiTianYiSe' were selected as plant materials [2]. The plants were potted at the Hydrangea Germplasm Resources Conservation Center of the Jiangsu Academy of Agricultural Sciences, China (32°04′N, 118°88′E). Prior to the experiment, cuttings from the different varieties were rooted in perlite for four weeks in a greenhouse maintained at 22–28 °C with a 16/8 h (light/dark) cycle. Afterward, the seedlings were transferred to pots for one month. The pathogen *C. cassiicola* was isolated and identified from the diseased leaves of the bigleaf hydrangea variety 'Bailer', which is also known as 'Endless Summer' [3].

Screening the resistance of *H. macrophyllavarieties* to leaf spot disease

Healthy leaves from the fourth leaf position of each hydrangea variety were collected. The leaves were sterilized by washing them with flowing water for 30 min, followed by exposure to 75% ethanol for 10 s and 15% hydrogen peroxide (H_2O_2) for 10 min. Subsequently, the leaves were rinsed three times with sterile water for 5 min and allowed to air dry. The sterilized leaves were placed in a petri dish between two layers of wet filter paper (moistened with sterile water). *C. cassiicola* was cultured on PDA medium for 5 days. Circular pieces, approximately 5 mm in diameter, of PDA medium with or without (control) *C. cassiicola* were obtained from the plate and inoculated onto the surface of the sampled leaves. The petri dishes containing the inoculated leaves were then placed in an incubator at 25 °C in the dark for 48 h before being transferred to a 16/8 h (light/dark) condition. The infected leaves were observed and photographed at 7 days post inoculation (DPI). The lesion area was quantified using ImageJ software (version 1.8.0).

Determination of physiological indexes of *H. macrophyllainfected* by *C. cassiicola*

The strain *C. cassiicola* was inoculated into the leaves of 'White Angel' and 'Ocean Heart' with conidial suspensions in vivo. The conidial suspensions were adjusted to a concentration of 1×10^6 conidia mL^{-1} with a hemocytometer, and were sprayed on the fourth leaf from the top. After incubating at 25℃ for 0 h and 24 h, respectively, the inoculated leaves were removed from plants and used to determine physiological characteristics. The activities of SOD, POD and CAT, and the contents of MDA, SS, and Pro were determined using the Assay Kit (Suzhou Keming Biotechnology Co. Ltd, Suzhou, China), according to the manufacturer's protocol. The contents of SA, JA, ET, and ABA were determined using enzyme-linked immunosorbent assay by Qingdao Kechuang Quality Testing Co. Ltd. All experiments were conducted with three biological replicates.

Exogenous ABA treatment of hydrangea leaves

Each two-year old hydrangea ('Ocean Heart' and 'White Angel') plant was exogenous sprayed with 100 mL of ABA solution (ABA was purchased from Caisson labs, New Jersey, USA), with the concentration being 100 μM. The CK was exogenous sprayed with equal volume of distilled water. The experiments were repeated three times. After 24 h of treatment, the healthy leaves at the fourth leaf position were collected. Then, the round pieces about 5 mm in diameter of PDA medium with *C. cassiicola* were inoculated on the surface of leaves. The petri dishes with leaves were placed in an incubator at 25℃ in dark for 48 h and were shifted to 16 /8 h (light/dark). The infected leaves were observed and photographed at 3 DPI. Lesion area was quantified using ImageJ software (v1.8.0).

RNA extraction, library construction, and sequencing

Total RNA was extracted from the sampled leaves of 'White Angel' and 'Ocean Heart' at 0 and 24 h post inoculation (HPI) with *C. cassiicola* using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol. The methods for library construction and sequencing were consistent with those used in previous research [2]. The samples were sequenced using the Illumina HiSeq2500 platform (Gene Denovo Biotechnology Co. Ltd., Guangzhou, China). Three biological replicates were employed for RNA-Seq analysis.

Bioinformatic analysis

Differential expression analysis between the two groups was conducted using DESeq2 software [24]. Genes exhibiting a false discovery rate (FDR) below 0.05 and an absolute fold change of ≥ 2 were deemed as DEGs. The KEGG enrichment analysis of DEGs was performed to identify enriched biological functions using KOBAS software [25].

qRT-PCR verification

The synthesis of cDNA was performed using the PrimeScript™ RT Reagent Kit (Takara, Shiga, Japan) in accordance with the manufacturer's protocol. The quantitative reverse transcription polymerase chain reaction (qRT-PCR) followed the methodology outlined in a previous report [26]. Transcript abundance estimates were derived from the mean of three biological replicates and calculated using the $2^{-\Delta\Delta Ct}$ method [27]. The reference sequences included *Hmβ-TUB* and *HmUPL7* [26]. All primers used for qRT-PCR were listed in Table S1.

Data analysis

All calculations were conducted using Microsoft Excel 2019, while statistical analyses were performed with the IBM SPSS Statistics software package version 20 (IBM Corporation, New York, USA). Data from each treatment were analyzed using one-way analysis of variance (ANOVA), and the Duncan multiple range test was employed to assess the significance of differences ($p < 0.05$) between treatments.

Results

Disease resistance of *H. macrophyllavarieties*

On 7 DPI, the disease symptoms on isolated leaves were observed, and the lesion area of leaf spots was measured (Fig. 1a and b). The results indicated that 'Ocean Heart' exhibited the most severe symptoms, with the largest lesion area measuring 25.73 cm^2, thereby categorizing it as the most susceptible variety. Conversely, 'White Angel' displayed the slightest symptoms and the smallest lesion area of 1.76 cm^2, indicating it as a disease-resistant variety. The resistance of the varieties, ranked from low to high based on lesion area, is as follows: 'Ocean Heart' < 'Girl in Red' < 'Wish You Peace' < 'Blue Danube' < 'Sweet Fantasy' < 'Cocktail' < 'You and Me Emotion' < 'Shui Tian Yi Se' < 'Spring Bird' < 'White Angel'.

Physiological indexes of 'White Angel' and 'Ocean Heart' in response to *C. cassiicola*

This study further investigates the physiological responses of different hydrangea germplasms to *C. cassiicola* infection, focusing on the resistant variety 'White Angel' and the susceptible variety 'Ocean Heart'. Physiological indices were measured to assess these responses. The activities of CAT and POD significantly increased 24 HPI in both 'White Angel' and 'Ocean Heart' (Fig. 2a and b). In the former, CAT and POD activities post inoculation were 5.63 and 3.67 times higher than that before inoculation, respectively. In contrast, in latter, CAT and POD activities post inoculation were 4.79 and 2.40 times higher than that before inoculation. Notably, the change of SOD activity in 'White Angel' leaves was not significant, but it increased significantly in 'Ocean Heart' (Fig. 2c). Additionally, the contents of MDA and SS significantly increased at 24 HPI in both varieties. In 'White Angel', the contents of MDA, SS, and Pro were of 1.16, 4.37, and 2.11 times higher than those before inoculation, respectively (Fig. 2d and e, and f). However, the increase of Pro content was not significant in 'Ocean Heart' leaves.

The infection of *C. cassiicola* resulted in a significant decrease of SA contents, but a significant increase in JA and ABA contents in both varieties (Fig. 3a, b and c). Notably, the increase of ABA content after inoculation in 'Ocean Heart' was significantly higher than that in 'White Angel'. Furthermore, the ET content in 'White Angel' was higher than in 'Ocean Heart' (Fig. 3d) post inoculation, but no significant differences were examined between two varieties. Among these hormones, the variation of ABA contents was the most pronounced one in both varieties.

Exogenous ABA treatment decreased hydrangea resistance to *C. cassiicola*

Exogenous ABA treatment decreased the resistance of hydrangea 'Ocean Heart' and 'White Angel' to *C. cassiicola*. At 3 DPI, the isolated leaf symptoms of both varieties were more severe under 100 μM exogenous ABA treatment compared to the CK. At the same time, the exogenous ABA treatment significantly increased the lesion area in both varieties (Fig. 4). Therefore, exogenous ABA treatment not only decreased the resistance to *C. cassiicola* of resistant hydrangea variety, but also increased the susceptibility of susceptible variety, highlighting the critical role of ABA in mediating this resistance.

Quality control of RNA-Seq data and sample relationship analysis

The total lengths of the raw reads and clean reads respectively ranged from 39,985,216 to 56,659,824 bases and 36,416,442 to 50,915,974 bases. The Q20 and Q30 percentages for all 12 libraries exceeded 98.02% and 94.71%, respectively. The proportion of ambiguous bases across the 12 libraries ranged from 0.000621 to 0.000714%. According to the absence of reference genomes, clean read transcripts were assembled using Trinity software for subsequent analysis. The total lengths of the transcript and unigene were 330,620,216 bp and 111,165,737 bp, respectively. The GC content of the transcript and unigene was 39.63% and 38.71%, respectively (Table S2).

Principal component analysis (PCA) showed that the 12 samples can be separated into four groups (Fig. 5a). Pearson correlation coefficient (PCC) of each group lays between 0.80 and 1.00, indicating a high degree of correlation in each group (Fig. 5b). The PCA and PCC results demonstrated a clear separation of transcriptional signatures induced by *C. cassiicola*, and confirmed a retrieval of high-quality RNA-Seq data for further analysis.

Identification of DEGs in response to *C. cassiicola*

The results of differential expression analysis showed that 5,802 genes were upregulated and 6,202 were downregulated in 'White Angel' (Fig. 6a), while 5,729 genes were upregulated and 6,163 were downregulated in 'Ocean Heart'. Additionally, a total of 16,311 DEGs were identified between 'White Angel' and 'Ocean Heart' before *C. cassiicola* inoculation, and 17,087

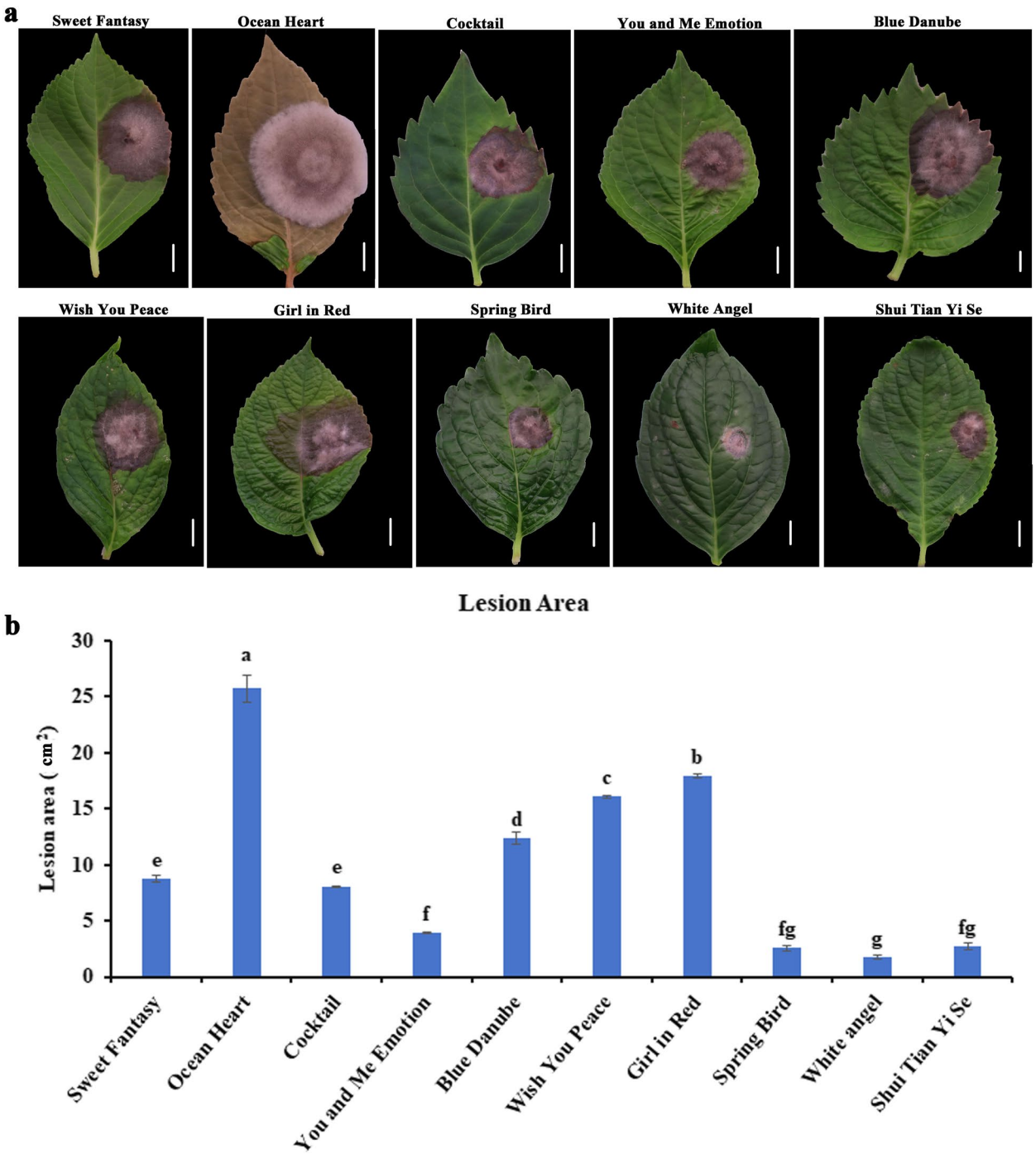

Fig. 1 The symptom (**a**) and leaf spot disease lesion area (**b**) of 10 *H. macrophylla* varieties on the 7 d post inoculation of *C. cassiicola*, the bar = 1 cm. Different letters on the bar chart indicate significant differences ($p < 0.05$) between treatments

DEGs were identified after inoculation. The number of DEGs between 'White Angel' and 'Ocean Heart' at 24 HPI was higher than that of 0 HPI, indicating that *C. cassiicola* infection altered gene expression in hydrangea leaves.

There were 9,092 co-expressed DEGs between the SCK-vs-RCK group and the ST-vs-RT group, and 5,128 co-expressed DEGs between the SCK-vs-ST group and the RCK-vs-RT group (Fig. 6b). The number of co-expressed DEGs between 'White Angel' and 'Ocean

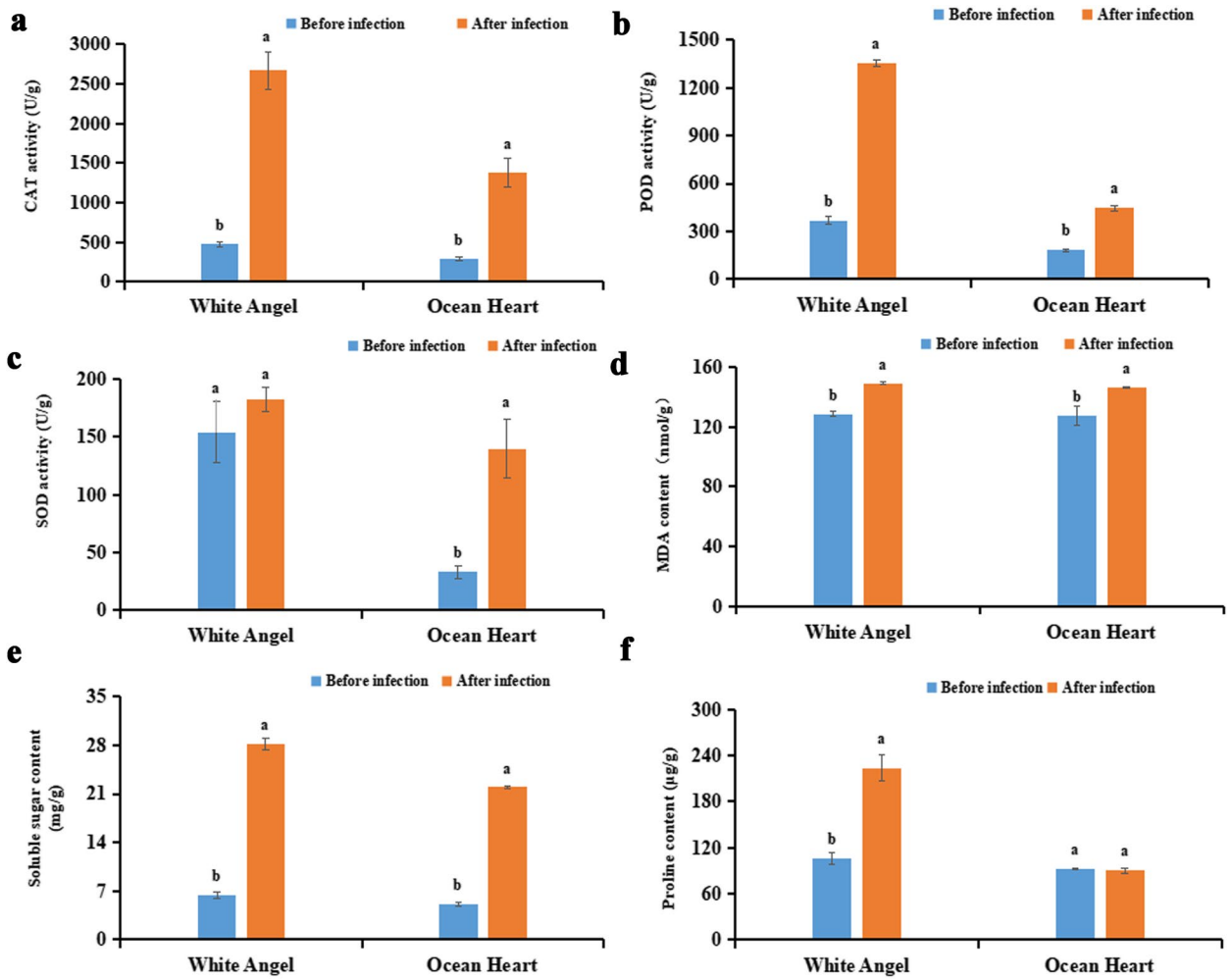

Fig. 2 The physiological indices in the leaves of the resistant variety 'White Angel' and the susceptible variety 'Ocean Heart' at 0 hours (before infection) and 24 hours (after infection) following the inoculation of *C. cassiicola*. **a** catalase (CAT) activity; **b** peroxidase (POD) activity; **c**superoxide (SOD) dismutase; **d** malondialdehyde (MDA) content; **e** soluble sugar (SS) content; **f** proline (Pro) content. Different letters on the bar chart indicate significant differences ($p<0.05$) between treatments

Heart' before *C. cassiicola* inoculation exceeded that of post inoculation, suggesting that the infection of *C. cassiicola* reduced the number of co-expressed genes in hydrangea leaves.

Analysis of DEGs in response to *C. cassiicola*

The top five KEGG pathways with the most significant Q values in RCK-vs-RT group were of plant hormone signal transduction, starch and sucrose metabolism, amino sugar and nucleotide sugar metabolism, glyoxylate and dicarboxylate metabolism, and peroxisome (Fig. 7a). The top five KEGG pathways with the most significant Q values in SCK-vs-ST group were of plant hormone signal transduction, phenylpropanoid biosynthesis, glyoxylate and dicarboxylate metabolism, amino sugar and nucleotide sugar metabolism, and MAPK signaling pathway-plant (Fig. 7b). Among them, plant hormone signal transduction, glyoxylate and dicarboxylate metabolism, and amino sugar and nucleotide sugar metabolism were significantly enriched in both varieties, whereas the plant hormone signal transduction was the sole pathway with the mostly significant FDR value (0.0043) in RCK-vs-RT. These results indicated that the response of hydrangea to *C. cassiicola* is a very complex biological process, and plant hormone signal transduction may play an important role in the response to *C. cassiicola*.

Expression analysis of ABA related DEGs in response to *C. cassiicola*

The change in ABA levels was the most significant aspect of the endogenous hormone in response to *C.*

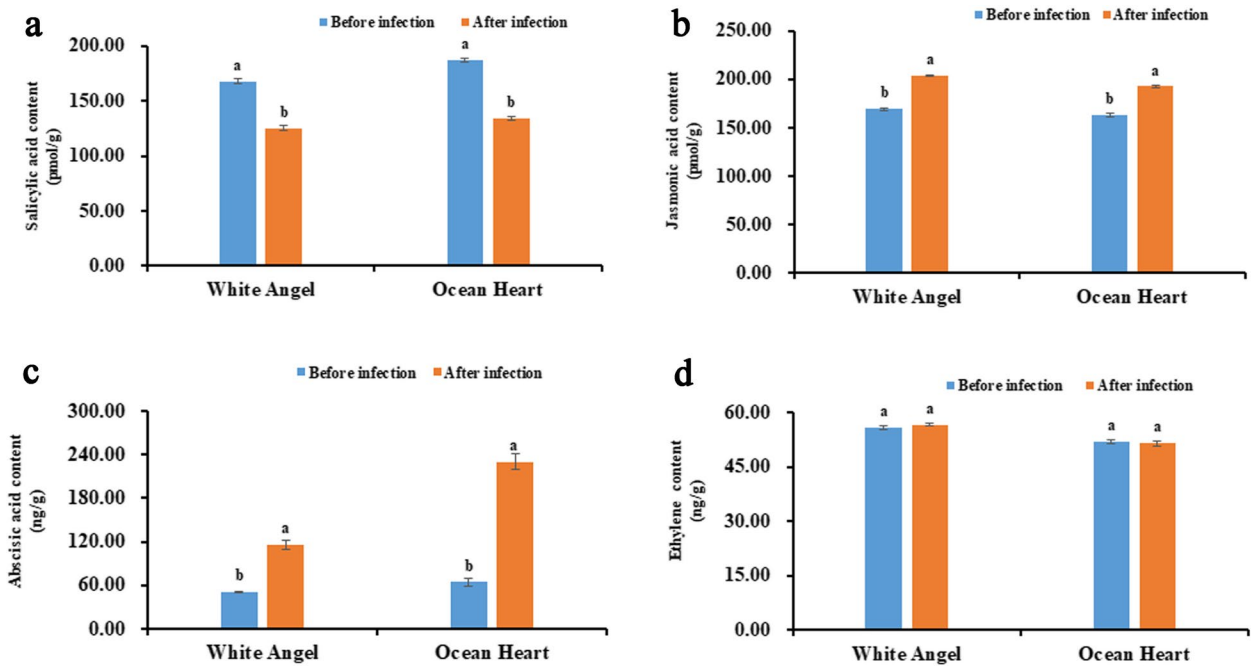

Fig. 3 Plant hormone content in leaves of the resistant variety 'White Angel' and the susceptible variety 'Ocean Heart' at 0 hours (before infection) and 24 hours (after infection) following the inoculation of *C. cassiicola*. **a** Salicylic acid (SA), **b** Jasmonic acid (JA), **c** Abscisic acid (ABA) and (**d**) Ethylene (ET). Different letters on the bar chart indicate significant differences ($p<0.05$) between treatments

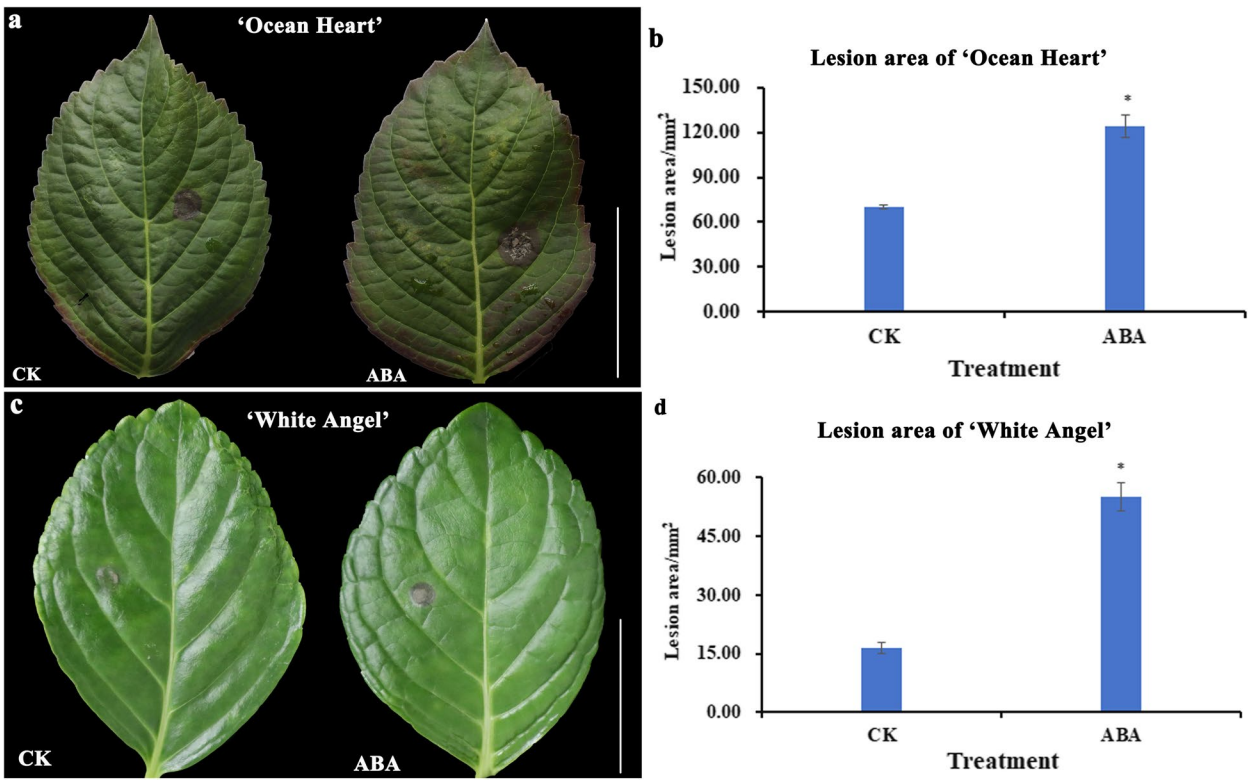

Fig. 4 The symptom (**a**) and leaf spot disease lesion area (**b**) of 'Ocean Heart' on the 3 d post inoculation of *C. cassiicola* under exogenous ABA treatment, the bar = 5 cm**.** The * on the bar chart indicate significant differences ($p<0.05$) between treatments

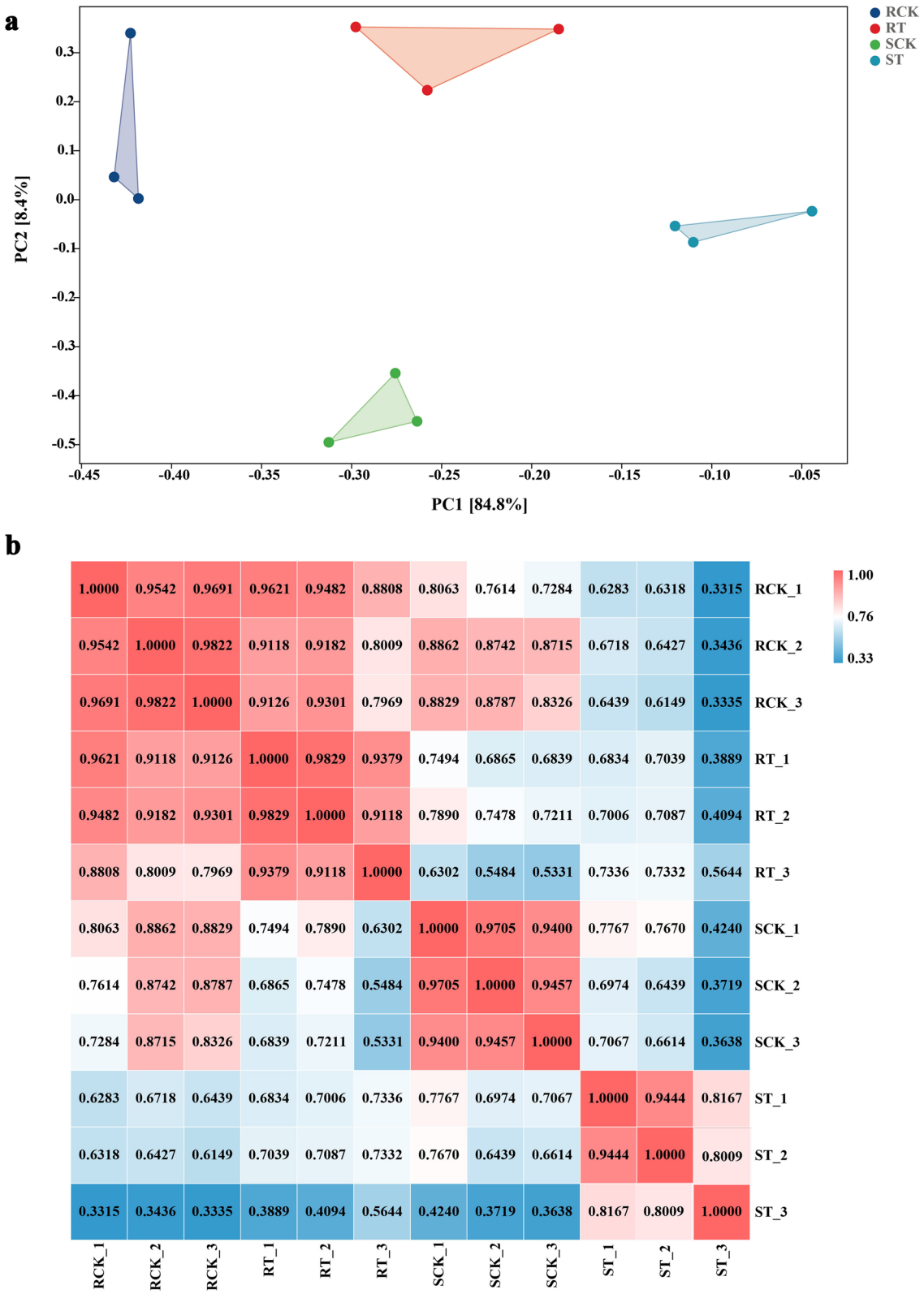

Fig. 5 Sample correlation analysis of RNA-Seq data. **a** Principal component analysis of samples. **b** Pearson correlation analysis between samples. SCK: the susceptible variety 'Ocean Heart' at 0 h post inoculation of *C. cassiicola*; RCK: the disease resistant variety 'White Angel' at 0 h post inoculation of *C. cassiicola*; ST: the susceptible variety 'Ocean Heart' under *C. cassiicola* at 24 h post inoculation of *C. cassiicola*; RT: the disease resistant variety 'White Angel' at 24 h post inoculation of *C. cassiicola*

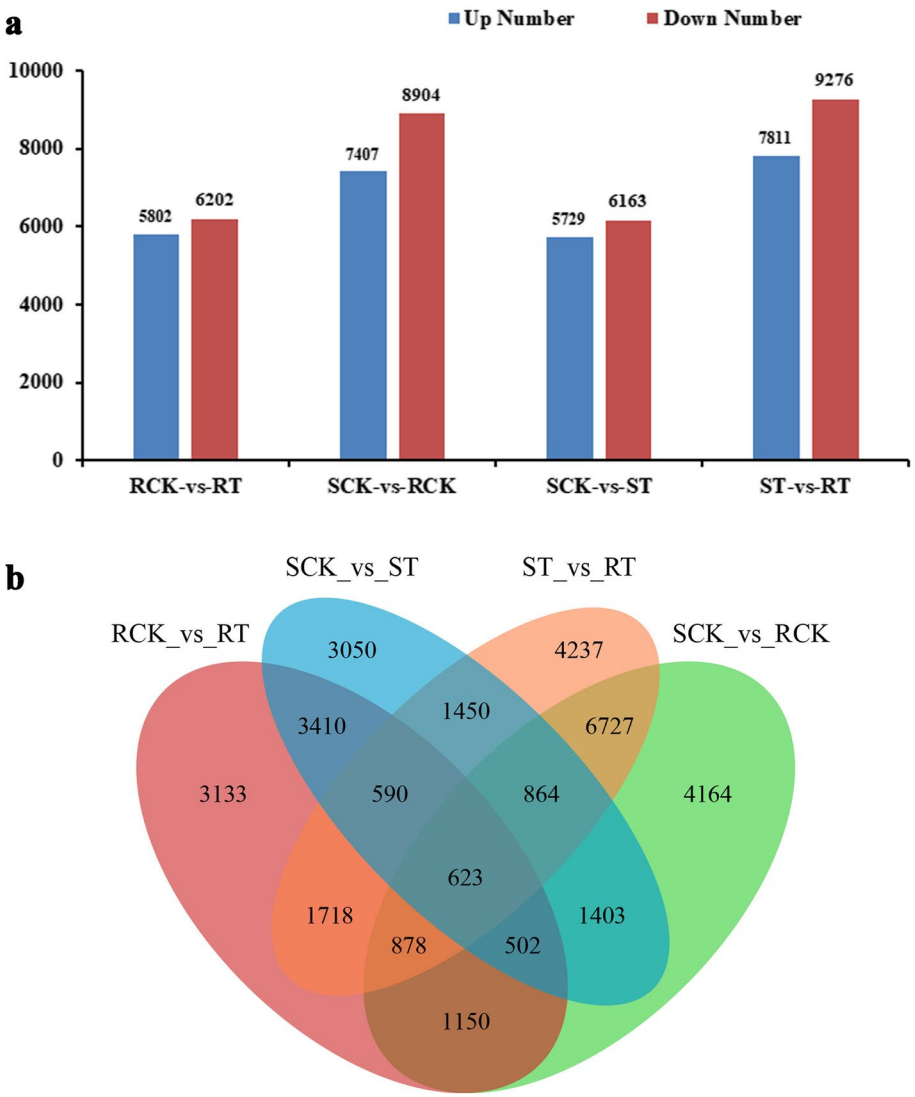

Fig. 6 Number of DEGs (**a**) and Venn diagram of DEGs (**b**) in leaves of 'White Angel' and 'Ocean Heart' in response to *C. cassiicola*. SCK: the susceptible variety 'Ocean Heart' at 0 h post inoculation of *C. cassiicola*; RCK: the disease resistant variety 'White Angel' at 0 h post inoculation of *C. cassiicola*; ST: the susceptible variety 'Ocean Heart' at 24 h post inoculation of *C. cassiicola*; RT: the disease resistant variety 'White Angel' at 24 h post inoculation of *C. cassiicola. Only two affiliated institutions can currently be included per author*

cassiicola. Combined with KEGG analysis, the results showed that plant hormone signal transduction pathways were significantly enriched in both varieties. Consequently, we focused on annotating DEGs related to ABA signal transduction and metabolism. In the ABA biosynthetic pathway, the expression of the genes *HmZEP3* (TRINITY_DN2183_c3_g1), *HmABA2* (TRINITY_DN34855_c0_g1), and *HmAAO3* (TRINITY_D95_c0_g1), which encode zeaxanthin epoxidase, was upregulated in response to *C. cassiicola* in both varieties (Fig. 8a). In plants, ABA 8'-hydroxylation is believed to play a predominant role in ABA catabolism [28]. The

(See figure on next page.)

Fig. 7 Analysis of DEGs in leaves of 'White Angel' and 'Ocean Heart' in response to *C. cassiicola*. **a** The top 20 KEGG pathways with the most significant Q value in 'Ocean Heart'. **b** The top 20 KEGG pathways with the most significant Q value in 'White Angel'. SCK: the susceptible variety 'Ocean Heart' at 0 h post inoculation of *C. cassiicola*; RCK: the disease resistant variety 'White Angel' at 0 h post inoculation of *C. cassiicola*; ST: the susceptible variety 'Ocean Heart' under *C. cassiicola* at 24 h post inoculation of *C. cassiicola*; RT: the disease resistant variety 'White Angel' at 24 h post inoculation of *C. cassiicola*

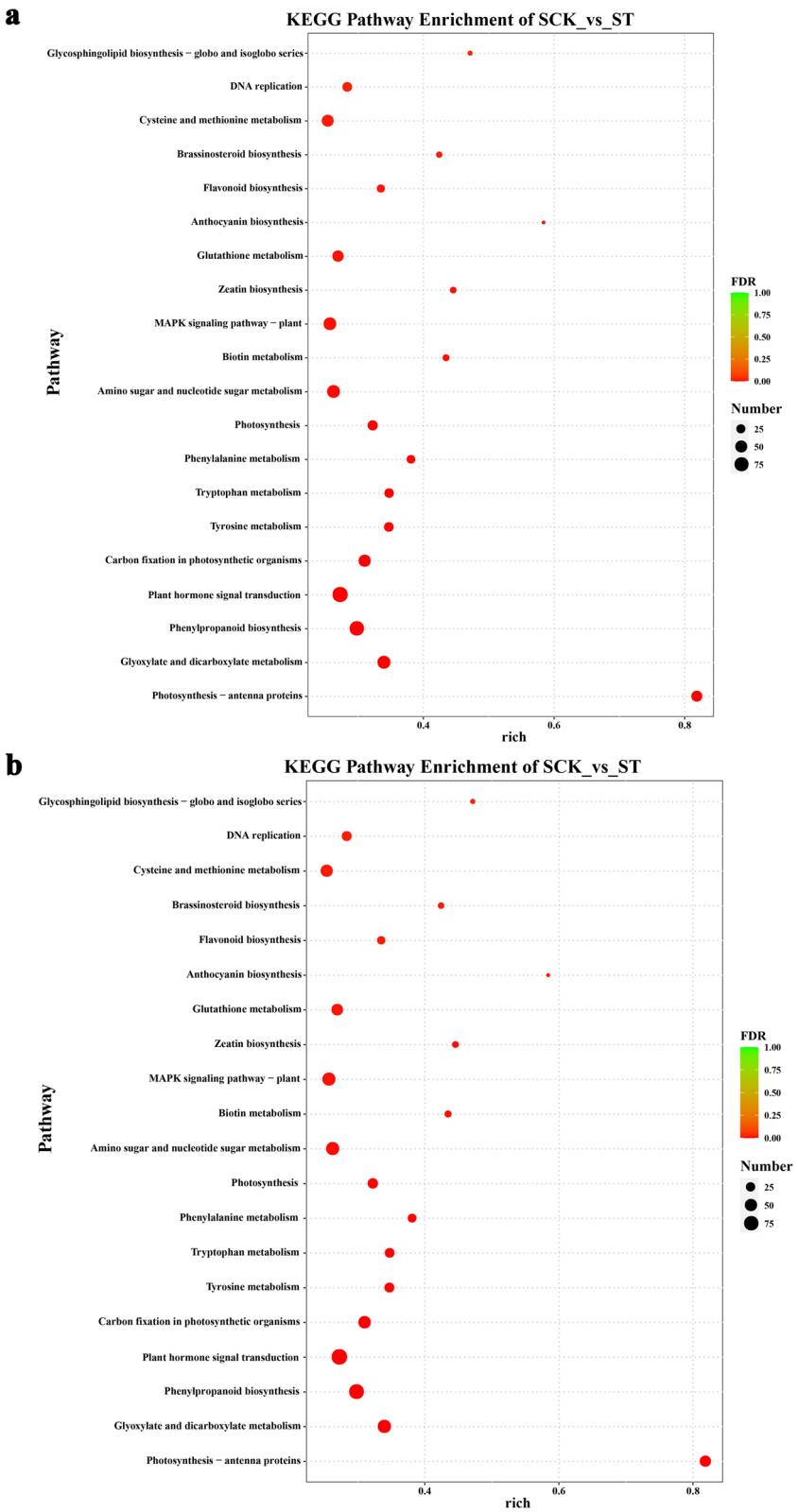

Fig. 7 (See legend on previous page.)

expression of *HmCYP707A4* (TRINITY_DN878_c11_g1), which encodes ABA 8'-hydroxylase, was significantly upregulated in the disease resistant variety 'White Angel', whereas it was slightly downregulated in the disease susceptible variety 'Ocean Heart' in response to *C. cassiicola* (Fig. 8b). In the ABA signal transduction pathway, the expression of the protein phosphatase *HmPP2C* (TRINITY_DN5318_c0_g1; TRINITY_DN1822_c0_g1) was also upregulated in response to *C. cassiicola* in both varieties (Fig. 8c). Furthermore, ABA-responsive element-binding transcription factors, *HmABFs* (TRINITY_DN598_c9_g1; TRINITY_DN3514_c0_g2; TRINITY_DN3514_c0_g1), were similarly upregulated in both varieties responding to *C. cassiicola*.

Validation of gene expression of selected genes by qRT-PCR

The quantitative reverse transcription polymerase chain reaction (qRT-PCR) data for the ten DEGs were consistent with the expression trends observed in the RNA-Seq data, thereby affirming the reliability of the RNA-Seq results (Fig. 9). The expressions of TRINITY_DN7759_c0_g1, TRINITY_DN27820_c0_g1, and TRINITY_DN878_c11_g1 were upregulated in response to *C. cassiicola* in the variety 'White Angel', while they were downregulated in 'Ocean Heart'. The expressions of TRINITY_DN25061_c0_g1 and TRINITY_DN44105_c0_g1 were upregulated in response to *C. cassiicola* in both varieties. Additionally, the expressions of TRINITY_DN529_c3_g1, TRINITY_DN25573_c0_g1, TRINITY_DN18907_c0_g1, TRINITY_DN43473_c0_g1, and TRINITY_DN9023_c0_g1 were upregulated in response to *C. cassiicola* in 'White Angel', with no significant changes in 'Ocean Heart'. Notably, the expression of TRINITY_DN878_c11_g1 (*HmCYP707A4*) in 'White Angel' at 24 HPI was 7.85 times higher than that at 0 HPI, while the expression level at 24 HPI in 'Ocean Heart' was only 51.68% of that at 0 HPI. Furthermore, the expression of *HmCYP707A4* in 'White Angel' was 15 times higher than that in 'Ocean Heart' at 24 HPI.

Discussion

The pathogenic fungus *C. cassiicola* is a necrotrophic pathogen that infects several economically important plants, including cucumber, tomato, and hydrangea [29]. A previous study demonstrated that the ITS sequence of *C. cassiicola* could be amplified in both resistant and susceptible cucumber varieties by PCR at approximately 6 HPI; however, physiological differences between the varieties began to happen until 12 to 24 HPI [30]. The present study also observed significant differences in physiological indices between resistant and susceptible hydrangea

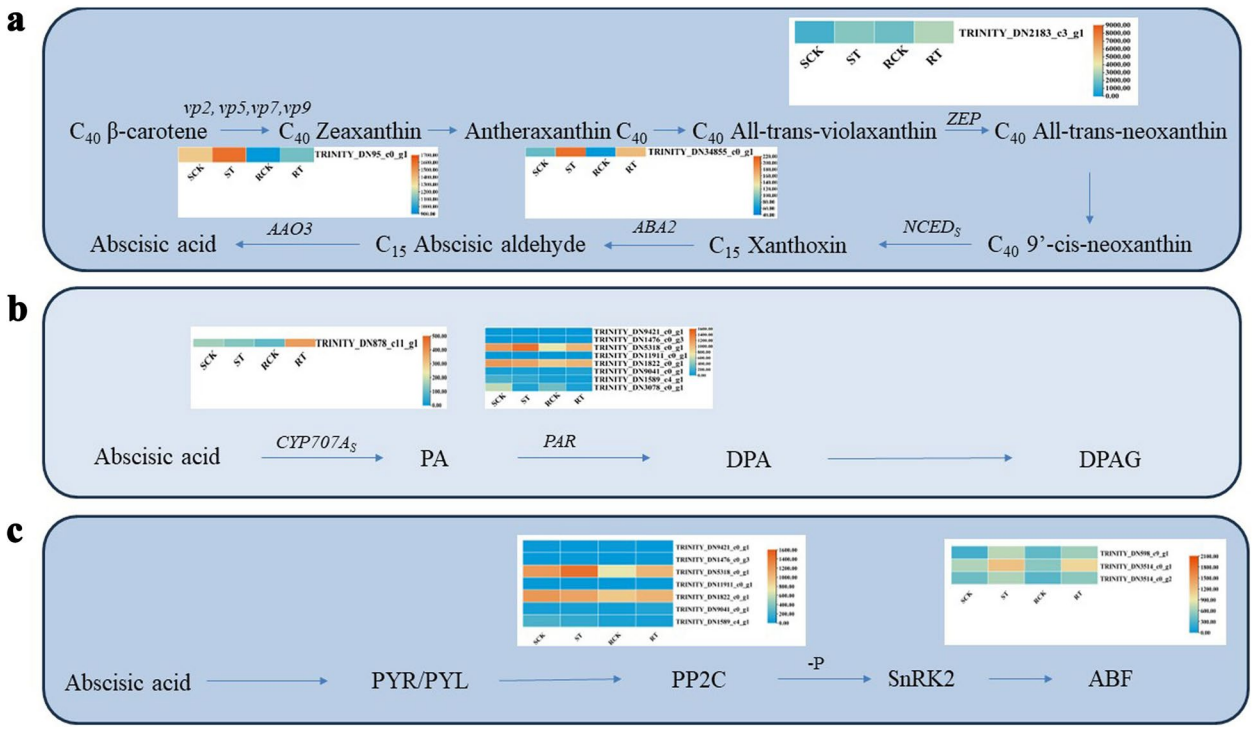

Fig. 8 Expression analysis of DEGs related to biosynthesis, catabolism, signal transduction pathways of abscisic acid by RNA-Seq in leaves of 'White Angel' and 'Ocean Heart' response to *C. cassiicola*. **a** ABA biosynthesis, **b** ABA catabolism, **c** ABA signal transduction

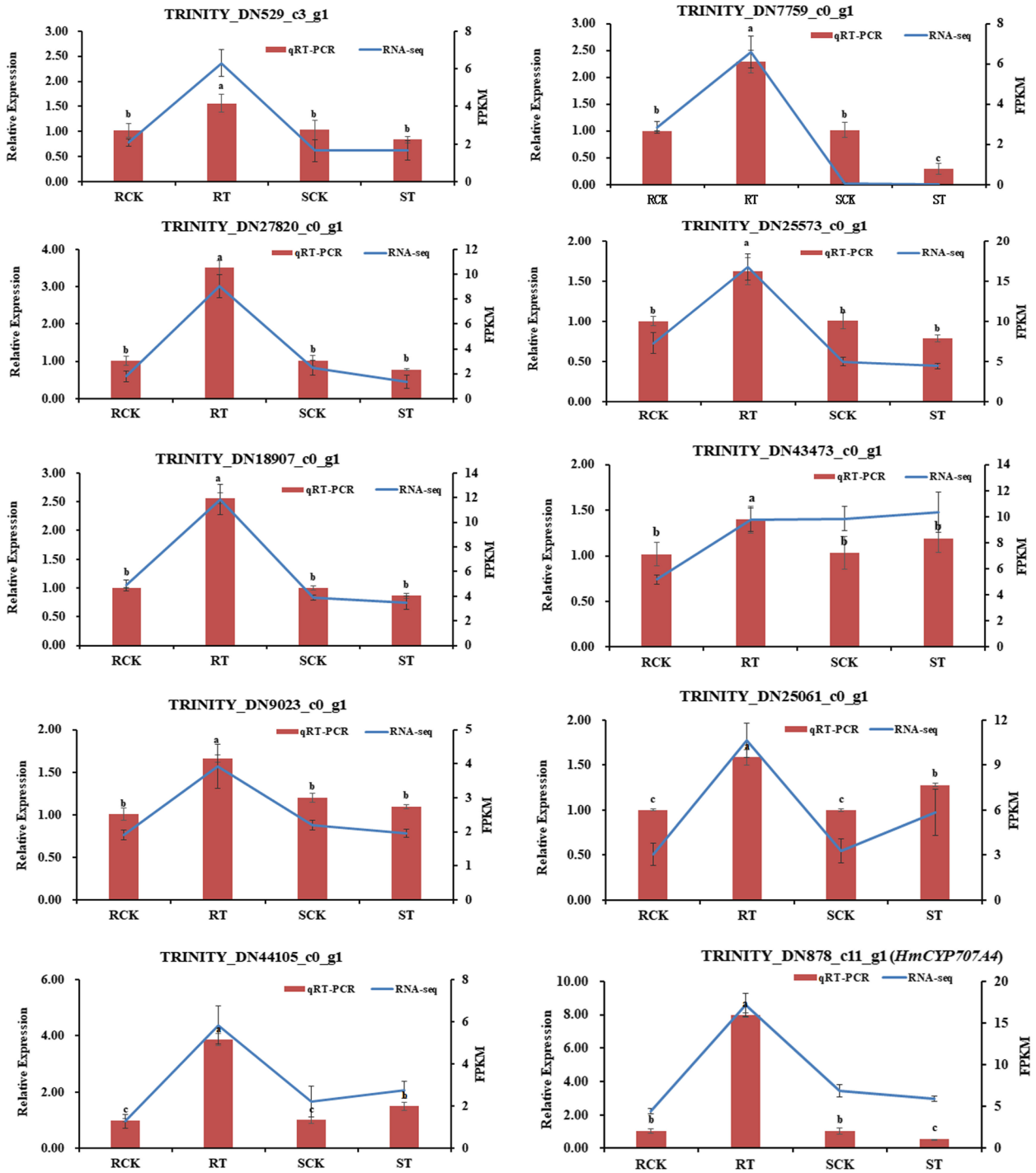

Fig. 9 Confirmation of transcript level of DEGs in leaves of 'White Angel' and 'Ocean Heart' response to *C. cassiicola*. SCK: the susceptible variety 'Ocean Heart' at 0 h post inoculation of *C. cassiicola*; RCK: the disease resistant variety 'White Angel' at 0 h post inoculation of *C. cassiicola*; ST: the susceptible variety 'Ocean Heart' under *C. cassiicola* at 24 h post inoculation of *C. cassiicola*; RT: the disease resistant variety 'White Angel' at 24 h post inoculation of *C. cassiicola*

varieties at 24 HPI. It was found that pathogen inoculation initially increased the activities of SOD, POD and CAT, with higher values observed in the resistant variety compared to the susceptible one [21]. Our findings align with these observations.

Plant hormone signaling molecules are crucial in mediating plant defense responses [31]. In this study, the

results indicated that plant hormone signal transduction was significantly enriched in both varieties; however, the increase of ABA content in the susceptible variety 'Ocean Heart' was markedly higher than that in the resistant variety 'White Angel'. ABA is the most important endogenous hormone in response to *C. cassiicola*, and the application of exogenous ABA can diminish resistance to *C. cassiicola* in both 'Ocean Heart' and 'White Angel'. Therefore, it can be reasonably concluded that ABA plays a pivotal role in the resistance of *H. macrophylla* to *C. cassiicola*.

ABA is commonly referred to as the "stress hormone" due to its role in responding to environmental stresses such as drought, thermal or heat stress, high salinity, low temperatures, and radiation stress [32]. It is the primary phytohormone that governs plant survival in harsh and variable environments [33, 34]. Numerous studies have demonstrated that ABA directly contributes to plant disease resistance in response to biological stress, and functions as a signal transduction factor that interacts with other hormones to modulate plant disease defense mechanisms [35, 36]. According to previous research, the hijacking of ABA signaling and stomatal closure by *Pseudomonas syringae* effectors constitutes a key mechanism of disease susceptibility [15]. Additionally, it has been found that ABA-inducible SnRK2-type kinase SAPK10 phosphorylates WRKY72 at Thr129, reducing the DNA binding ability of AOS1 and leading to the inhibition of AOS1 expression, which in turn alleviates JA synthesis. Consequently, the defense mechanism against rice white leaf wilt is mediated through an interaction with JA [37]. Moreover, research on *Arabidopsis* has shown that exogenous ABA inhibits the basal transcription of *Fusarium oxysporum* related defense genes and the transcription activated by JA-ET. By using ET and ABA signaling mutants, it has been demonstrated that the interaction between ABA and the ET signaling pathway is antagonistic in vegetative tissues. The antagonistic interactions between ABA and several components within the JA-ET signaling pathway have clarified how gene expression related to disease resistance and stress responses is regulated [38]. However, it remains unclear whether ABA directly participates in leaf spot disease resistance or only serves as a signal transduction factor that interacts with other hormones.

There are two primary pathways for ABA metabolism: cytochrome P450 monooxygenase (*CYP707As*) mediated ABA hydroxylation and glucosyltransferase-mediated ABA glycosylation. Hydroxylation of ABA can occur at three distinct methyl groups (C-7', C-8', and C-9'), with C-8' serving as the principal hydroxylation site. The hydroxylation of ABA predominantly occurs via the 8'-methyl hydroxylation pathway, which represents the primary mode of ABA catabolism in higher plants [39]. The 8'-hydroxylase, composed of cytochrome P450 (CYP) 707 A-mediated proteins (CYP707A1, CYP707A2, CYP707A3, and CYP707A4), is the key enzyme in this metabolic pathway. The *CYP707A* genes are crucial for ABA metabolism [28, 40]. These four genes exhibit distinct spatio-temporal expression patterns, suggesting that each gene product may act in different physiological or developmental processes, collectively regulating ABA breakdown [37]. Previous studies have shown that *CYP707A1* and *CYP707A2* are key genes controlling seed dormancy and germination in *Arabidopsis thaliana* [41]. Furthermore, *CYP707A3* expression is strongly induced during both dehydration and subsequent rehydration in *A. thaliana* [42]. In tomatoes, *SlCYP707A2* plays a critical role in ABA metabolism, regulating fruit ripening through its up-regulation and down-regulation [43]. These findings suggest that *CYP707As* play an important role in regulating the homeostasis of endogenous ABA, influencing plant growth and development, and enhancing plant resistance to stress. In the present study, the molecular mechanism by which *HmCYP707A4* regulates leaf spot disease in hydrangea remains to be explored further. Nevertheless, our results will provide a foundation for more detailed investigations into how *HmCYP707A4* regulates leaf spot resistance and will help accelerate the breeding of hydrangea varieties resistant to this disease in the future.

Conclusion

The present study evaluated the resistance to leaf spot disease in ten varieties of *H. macrophylla*. Among them, 'White Angel' was identified as a representative variety exhibiting resistance, while 'Ocean Heart' demonstrated susceptibility. Physiological results, RNA-Seq data analysis, and phenotypic observations from exogenous spraying of ABA clearly indicated that it plays a critical role in regulating resistance to leaf spot disease in hydrangea. Furthermore, *HmCYP707A4* has been identified as a key candidate gene for this regulation, which may facilitate future breeding efforts aimed at enhancing resistance in hydrangea.

Supplementary Information

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Supplementary Material 1.

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None.

Authors' contributions
Y.D. and H.C. conceived the project. H.C., X.L., X.Q., S.C., J.F., Y.J., and J.M. cultivated plants, collected samples and performed experiments. H.C. and X.L. visualized the data, performed the bioinformatic analysis and drafted the manuscript. H.C., M.Z.A., M.S. and Y.D. revised the manuscript. All authors reviewed and approved the manuscript.

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Data availability
The relevant data of this study were included in this article and the supplementary files. The transcriptome data have been uploaded to the National Center for Biotechnology Information under accession number PRJNA 1062999. The corresponding author can provide the data on request.

Declarations

Ethics approval and consent to participate
Not applicable. Our research did not involve any human or animal subjects, material, or data. We declare that the plant material in the experiment was collected and studied in accordance with relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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