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Volatiles emitted by *Pseudomonas aurantiaca* ST-TJ4 trigger systemic plant resistance to *Verticillium dahliae*

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ABSTRACT

Verticillium dahliae is among the most devastating fungal pathogens, causing significant economic harm to agriculture and forestry. To address this problem, researchers have focused on eliciting systemic resistance in host plants through utilizing volatile organic compounds (VOCs) produced by biological control agents. Herein, we meticulously measured the quantity of *V. dahliae* pathogens in plants via RT—qPCR, as well as the levels of defensive enzymes and pathogenesis-related (PR) proteins within plants. Finally, the efficacy of VOCs in controlling *Verticillium* wilt in cotton was evaluated. Following treatment with *Pseudomonas aurantiaca* ST-TJ4, the expression of specific *VdEF1-α* genes in cotton decreased significantly. The incidence and disease indices also decreased following VOC treatment. In cotton, the salicylic acid (SA) signal was strongly activated 24 h post-treatment; then, hydrogen peroxide (H_2O_2) levels increased at 48 h, and peroxidase (POD) and catalase (CAT) activities increased to varying degrees at different time points. The malondialdehyde (MDA) content and electrolyte leakage in cotton treated with VOCs were lower than those in the control group, and the expression levels of chitinase (CHI) and PR genes (*PR10* and *PR17*), increased at various time points under the ST-TJ4 treatment. The activity of phenylalanine ammonia lyase (PAL) enzymes in cotton treated with VOCs was approximately 1.26 times greater than that in control plants at 24 h , while the contents of phenols and flavonoids increased significantly in the later stage. Additionally, 2-undecanone and 1-nonanol can induce a response in plants that enhances disease resistance. Collectively, these findings strongly suggest that VOCs from ST-TJ4 act as elicitors of plant defence and are valuable natural products for controlling *Verticillium* wilt.

1. Introduction

Verticillium dahliae is a highly destructive soil-borne pathogenic fungus that causes *Verticillium* wilt in temperate and subtropical climates. This fungus exhibits significant genetic variability and can infect more than 660 plant species, including economically important crops such as cotton, tomato, potato, and eggplant (Hanson et al., 2021; Sun et al., 2024). Additionally, the fungus affects valuable tree species, such as *Cotinus coggygria*, *Sophora japonica*, and *Ulmus pumila*. Symptoms of *V. dahliae* infection in plants include wilting, leaf drop, necrosis, V-shaped spots, and yellowing of leaves (Van et al., 2021; Zhang et al., 2021; Godena et al., 2022), with severe cases leading to plant death and substantial economic losses in agriculture and forestry. Consequently, researchers worldwide are actively investigating effective strategies for controlling *Verticillium* wilt caused by *V. dahliae*.

Various approaches have been employed to mitigate the occurrence of *Verticillium* wilt, including soil amendment, soil fumigation, soil solarization, tillage, and the development of resistant plant varieties (Long et al., 2018; Castello et al., 2022). However, the longevity of *V. dahliae* in soil as microsclerotia in the absence of a host diminishes the efficacy of these methods in controlling *V. dahliae* (Shan et al., 2024). Furthermore, the use of fungicides and other chemical methods may have adverse effects on human health and the environment. Biological control is the most promising method of control due to its potential to reduce environmental pollution, achieve broad control spectrum, and generate long-lasting effects (Liu et al., 2023; Song et al., 2024).

Volatile organic compounds (VOCs) released by plant growth-promoting rhizobacteria (PGPRs) are low-molecular-weight, gaseous,

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metabolic substances that bacterial cells emit under normal circumstances (Raza et al., 2016). The VOCs produced by PGPRs perform several important functions, including controlling plant pathogens, promoting plant growth, and inducing systemic resistance, and exhibit biological activity even at low concentrations (Zou et al., 2010; Lee et al., 2012; Park et al., 2015). The mechanism by which VOCs exhibit activity is also advantageous over other methods of biocontrol and growth regulation, as VOCs do not require physical contact with pathogens or plant parts; in contrast, many other processes used to control phytopathogens and promote plant growth necessitate physical contact and close proximity (Anuj et al., 2024).

Numerous bacterial species, such as those belonging to the genera *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Serratia* and *Streptomyces*, have been shown to help promote plant growth and induce systemic resistance (Khamdan et al., 2020; Rani et al., 2022; Rohatgi et al., 2023). For instance, *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a were identified by Ryu et al. (2003) as producers of plant growth-promoting VOCs, such as 2,3-butanediol and acetoin. Additionally, the VOC 2-pentylfuran emitted by *B. megaterium* XTBG34 was found to stimulate the growth of *Arabidopsis thaliana* (Zou et al., 2010), while *P. fluorescens* SS101 produced 13-tetradecadien-1-ol, 2-butanone, and 2-methyl-n-1-tridecene, which promoted the growth of *Nicotiana tabacum* (Park et al., 2015). In addition to their growth-promoting effects, these VOCs induce plant tolerance against biotic and abiotic factors by eliciting systemic resistance (Tahir et al., 2017).

Several studies have investigated the process by which bacterial VOCs enhance plant growth and trigger systemic resistance by interacting with plant hormones. For example, Xie et al. (2009) reported increased photosynthetic activity and chlorophyll content in Arabidopsis plants exposed to VOCs from *B. subtilis* GBO3. Transcriptomic analysis of Arabidopsis plants exposed to GBO3-VOCs indicated that these compounds regulated auxin levels, leading to growth promotion (Zhang et al., 2007). Additionally, VOCs were found to differentially regulate the transcription of genes involved in the ethylene response and biosynthesis (Kwon et al., 2010).

Pseudomonas chlororaphis subsp. *aurantiaca* ST-TJ4 was obtained from the rhizosphere soil of poplar and was shown to possess a broad spectrum of antifungal properties (Kong et al., 2020a). Analysis of the inhibitory substances revealed that the VOCs produced by ST-TJ4 exhibited stronger inhibitory effects than those of diffusible substances against *V. dahliae* (Ni et al., 2022). Nevertheless, whether the VOCs produced by the ST-TJ4 strain can elicit host resistance against *V. dahliae* remains unknown. Therefore, this study was performed to address this gap in knowledge.

2. Materials and methods

2.1. Strains and culture conditions

P. chlororaphis subsp. *aurantiaca* ST-TJ4 was obtained from the interrhizosphere soil of poplar trees in Tianjin, China, in 2018 (CCTCC, NO: M2020435) (Kong et al., 2020a). The ST-TJ4 strain was maintained in King's B media supplemented with 50 % (v/v) glycerol at −80 °C for long-term storage (Johnsen and Nielsen, 1999). The pathogen *V. dahliae* was previously isolated from *Acer truncatum* in Jining, Shandong, China (Li et al., 2018), and preserved in the Forest Pathology Laboratory of Nanjing Forestry University. The fungus was cultured on potato dextrose agar (PDA) media for 10 days at 25 °C.

2.2. Experiment on the interaction between ST-TJ4 VOCs and cotton plants

Ten microlitres of ST-TJ4 were placed on a petri dish (35 mm) containing KB solid medium, which was then placed at the bottom of a tissue culture bottle. Cotton plants that had been cultured were transferred to a plastic cup (6 cm × 3 cm) with holes at the bottom. The spores of *V. dahliae* were mixed with soil (peat:vermiculite:perlite = 5:1:1, V/V) and added to the cup. The plastic cup with the cotton seedlings was attached to the tissue culture bottle that contained the ST-TJ4 strain VOCs and sealed with a film to prevent the volatiles from escaping. A control was set up using solid KB medium without the ST-TJ4 solution, and both were cultured at 25 ℃ with a 12-hour light/12-hour dark cycle for 20 days (Liu et al., 2021b). The experiment was replicated three times with 10 plants in each replicate.

2.3. Determination of pathogen colonization in plants treated with ST-TJ4 VOCs

Fungal plugs were affixed to the base of cotton rhizomes and exposed to a 100 μL solution of ST-TJ4 for 72 h. The base of the cotton rhizome, which is the attachment point, was cleaned, cut, and thoroughly mixed before being frozen in liquid nitrogen and stored at −80 ℃ for future use. DNA was extracted from the leaves using a SteadyPure Plant Genomic DNA Extraction Kit (AG21011, Accurate Biology, Hunan, China) and quantified by spectrophotometry, and 20 ng of DNA from each sample was used for RT–qPCR. RT–qPCR was performed using genomic DNA as the template and Hieff RT–qPCR SYBR Green Master Mix (CAT: 11202ES08; Yeasen, Shanghai, China) under the following conditions: an initial denaturation step of 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The *V. dahliae* target gene *VdEF1-α* was used for the quantification of fungal colonization. The 18 S rRNA gene of cotton was used as the endogenous reference gene (Kong et al., 2022). The specific sequences of the primers used for quantification are detailed in Table 1.

2.4. Determination of salicylic acid (SA) content in plants

The method used to inoculate pathogenic fungi was as described in Section 2.3. Cotton roots and stems (0.1 g) subjected to VOCs or not exposed to VOCs were weighed at 24, 48, and 72 h. Subsequently, the SA content of the plants was determined in accordance with the guidelines provided by Merck Biological Instructions.

2.5. Accumulation of reactive oxygen species in plants

The *V. dahliae* discs were inoculated onto the surface of the cotton rhizome and covered with sterile cellophane. Subsequently, 0.1 g of cotton rhizome exposed to VOCs or not exposed to VOCs was weighed at 24, 48, or 72 h. One millilitre of extract was added, and homogenization was performed in an ice bath. After centrifugation at 4 ℃ and 8000 rpm for 10 minutes, the supernatant was collected and placed on ice for analysis. Each treatment was replicated three times, and the H_2O_2 enzyme activity was assessed using the protocol for the reagent kit from Suzhou Keming Biotechnology Co., Ltd.

2.6. Determination of malondialdehyde (MDA) and electrolyte leakage (EL) levels

Pathogenic fungal inoculation was conducted in a manner consistent with the procedure outlined in Section 2.2. Specifically, 0.5 g of cotton rhizome was exposed to VOCs and compared with a sample that was not exposed to VOCs at 24, 48, and 72 h post inoculation. The samples were then processed by adding a specific quantity of quartz sand and 2

Table 1
Primers used to measure relative fungal biomass in cotton.

Primer	(5′–3′) Sequence
18S-F	CGGCTACCACATCCAAGGAA
18S-R	TGTCACTACCTCCCCGTGTCA
VdEF1-α-F	TGAGTTCGAGGCTGGTATCT
VdEF1-α-R	CACTTGGTGGTGTCCATCTT

Table 2
Sequences of primers used in this study.

Gene name	Forwards	Reverse
POD	CCGCATAACCATCACAAG	ACTCTCATCACCTTCAACA
PPO	ATATCCTTGTTCTGTCTGCTA	CTCCTTCTACCGTCTCTTC
PAL	TGGTGGCTGAGTTTAGGAAA	TGAGTGAGGCAATGTGTGA
PR10	ATGATTGAAGGTCGGCCTTTAGGG	CAGCTGCCACAAACTGGTTCTCAT
PR17	GGCTTTTCACCTGCCTGCTAT	CTGTCGGATGGGTTGGTTTGT
Ubiquitin	GAGTCTTCGGACACCATTG	CTTGACCTTCTTCTTCTTGTGC

millilitres of 10 % trichloroacetic acid, followed by thorough homogenization. Subsequently, 8 millilitres of 10 % trichloroacetic acid were added, followed by further grinding, and the resulting mixture was centrifuged at 4000 revolutions per minute for 10 minutes. The supernatant obtained from this process was utilized as an extract. A 2-millilitre portion of the supernatant was transferred to a clean test tube, while the control tube was replaced with 2 millilitres of distilled water. Two millilitres of 0.6 % thiobarbituric acid solution was added to each tube, and the contents were thoroughly mixed. The reaction was then conducted in a boiling water bath for 15 minutes, followed by rapid cooling and subsequent centrifugation. The absorbance (A) of the final supernatant was measured at wavelengths of 532, 600, and 450 nanometres using the following formula: c (MDA) (μM) = 6.45 (A532-A600) - 0.56A450 (Kong et al., 2020b).

At 24, 48, and 72 h post inoculation, segments of cotton roots and stems measuring approximately 5 mm in length were obtained and thoroughly rinsed with distilled water. Subsequently, these segments were placed in a centrifuge tube containing 10 mL of deionized water and placed in the dark for 24 h. The electrical conductivity of the roots and stems was then determined using a conductivity meter and recorded as EC1. Next, the sample was heated with boiling water for 20 minutes and allowed to cool to room temperature, after which the conductivity was measured as EC2. The relative conductivity, denoted as EL (%), was calculated using the formula EL (%) = (EC1/EC2) × 100 (Clavijo et al., 2012).

2.7. Measurement of plant defensive enzyme activity

The harmful fungus was introduced into a host in the same manner as described in Section 2.2. Cotton rhizomes (0.1 g) exposed to VOCs or not exposed to VOCs were weighed at 24, 48, and 72 h. Subsequently, these samples were analysed using a reagent kit (Keming Biology, Suzhou) to determine the activities of peroxidase (POD), phenylalanine ammonia lyase (PAL), catalase (CAT), β-1,3 glucanase (β-1,3-GLU), and chitinase (CHI).

2.8. Plant RNA extraction and quantitative gene expression assay

The pathogenic fungal inoculation was performed as described in Section 2.2. RT—qPCR was used to assess the expression of five stress resistance-related genes in cotton following exposure to VOCs and in the absence of VOCs. These genes included three plant defence-related genes (*POD* and *PAL*) and two disease progression protein-related genes (*PR10* and *PR17*) (Wubben et al., 2008). Total RNA was extracted using a Trazol up Plus RNA Kit (Takara Biotech., Beijing, China). Ubiquitin was utilized as a reference gene, and the relative expression levels were determined using the $2^{-\Delta\Delta Ct}$ method.

2.9. Measurement of total flavonoid content in plants

Cotton root samples (0.1 g) that had been subjected to VOCs and a control group without VOC exposure were weighed. The samples were ground into a powder using liquid nitrogen, and then 5 millilitres of 60 % ethanol were added. The mixture was placed in a water bath and refluxed at 70 °C for 60 minutes. The solution was filtered, and the volume was adjusted to 5 millilitres. Two millilitres of the resulting sample was added to 0.5 millilitres of 5 % sodium nitrite solution and allowed to stand for 6 minutes. Subsequently, 0.5 millilitres of 10 % aluminium nitrate solution was added, and the mixture was allowed to stand for 6 minutes. Then, 4 millilitres of 4 % NaOH was added, the solution was shaken well, and the volume was adjusted to 10 millilitres with 60 % ethanol. The solution was left to stand for 15 minutes, after which the absorbance was measured at 510 nm (Wang et al., 2022).

2.10. Measurement of phenolic content in plants

The Folin-phenol method was utilized to quantify light absorption at an OD of 765 nm and establish a standard curve. A 1 g sample of cotton root was pulverized to powder using liquid nitrogen in a mortar. Subsequently, 8 mL of 80 % methyl alcohol was added to the sample, which was subsequently transferred to a 15 mL centrifuge tube. The mixture was subjected to ultrasonication for 25 minutes and then centrifuged at 5000 rpm/min for 10 minutes. Following centrifugation, 800 μL of the supernatant was extracted and combined with 500 μL of Folin reagent and 2 mL of 20 % $NaCO_3$ solution. The resulting mixture was left at room temperature for 30 minutes. The OD at 765 nm was then determined, and the corresponding total phenol content was calculated using a standard curve (Wang et al., 2022). All experiments were repeated twice, and each treatment was conducted in triplicate.

2.11. Statistical analysis

The data were analysed by analysis of variance and Duncan's multiple comparison test with SPSS 17.0 software (IBM, Armonk, NY, USA), and the standard errors of all mean values were calculated ($p < 0.05$). Prism 8.0 software (Prism Software, Irvine, CA, USA) was used for drawing.

3. Results

3.1. ST-TJ4 VOCs alleviate cotton wilt disease and reduce the amount of *V. dahliae* in plants

The application of ST-TJ4 volatiles to cotton plants significantly reduced leaf yellowing, necrosis, defoliation and decreased disease severity (Fig. 1A). The untreated cotton exhibited an incidence rate of 67.73 % and a disease index of 68.33, whereas the cotton treated with ST-TJ4 VOCs had an incidence rate of 30.12 % and a disease index of 58.33 (Fig. 1B). The relative copy number at the base of the cotton rhizome was utilized to determine the biomass of *V. dahliae* in the host root. The relative expression levels of the target genes (*VdEF1-α*) in plants without VOC treatment were significantly greater than those in plants treated with ST-TJ4 VOCs, with the latter showing an approximately 3.47-fold lower expression level (Fig. 1C). This result suggested that the VOCs of ST-TJ4 induce discernible alleviation of cotton *Verticillium* wilt.

B

Treatment	Disease rate (%)	Disease index
Vd	67.73±7.30	68.33±5.77
VOCs	30.12±7.10	58.33±36.17

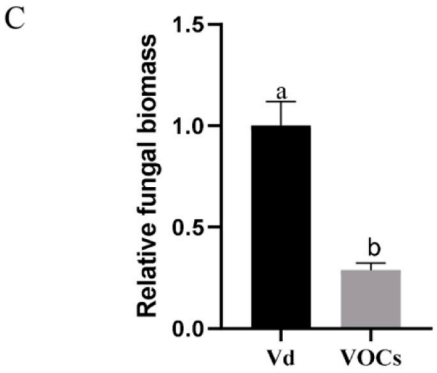

Fig. 1. Control effect of ST-TJ4 VOCs on *Verticillium* wilt in cotton. (A) Phenotype, (B) disease rate and disease index and (C) amount of fungal colonization. CK: Control; Vd: inoculation with pathogens only; VOCs: inoculation with pathogens and exposure to VOCs. Vertical bars represent the standard deviation of the average (n = 3). Different lowercase letters represent significant differences (p <0.05).

3.2. Increased resistance to ST-TJ4 VOCs is related to the elicitation of SA and H_2O_2 signals

We focused on examining the potential roles of H_2O_2 and SA signals in the triggering of VOCs and host resistance to *V. dahliae*. Our experiment revealed that significantly more SA in cotton was activated after 24 h of treatment than in the control (Fig. 2A), suggesting that the VOCs of ST-TJ4 play a role in initiating the host immune response through SA signalling during the initial stages of treatment.

For the oxidation signal H_2O_2 (Fig. 2B), there was no notable alteration in H_2O_2 levels in cotton subjected to ST-TJ4 VOCs compared to untreated cotton at the 24-h mark. However, following 48 and 72 h of VOC treatment, the H_2O_2 content in cotton rapidly increased, suggesting that ST-TJ4 VOCs enhanced the oxidation reaction to combat pathogen infection.

3.3. ST-TJ4 VOCs amplify plant resistance by regulating antioxidant defence systems

Following treatment with VOCs, the enzyme activity of POD in cotton exhibited an increasing pattern, surpassing the control levels across all periods (Fig. 3A). The alteration in POD gene expression mirrored the trend in POD enzyme activity (Fig. 3B). Similarly, the activity of CAT initially increased, peaked at 48 h, and subsequently stabilized at 72 h, with no notable difference from that of the control (Fig. 3C).

The alterations in MDA content within plants are depicted in Fig. 3D. The findings revealed that following treatment with the ST-TJ4 VOCs, the MDA content in plants was consistently lower than that in the control group across all periods (Fig. 3D), with statistically significant disparities. Concurrently, the EL in the plants exhibited a decrease to approximately 0.68 times less than that of the control group after 72 h (Fig. 3E).

3.4. ST-TJ4 VOCs increased the activity of defence-related PR proteins against V. dahliae

The enzymes CHI and β-1,3-GLU are involved in the breakdown of fungal cell walls. This study revealed a notable increase in CHI enzyme activity in plants exposed to ST-TJ4 VOCs after 24 h of treatment, which significantly differed from that in the control. Although the CHI levels decreased over time, they remained higher than those in the control at the 72-h mark (Fig. 4B). Conversely, the changes in β-1,3-GLU activity appeared to be gradual at all time points, with no significant difference compared to the control (Fig. 4A).

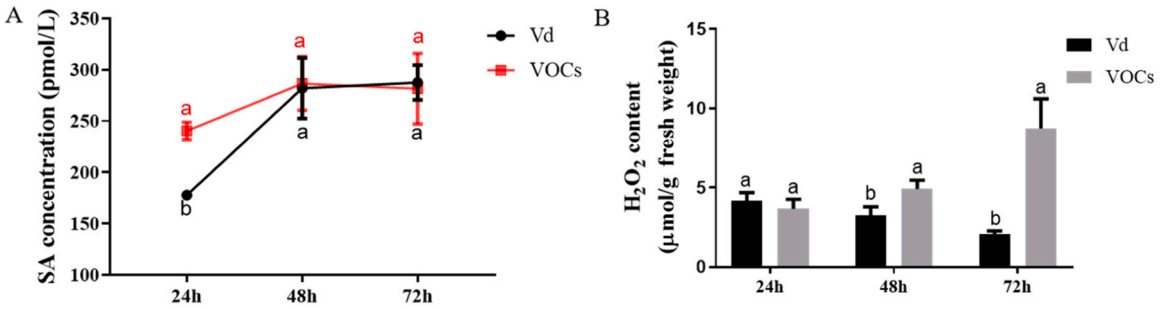

Fig. 2. ST-TJ4 VOCs activate host SA and H_2O_2 signals to confer resistance to *V. dahliae* infection. (A) SA content and (B) H_2O_2 content. Different lowercase letters represent significant differences (p <0.05) between Vd and VOCs at the same point.

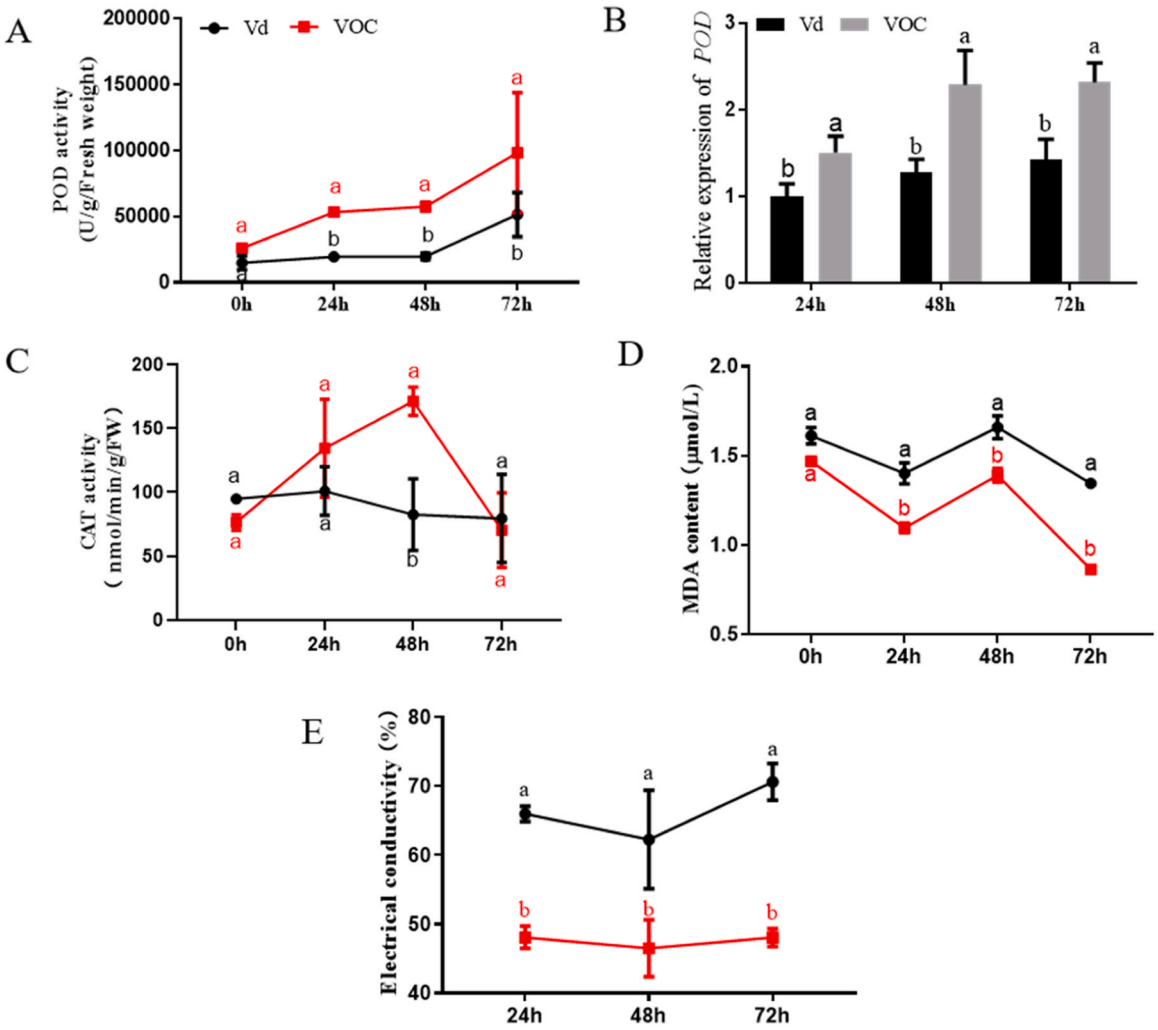

Fig. 3. ST-TJ4 VOCs regulated antioxidant defence in cotton. (A) POD activity, (B) *POD* gene expression, (C) CAT activity, (D) malondialdehyde content and (E) electrical conductivity. Different lowercase letters represent significant differences (p <0.05) between Vd and VOCs at the same point.

The proteins *PR10* and *PR17* are associated with plant disease. The protein encoded by the *PR10* gene exhibits nuclease activity, enabling host resistance to be bolstered by the degradation of nucleic acid in the pathogen. Moreover, *PR17* can directly interact with pathogen effectors to impede pathogen infection. RT—qPCR revealed that the *PR10* gene in plants treated with ST-TJ4 VOCs was significantly induced at 48 h, and its expression continued to increase over time. By 72 h, the expression level of the *PR10* gene was approximately 2.67 times greater than that in the control (Fig. 4C). Similarly, expression of the *PR17* gene was strongly induced during the initial stage, and by 72 h, its expression level was approximately 1.33 times that in the control (Fig. 4D).

3.5. *Involvement of the PAL metabolism pathway in ST-TJ4 VOC-induced resistance in cotton*

PAL is a group of enzymes closely associated with the biosynthesis of flavonoid pigments. The PAL enzyme activity in cotton rapidly increased 24 h after exposure to ST-TJ4 VOCs and significantly differed from that of the control, with a 1.26-fold increase (Fig. 5A). The pattern of change in *PAL* gene expression mirrored that of PAL enzyme activity (Fig. 5B). Concurrently, the total flavone content in cotton was assessed and found to be 1.36-fold greater than that of the control after 72 h of treatment (Fig. 5C). The changes in phenolic substances followed a trend similar to that of total flavonoids (Fig. 5D). Notably, the phenolic content in cotton significantly increased to approximately 2.17 times that of the control after 72 h of VOC treatment.

3.6. *2-Undecanone and 1-nonanol primarily contribute to ST-TJ4-induced resistance against cotton wilt disease*

Based on the volatile profile of the ST-TJ4 strain as reported in prior research (Ni et al., 2022), we screened potential elicitors. The results showed that the entire young roots of cotton plants infected with *V. dahliae* became black. However, when cotton roots were treated with varying concentrations of 2-undecanone and 1-nonol, the symptoms were noticeably alleviated (Fig. 6 A). Notably, the optimal concentration of 2-undecanone ranged from 10 to 200 μg/mL without significant variation (Fig. 6B), while the most effective concentration of 1-nonanol was determined to be 100 μg/mL (Fig. 6 C). These findings indicated that these two substances induced resistance to *V. dahliae* in cottons.

4. Discussion

According to various sources, VOCs produced by microorganisms in the rhizosphere of plants exert positive environmental effects. These compounds facilitate beneficial interactions between PGPR and plants, leading to the systemic resistance of plants to biological and abiotic stressors. Additionally, these compounds inhibit fungal and bacterial pathogens and promote plant growth. This study aimed to explore the interactions between VOCs produced by *P. aurantiaca* ST-TJ4 and plants.

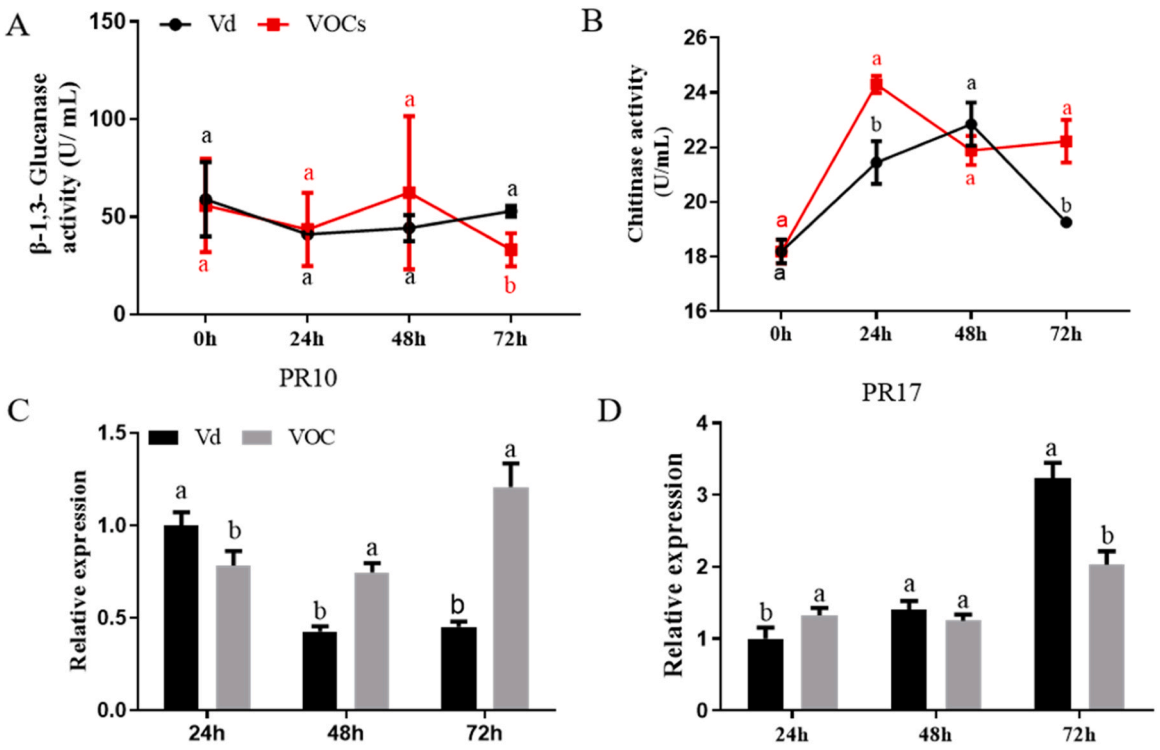

Fig. 4. ST-TJ4 VOCs increased the activity of plant defence-related PR proteins. (A) β-1,3-glucanase and (B) chitinase levels, (C, D) Relative expression of the *PR10* and *PR17* genes. Different lowercase letters represent significant differences (p <0.05) between Vd and VOCs at the same point.

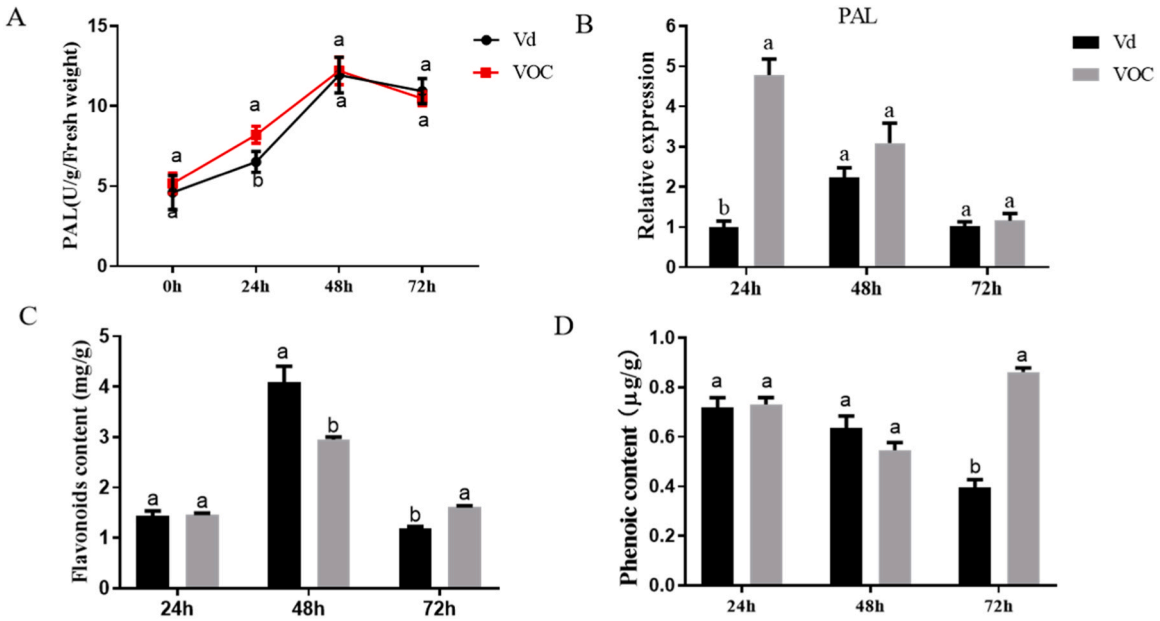

Fig. 5. ST-TJ4 VOCs activated the phenylpropanoid metabolic pathway. (A) PAL activity, (B) relative PAL expression, (C) flavonoid content and (D) phenolic content. Different lowercase letters represent significant differences (p <0.05) between Vd and VOCs at the same point.

Assessing the pathogen content in plants and the occurrence of disease is crucial for evaluating the efficacy of biological control techniques. Previous research has shown that the specific gene expression of *V. dahliae* in tobacco and cotton stem bases is downregulated after treatment with the *B. velezensis* BvR001 wettable powder WPBvR001 (Ji et al., 2021). In this study, the expression of the specific gene *VdEF1-α* was also significantly reduced in cotton after treatment with VOCs from ST-TJ4. The length of cotton root disease spots was lower than that in the control, and the incidence rate and disease index also decreased, indicating that the ST-TJ4 VOCs induced a resistance response in the host.

The interplay between SA and ROS in the defence response to biotic stress has been comprehensively demonstrated (Herrera-Vsquez et al., 2017). The SA-dependent pathway in tomato is triggered by the beneficial fungus *Trichoderma virens*, leading to an enhancement in the resistance of tomato plants to *Fusarium* (Jogaiah et al., 2018). Furthermore, a recent investigation indicated that *T. harzianum* augments its defence against *Fusarium* by modulating the generation of ROS in

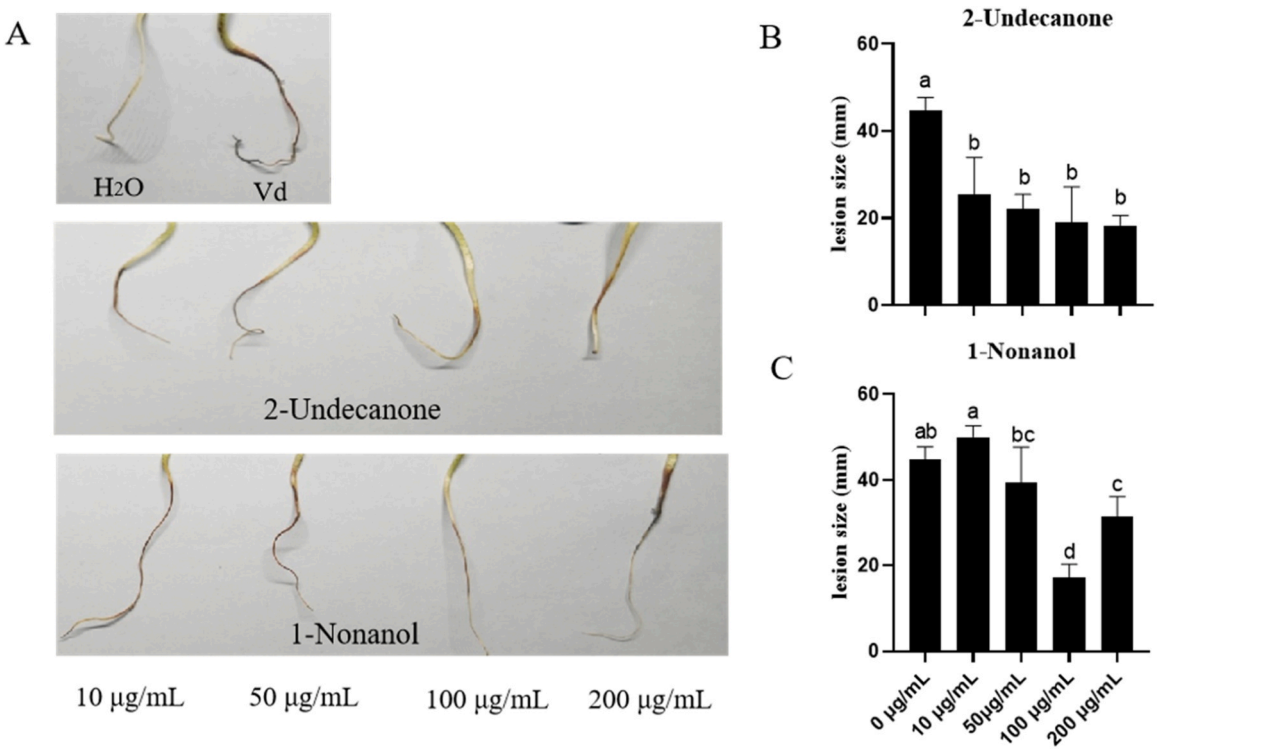

Fig. 6. 2-Undecanone and 1-nonanol induce systemic resistance in plants. (A) Cotton root disease phenotype and (B, C) size of cotton root lesions induced by 2-undecanone and 1-nonanol.

cucumber roots (Chen et al., 2019). In the present study, the SA content of plants treated with ST-TJ4 VOCs rapidly increased in the initial stage (24 h); then, the H_2O_2 content rapidly increased at 48 h, with notable disparities compared to those of the control. These findings demonstrate that VOCs from ST-TJ4 bolster host resistance by activating host SA and H_2O_2 signals.

Antioxidant enzyme systems and nonenzymatic antioxidants are crucial defence mechanisms that can effectively mitigate oxidative stress in plants subjected to environmental stress (Ma et al., 2023). In this study, the VOCs produced by ST-TJ4 were found to enhance the activity of POD and CAT in pathogen-infested plants, thereby helping the host bolster its defence mechanisms. MDA, the end product of lipid peroxidation, is commonly utilized as an indicator of oxidative cellular damage under stress conditions (Hong et al., 2021). The results of this experiment revealed that the MDA content and electrolyte leakage in cotton treated with ST-TJ4 VOCs were significantly lower than those in the control group, indicating that the VOCs alleviated oxidative damage to plant cell membranes. These findings demonstrate that ST-TJ4 VOCs can modulate the host antioxidant system to induce resistance in the host. PR proteins constitute a group of proteins that play a pivotal role in the disease resistance response in plants. For instance, researchers have reported that *PR10.5D1* mediated lipid metabolism pathway to strengthen structural defense and activate defense signaling, which largely released the repression of cell growth caused by *V. dahliae* infection (Guo et al., 2022). The PmPR10 recombinant protein purified from *Pinus monticola* has inhibitory effects on both the mycelial growth and spore germination of *Cronartium ribicola*, *Phoma exigua*, and *Phoma argillacea* (Liu et al., 2021a). The silencing of NbPRp27, a secretory protein from the PR17 family in *Nicotiana benthamiana*, can diminish the tobacco's innate resistance to *Pseudomonas syringae* pv. *tabaci*, thereby permitting a greater bacterial infestation (Xie and Goodwin, 2009). In the present study, plants treated with VOCs from ST-TJ4 exhibited overexpression of the *PR10* gene at a later stage (72 h), while the *PR17* gene was more highly expressed than in the control at the beginning of the treatment. These findings indicate that these two PR proteins promoted resistance at different stages in plants treated with ST-TJ4 VOCs.

Both β-1,3-GLU and CHI, which are composed of β-1,3-glucan and chitin, can target the cell walls of plant pathogenic fungi. β-1,3-GLU can breakdown β-1,3-glucan, leading to loosening of the fungal cell wall and inhibiting fungal pathogen growth (Sun et al., 2021). On the other hand, CHI can catalyse the hydrolysis of chitin in the cell wall of pathogenic fungi, resulting in the production of N-acetylglucosamine, which can directly damage the cell wall of pathogenic fungi. The combined action of these enzymes can compromise the integrity of the fungal cell wall, causing protoplasm leakage and mycelium breakage (Yuan, et al., 2023; Negin et al., 2024; Naresh et al., 2024). In our research, the activity of CHI in plants significantly increased following treatment with VOCs, showing a similar pattern to that of antioxidant enzymes. This increased activity may effectively target the fungal cell wall and prevent fungal infection. Conversely, the activity of β-1,3-GLU did not significantly differ after VOC treatment compared to that of the control, possibly due to the varying degrees of pathogen regulation of host innate immunity.

Metabolites derived from the phenylpropane pathway, including phenols, flavonoids, and lignin, play a crucial role in plant defence against pathogens (Roy et al., 2016). The heightened enzymatic activity within this metabolic pathway is a common response in plants to combat diseases. PAL, an essential enzyme, initiates the phenylalanine metabolism pathway by converting phenylalanine from the hydrolysis pathway to cinnamic acid. Phenolic compounds, characterized by hydroxyl and aromatic hydrocarbon groups, are synthesized by cottons and contribute to *Verticillium* wilt resistance (Xiong et al., 2021). Flavonoids, a category of secondary metabolites in plants, confer resistance to pathogen infections (Xu et al., 2023). Lignin, a complex organic polymer, performs various functions, such as providing structural support to cell walls, facilitating water transport, and participating in defence mechanisms (Yu et al., 2022). Our findings demonstrated that the activity of PAL in plants treated with VOCs rapidly increased in the initial stage, reaching approximately 1.26 times that in the control group, and the expression level of the *PAL* gene exhibited a trend similar

to that of the enzyme activity. Furthermore, the levels of flavonoids and total phenols notably increased within 72 h. These results indicate that the VOCs produced by ST-TJ4 activate the host phenylpropanoid metabolism pathway.

5. Conclusion

In summary, this study demonstrated that *P. aurantiaca* ST-TJ4 can induce systemic resistance against *V. dahliae* in the host through emitting VOCs. The ST-TJ4 volatiles can impact *V. dahliae* colonization in plants by inducing the activation of SA and H_2O_2 signalling molecules inside the host. After exposure to VOCs, the levels of MDA decreased, while the activities of POD and CAT increased over a predetermined period. This result implies that VOCs reduce damage to membrane lipids and stimulate the antioxidant system of the host plant. In addition, the plant resistance-related proteins *PR10* and *PR17* were active, and host chitinase activity increased, hastening the breakdown of pathogenic fungal cell walls. Concurrently, the host's phenylpropanoid metabolic pathway was enhanced by VOCs, which promoted the accumulation of secondary metabolites, such as flavonoids and phenols. The main chemicals that induce resistance in ST-TJ4 are 1-nonanol and 2-undecanone. Further investigation is required to determine the signal pathways through which host defence responses are activated by these two elicitors, ascertain the presence of any synergistic interactions between them, and assess their practical application effect in real agricultural filed.

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CRediT authorship contribution statement

Hang Ni: Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Wei-Liang Kong:** Writing – review & editing, Resources, Methodology, Data curation, Conceptualization. **Qiao-Qiao Zhang:** Writing – review & editing, Methodology, Formal analysis. **Xiao-Qin Wu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no competing interests

Data availability

No data was used for the research described in the article.

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