

RESEARCH

Open Access



Induce defense response of DADS in eggplants during the biotrophic phase of *Verticillium dahliae*

Muhammad Ali, Husain Ahmad, Bakht Amin, Muhammad Jawaad Atif and Zhihui Cheng*

Abstract

Purpose: *Verticillium* wilt is a destructive vascular disease in eggplants. The complex defensive mechanisms of eggplant against this disease are very limited.

Methods: Our work examined the bioactive properties of garlic allelochemical diallyl disulfide (DADS) as potential biostimulants for defense against *V. dahliae* in eggplant seedlings. We, therefore, foliar sprayed DADS on eggplants to study the defense response during the early biotrophic phase of *V. dahliae* (a hemibiotroph).

Results: DADS application significantly increased root peroxidase (POD), phenylalanine-ammonia lyase (PAL) enzyme activity, and reduced H₂O₂ levels after 24 h of fungal inoculation. Salicylic acid (SA) in leaves and roots was significantly increased while, the jasmonic acid (JA), indole acetic acid (IAA), and abscisic acid (ABA) levels were decreased. The microscopic examinations of *V. dahliae* infection in roots displayed that the progression of infection was restricted in DADS-treated plants. Depositions of lignin and phenolic compounds such as ferulic acid, *p*-coumaric acid, and caffeic acid content were significantly higher in DADS-treated plants at 48 h post-inoculation. Similarly, the DADS application up-regulated pathogenesis-related (PR1, PR2, and PR5), mitogen-activated protein kinase (MPK1), and lipoxygenase (LOX) genes. Furthermore, DADS-treated plants exhibited a lower disease severity index (23.3% vs. 57.0% in controls), indicating successful defense against *V. dahliae*.

Conclusions: Our findings concluded that the biological function of garlic allelochemical DADS has a prominent role in the higher defense resistance of eggplants during the early infection of *V. dahliae*.

Keywords: Diallyl disulfide, PR-genes, Plant hormones, Phenolic compounds, *V. Dahliae*

Background

Eggplant (*Solanum melongena* L.) is an economically important crop and is often encountered by pathogenic attacks that adversely affect its yield. In China, eggplant is mainly grown inside plastic tunnels where it is highly vulnerable to pathogenic attack, particularly to *Verticillium dahliae*-caused *Verticillium* wilt. It is a potent soil-borne fungal disease that infects eggplants at any growth

stage, with the most prominent symptoms being leaf necrosis, wilted areas distinguished by yellow bronze coloration seen between leaf veins, stunted growth, and vascular discoloration [1, 2]. It infects the plants through the roots, and its resting structure (microsclerotia) can survive in the soil for 14 years, even in the absence of a host under cooler soil conditions [3, 4]. Its complete control is very difficult because the source of infection occurs in the soil and the fungus invades internally throughout the plant body.

Synthetic fungicides are being routinely used in developed agricultural systems to control disease and secure

*Correspondence: chengzh@nwfau.edu.cn
Department of Vegetable Science, College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

crop yields [5]. However, their residue may cause adverse effects on the environment [6]. Therefore, several horticultural practices are still practiced to ascertain the harm and ineffectiveness posed by chemical pesticide application [7]. Past trials have been attempted on eggplant for variety improvements by resistance induction through trait exploitation in wild varieties with limited success [8]. Subsequently, transformation experiments have been performed by inducing *Agrobacterium*-mediated genetic modification while inoculating seedling explants (hypocotyls and cotyledons) and leaves [9]. Grafting attempts in eggplant and tomato have shown elevated tolerance but still susceptibility towards *V. dahliae* [10, 11]. Lately, miRNA, Mir395 from *Arabidopsis thaliana* elevating sulfate production was found to induce hypersensitivity in eggplants towards *V. dahliae* [12]. Nevertheless, these practices are very time-consuming and laborious. In place of chemicals, organic-based biostimulants have become a significant focus for sustainable agriculture. It is both cost-effective and promising for priming defense responses against fungal pathogenic invasions as well as improving crop quality and yield while being safe for the environment and health [13, 14]. Plants allelochemicals or extracts from garlic [15], moringa leaves [16], lemongrass [17], and seaweed [18] were reported to improve plant physiology and activate defense systems against pathogen infections. Moreover, plant-microbe interaction studies also show that antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD) are the first lines of defense to scavenge the ROS burden produced within the cells as a result of phytopathogen infections [19–21].

Generally, plants trigger several cellular-level defense mechanisms against pathogen invasion [22]. The plant cell wall is mainly composed of cellulose, pectin, and lignin, which represent the most important semi-permeable physical barriers [23]. The reinforcement of the cell wall occurs through the deposition of lignin and lignin-like phenolic compounds during the plant's defensive mechanisms [24, 25]. Plants were shown to respond to fungal invasion by rapid accumulation of phenolic compounds such as caffeic and ferulic acid in the cell wall, thereby inducing disease resistance to the fungal pathogen *V. dahliae* [24, 26]. Moreover, these phenolic compounds inhibit pathogenic enzymes, thereby restricting the degradation of the cell walls. Notably, plant cell wall perturbations often lead to altered hormonal production [27–29]. Salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) play a major role in regulating plant defense responses against various pathogens, pests, and abiotic stresses [22, 30]. In addition, high levels of SA and JA accumulation in pathogen-exposed tissues induce the expression of pathogenesis-related (PR) genes, which are

markers during the onset of systemic acquired resistance (SAR) [31].

Garlic has been recognized as a medicinal plant with a therapeutic effect through most of human history [32, 33]. It consists of several bioactive compounds, among which allicin, or diallyl disulfide (DADS), is widely known due to its bioactive nature [34]. These primal sulphur compounds are responsible for inhibiting a variety of pathogens, including *Phytophthora capsici*, *Botrytis cinerea*, *Verticillium dahliae*, and *Fusarium oxysporum* [35]. Numerous researchers have documented the antimicrobial activities of garlic for ages [36, 37]. However, little is known about its mechanism in soil-borne diseases. The allelopathic potency of garlic-derived compounds has a strong allelopathic influence on the cropping environment and may help to alleviate continuous cropping obstacles in eggplant [38], pepper [39], and cucumber [40]. Moreover, garlic straw containing sulphur compounds was found to be the most effective component in inhibiting *Meloidogyne incognita* growth [41]. Recent literature has revealed that garlic allelochemicals influence mitotic gene expression and plant hormonal balance in cucumber and tomato plants [42, 43]. However, very little scientific evidence is available on the use of garlic extract as a priming inducer against *Verticillium* in eggplants [44]. Nevertheless, the phenolic metabolism and molecular basis of eggplant's response to *V. dahliae* in a biotrophic phase are still unclear.

This work aimed to investigate the mechanism of induction of resistance on a molecular basis offered by DADS in eggplant against *V. dahliae*. Here we identify induced PR-genes upon pathogen infection, physiological assessment of the plant's primary and secondary metabolism such as phytohormonal regulation, peroxidase, phenylalanine-ammonia lyase (PAL) activity, H₂O₂ production, phenolic compounds, deposition of lignin, and histological observations to monitor fungal invasion in eggplant at a biotrophic phase of *V. dahliae*.

Materials and methods

Plant and fungal materials

Seeds of *V. dahliae* susceptible eggplant cultivar 'Cui Guan' (bought from Yufeng seeds company, Yangling, China), were surface-sterilized by immersion in 0.1% sodium hypochlorite solution for 1 min and thoroughly rinsed before sowing in commercial seedling medium-provided plastic trays. Seedlings were transferred from trays to pots (12 × 12 cm) after the emergence of true leaves and allowed to grow in a growth chamber with a 16-h-light/8-h-dark duration at 23°C. The garlic allelochemical Diallyl Disulfide (DADS) was purchased from Sigma Aldrich (Shanghai) and diluted to a final concentration of 0.4 mmol·L⁻¹ (foliar applied 25 mL to each

plant), which was previously revealed to be optimal for cucumber resistance to downy mildew [45]. The virulent, defoliating *V. dahliae* strain XJ2008 (originally isolated from cotton in Xinjiang Province, China) was obtained from the College of plant pathology, Northwest A&F University, Yangling, Shaanxi, China [46]. The spore suspensions were prepared according to Gayoso et al., (2010) [24], and cultured on a potato dextrose agar medium for 7–10 days at 25 °C in the dark. Conidia's concentration was finally adjusted to 10^7 conidia mL⁻¹ before inoculation.

Bioassay conditions, and fungal inoculation

A bioassay was performed to assess the induced defense response of DADS in the eggplant. At the 3–4th true leaf stage, seedlings were treated with the selected concentration of DADS through foliar application. A 25 mL solution was applied to each plant, and the same amount of distilled water was applied to control plants. After 48 h of DADS application, a fungal suspension of 20 mL was applied as a soil drench, and then plants were shifted to the growth chamber at 26 °C with 85% humidity and a 16/8 h light and dark duration. The experiment used a randomized complete block design with three biological replicates, for a total of 720 plants. Each treatment consists of 60 plants in a single replication. Treatments consist of Control (CK), and Diallyl disulfide (DADS) both with (+) and without (–) *V. dahliae* inoculation. Plant roots were collected at 0, 6, 12, 24, 48, 72, 144, and 240 hpi (hours post-inoculation) of *V. dahliae* for physiological analysis. At 24 and 48 hpi, the expression of PR genes, phenolic compounds, total lignin content, and differences in the fungal infection progression were examined to determine the role of DADS in eggplant defense mechanisms.

Statistical analysis

The treatments were subjected to one-way analysis of variance (ANOVA) and the differences in means were determined by using statistix software V.8.1, Tukey's HSD test ($P \leq 0.05$) was performed. All figures and pictures were created using the *ggplot* function in R 3.6.0 (R-Studio Team., 2019), and Photoshop CC.

Eggplant growth attributes

Growth attributes were recorded after 14 days of fungal inoculation. Plant height and fresh biomass were recorded using a measuring tape (cm) and a weight balance, and their dry weight was determined by drying the samples at 75 °C until the weight remained constant.

Measurement of enzymes activities and H₂O₂ content

The root samples were collected at 0, 6, 12, 24, 48, 72, 144, and 240 hpi from both control and DADS plants with (+) and without (–) inoculation of *V. dahliae*. Three biological replicates per time point were collected and immediately frozen in liquid nitrogen before stored at –80 °C for the estimation of enzymes activity.

POD measurement was determined using the guaiacol method, wherein the solution for determining activity was prepared by mixing 50 mL of 0.05 M phosphate buffer (pH 7.8), 28 μL of guaiacol, and 19 μL of 30% H₂O₂ (v/v). Then 3.5 mL of the reaction mixture solution was transferred into a 1 cm path length cuvette for absorbance measurement. After introducing 0.5 mL of enzyme extract into the overall mixture, absorbance was measured over 3 min at 30 s intervals using a wavelength of 470 nm. POD activity was expressed as (U·g⁻¹ FW min⁻¹) [47].

The total PAL enzyme was determined using the method of [48]. The root samples were homogenized and the supernatant was subjected to the Beaudoin-Eagan and Thorpe method. The extract was kept for incubation at 37 °C for 2 h in 10 mM L-phenylalanine, 0.5 M Tris-HCl at pH 8.0, and the reaction was halted by adding 5 M HCl. Spectrophotometric analysis was carried out at 290 nm to measure the resulting trans-cinnamic acid after centrifuging the reaction mixture. PAL activity was expressed as milligrams of cinnamic acid formed per gram of protein.

Hydrogen peroxide levels were measured by initially homogenizing root samples in an extraction buffer (Tris-acetate 50 mM, pH 5.0). The supernatant was collected in a clean tube by filtering the mixture through cheesecloth and centrifuging for half an hour at 14,000 g at 4 °C. After discarding the pellet, control and treated plants were quantified for H₂O₂ using the xylenol orange method. For this, 500 μL of root extract is mixed with 500 μL of the reaction mixture (500 μM ferrous ammonium sulfate, 50 mM H₂SO₄, 200 μM xylenol orange, and 200 mM sorbitol) [24].

Endogenous phytohormone extraction and purification

Plant endogenous hormones, salicylic acid (SA), jasmonic acid (JA), auxin (IAA), and abscisic acid (ABA), were determined as described by [49, 50]. An internal standard having 2 μL of H₂-JA (2 ng/μL), (40 ng of D₄-SA), 2 μL of d₅-IAA (2 ng/μL), and 25 μL of d₆-ABA (0.25 ng/μL) was added to each sample (Yuanye Biotechnology Co., Ltd., Shanghai, China). Samples from leaves and roots (fresh) were taken at 0, 12, 24, 48, and 72 h after inoculation of *V. dahliae* (three biological replicates per time point). Briefly, 0.5 g of sample was pulverized in liquid nitrogen,

and an extraction solvent of 0.5 mL (isopropanol: DH₂O: conc. HCl = 2:1:0.002, v/v/v) was added to the samples and shaken for 30 min at 4 °C at 100 rpm. Then, 1 mL of dichloromethane was added, and the mixture was shaken for 30 min. The solvent was concentrated using a rotary evaporator at 45 °C after centrifugation. Finally, the dry residue was re-dissolved in 1 mL of methanol and filtered through 0.22 µm membranes for LC-MS analysis. The samples were quantified using an Agilent SB-C18 column (50 × 4.6 mm, 1.8 µm) with a sample flow rate of 0.6 mL min⁻¹ in MRM (multiple-reaction monitoring) mode using an Agilent HPLC system 1260 and a mass spectrometer (an AB Qtrap 5500 triple quadrupole) coupled with an electrospray ionization source. Setting conditions were listed in supplementary materials (Table S1).

Analysis of quantitative real-time PCR (RT-qPCR)

The relative quantitation of pathogenesis-related PR1, β-1,3-glucanase (PR-2), chitinase (PR-3), Thaumatin-like proteins (PR5), lipoxygenase (LOX), and mitogen-activated protein kinase (MAPK1) genes were observed in eggplant roots (at 24 and 48 h with and without inoculation of *V. dahliae*). RNA was extracted using the E.Z.N.A Plant RNA kit (OMEGA) following the manufacturer's protocol. The RNA obtained was used to synthesize cDNA using a HiFiScript cDNA synthesis kit (CW2582M, CWBIO, China), in accordance with the manufacturer's guidelines. The real-time fluorogenic quantitative PCR was performed using SYBR-Green III mixture (E104-A001, JIEYI Biotech, China) in a 20 µL volume, in accordance with the manufacturer's guidelines. The primers of pathogenesis-related genes and the reference genes were listed in Table S2, obtained from the given references [51, 52]. PCR conditions consisted of an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C, and 60 s at 54 °C. Melting curves were programmed as 15 s at 95 °C and 60 s at 60 °C, followed by a gradual temperature increase to 95 °C at a rate of 0.3 °C s⁻¹. Analysis of relative expression levels was carried out using the 2^{-ΔΔCT} method [53]. The specificity for each primer pair was verified by melting curve analysis, which revealed that each gene had a single amplification peak (Fig. S1). The analysis was performed with three biological replicates and three technical replicates.

Analysis of phenolic compounds

Phenolic acids (caffeic acid, ferulic, and *p*-coumaric) were determined in eggplant roots at 24 and 48 h with and without *V. dahliae* inoculation (three biological replicates per time point). Approximately, root samples (150 mg of powder) were extracted with 6 mL of 70% methanol followed by sonication at 30 °C for 30 min. The samples were centrifuged for 7 min at 10,000 rpm at 4 °C. The

supernatant was concentrated to a final volume of about 1 mL by filtering and vacuum distillation on a rotary evaporator. Finally, 0.22 µm membranes are used to filter samples for LC-MS analysis [26].

Staining of eggplant roots with WGA-AF488 and fluorescent brightener 28

The fungal invasion during root colonization was examined using Wheat Germ Agglutinin-Alexa Fluor 488 (WGA-AF488) and fluorescence brightener 28 (FB28) (Wuhan Service Bio-Technology Co., Ltd.) staining. At 24 and 48 hpi of *V. dahliae* infection, eggplant roots were stained with WGA-AF488 to visualize fungal structures and FB28 for cell wall visualization. After clearing the colonized roots in pure ethanol, they were incubated in 10% KOH for 2–3 h at 85 °C. The samples were neutralized with 1 × PBS buffer (pH: 7.4) and 4–5 washing steps after incubation. Next, the WGA-AF488/FB28 staining solution (1 µg mL⁻¹ FB28, 10 µg mL⁻¹ WGA-AF488; 0.02% Tween 20 in PBS pH7.4) was vacuum infiltrated into samples for 4 times (5 min each) at 25 kPa using a desiccator. The WGA-AF488/FB28 staining roots were kept at 4 °C in the dark in PBS buffer until microscopic analysis. Stained root samples were imaged with the Nikon Eclipse E100 (Nikon Co., Ltd., Japan) with a Nikon DS-U3 image capture. WGA-AF488 excitation was at 488 nm and detection at 500–540 nm; FB28: excitation was at 365 nm and detection at 450 nm [54, 55].

Quantification of total lignin content

Cell walls were prepared using a Triton X-100 washing procedure that included, as the last steps, washing with ethanol (3 times) and diethyl ether (also three times) [56]. Total lignin content was determined using the acetyl bromide method as described in [57]. Alkaline nitrobenzene was used to oxidize lignin in cell walls, and acetyl bromide was measured using absorbance at 280 nm.

Histochemical analysis

Hypocotyl sections were selected at 48 hpi from 5 DADS-treated plants and 5 control plants (with (+) and without (-) *V. dahliae* infection) [58]. For 10 min, cross-sections were incubated in a phloroglucinol solution (2% (w/v) in 95% ethanol) or (95% ethanol as a staining control), then treated with 18% HCl for 5 min and observed directly with a fluorescence microscope (DM2500; Leica).

Disease index, and leaf chlorosis

The disease index (DI) was determined by grading symptoms on a 0–4 scale (0; no visible disease symptoms, 1: wilted leaves < 25%, especially in older leaves, 2: wilted leaves 25–50%, 3: wilted leaves 50–75%, and 4: wilted leaves 75–100%). The following formulas were

used to calculate the disease index and control efficacy: $DI = [(\sum \text{disease grades} \times \text{each grade's no. of infected plants}) / (\text{total number of plants examined} \times \text{maximum disease grade})] \times 100\%$, and control efficacy = $[(\text{mean DI of control} - \text{mean DI of treatment}) / (\text{mean DI of the control})] \times 100\%$ [59]. The severity of leaf chlorosis was graded on a scale of 0–4 [0; no symptoms, 1; up to 25% chlorotic leaves, 2; up to 50% chlorotic leaves, 3; up to 75% chlorotic leaves, and 4; up to 100% chlorotic leaves] [60]. Disease symptoms were scored 14 days after infection.

Results

Plant growth attributes are affected by the foliar application of DADS

Foliar application of DADS improved the plant growth attributes of eggplant, against *V. dahliae* as shown in Table 1. DADS treated plants significantly improved plant height, fresh shoot weight, dry shoot weight, and fresh and dry root weight as compared to those of Vd+/control plants. After *V. dahliae* inoculation, DADS-treated plants had the highest plant height (19.4 cm), fresh weight of shoot (42.00 g/plant), and dry weight of shoot (5.727 g/plant) compared to Vd+/control plants. Root attributes such as fresh root (14.59 g/plant) and dry weight (2.855 g/plant) were found to be significantly higher in DADS treated plants than in Vd+/control plants. At 14 dpi, DADS treated plants were healthier as compared to Vd+/control as shown in Fig. 1A. Furthermore, Vd+/control plants showed more chlorotic and necrotic leaves due to pathogen infection. The disease index value was significantly higher in Vd+/control (57.0%) compared to 23.3% in DADS-treated plants (Fig. 1B). Leaf chlorosis was more severe in Vd+/control plants (grade 3) compared with DADS-treated plants (grade 1) Fig. 1C. The Vd+/DADS plants had a 60% controlling effect on disease control Fig. 1D.

Progression of fungal infection is restricted within eggplant roots by DADS

The conidial germination and hyphal colonization in the root sections were examined at 24 and 48 h after *V. dahliae* inoculation (Fig. 2). In our results at 24 hpi,

V. dahliae conidia formed germ tubes in control plant roots, whereas in DADS treated plants, the hyphae growth was restricted (Fig. 2, A and C). By 48 hpi, the roots of control plants were highly colonized and formed dense fungal hyphae. In contrast, the cortical cells of the DADS-treated plants showed sparse hyphae (Fig. 2, B, and D).

Analysis profiles for enzyme's activities (PAL, POD), and H₂O₂ content

In Vd+/control, the PAL activity dramatically increased from 48 hpi, with the highest activity being observed at 144 hpi, while its activity declined significantly compared to the treated plants at 240 hpi as shown in Fig. 3A. The application of DADS-treated plants showed an increasing trend of PAL activity, with the maximum activity recorded at 12 hpi. However, its activity gradually declined after 24 hpi, and no significant difference was observed afterward (Fig. 3A).

The peroxidase (POD) activity of DADS-treated inoculated plants was maintained at a higher level from 12 hpi to 72 hpi, while its activity declined at 240 hpi. In Vd+/control plants, the POD activity had a delayed increase at 72 hpi; afterward, an increase in POD activity was detected at 240 hpi, which was the maximum as compared to the treated plants (Fig. 3B).

The production of H₂O₂ appeared to be stimulated from 0 hpi to 24 hpi in DADS treated plants after *V. dahliae* inoculation, but it accumulated to a relatively low degree from 48 hpi to 240 hpi when compared with Vd+/control plants. At 240 hpi, an increase in H₂O₂ content was detected in the roots of Vd+/control plants in comparison to other treatments (Fig. 3C).

Analysis of hormone accumulation in eggplant tissues

In shoots at 0 hpi, the levels of SA in response to DADS-treatment were higher by 3.56-folds when compared to Vd-/control plants. While in Vd+/control plants, it increased by 3.35 and 10.93-folds from 12 to 24 hpi, respectively. In contrast to DADS-treated inoculated plants, SA levels were maintained at a higher level from 12 to 24 hpi, resulting in 7.28- and 11.62-folds increases, respectively, when compared to Vd-/controls

Table 1 Foliar application of DADS affected eggplant growth attributes at 14 dpi of *V. dahliae*

Treatment		Plant height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	Vd-	21.4 ± 2.0a	48.57 ± 2.63ab	5.511 ± 0.239a	22.03 ± 1.70a	3.623 ± 0.382a
	Vd+	9.0 ± 1.4b	14.22 ± 2.10c	2.951 ± 0.295b	5.63 ± 0.41c	1.284 ± 0.190b
DADS	Vd-	24.7 ± 1.0a	67.02 ± 4.67a	6.819 ± 0.496a	28.22 ± 2.31a	4.142 ± 0.336a
	Vd+	19.4 ± 1.8a	42.00 ± 3.33b	5.727 ± 0.145a	14.59 ± 1.57b	2.855 ± 0.118a

Treatment values are presented as the mean of three replications (standard error: SE, having 10 plants/treatment in a single replication). Means followed by different letters are significantly different from the controls at (p ≤ 0.05) according to Tukey's HSD test

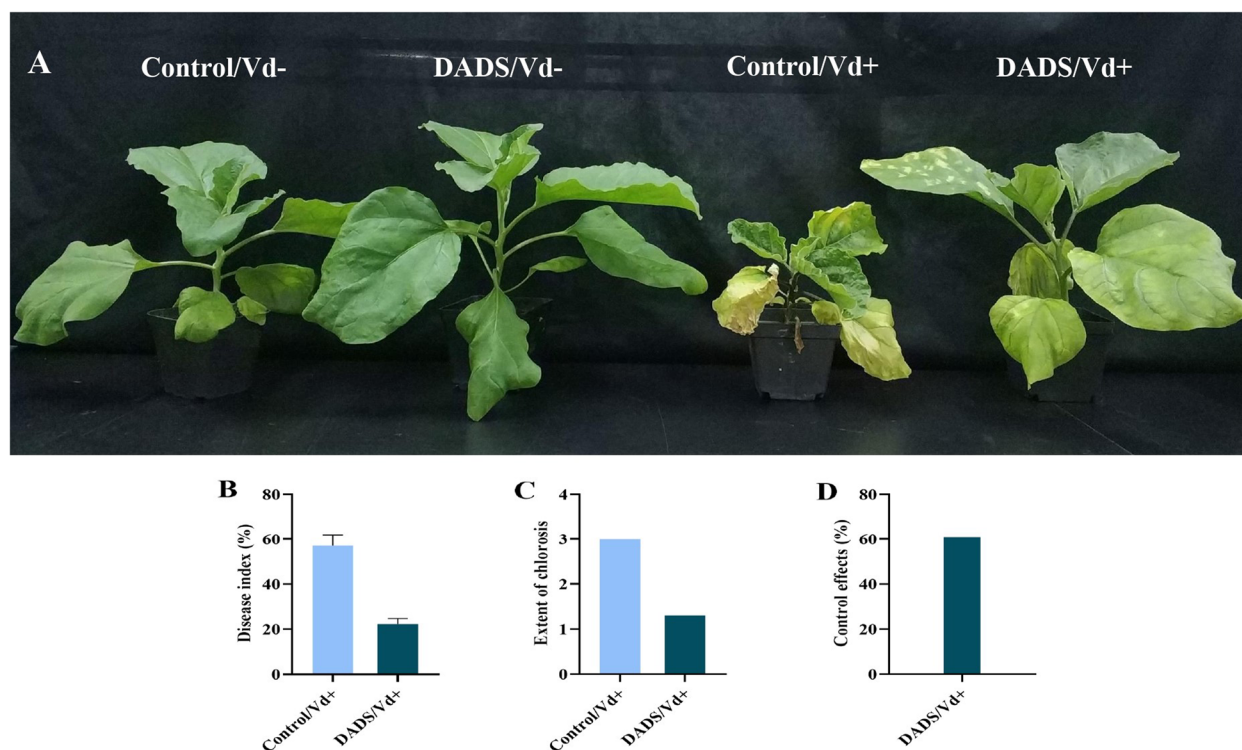


Fig. 1 Foliar application of DADS improves the resistance of eggplant against *V. dahliae*. **A**; At 14 days post-inoculation (dpi), the phenotypes of control and DADS-treated plants were photographed. **B**; Assessment of disease Index at 14 dpi. Data are the averages of three biological replications (20 randomly selected seedlings among 60 plants per replicate) in each treatment (\pm SE, standard error). **C**; Extent of leaf chlorosis symptoms in control and DADS-treated plants at 14 dpi. **D**; Control effect of DADS-treated plants under *V. dahliae* stress

plants. In root Vd+/control plants, SA concentrations sharply increased by 6.14 and 9.86-folds from 12 to 24 hpi, respectively. Whereas in DADS-treated inoculated plants, the SA levels were at a higher level from 12 to 24 hpi, resulting in 10.70 and 11.56-folds, respectively, as compared to Vd-/control plants (Fig. 4A).

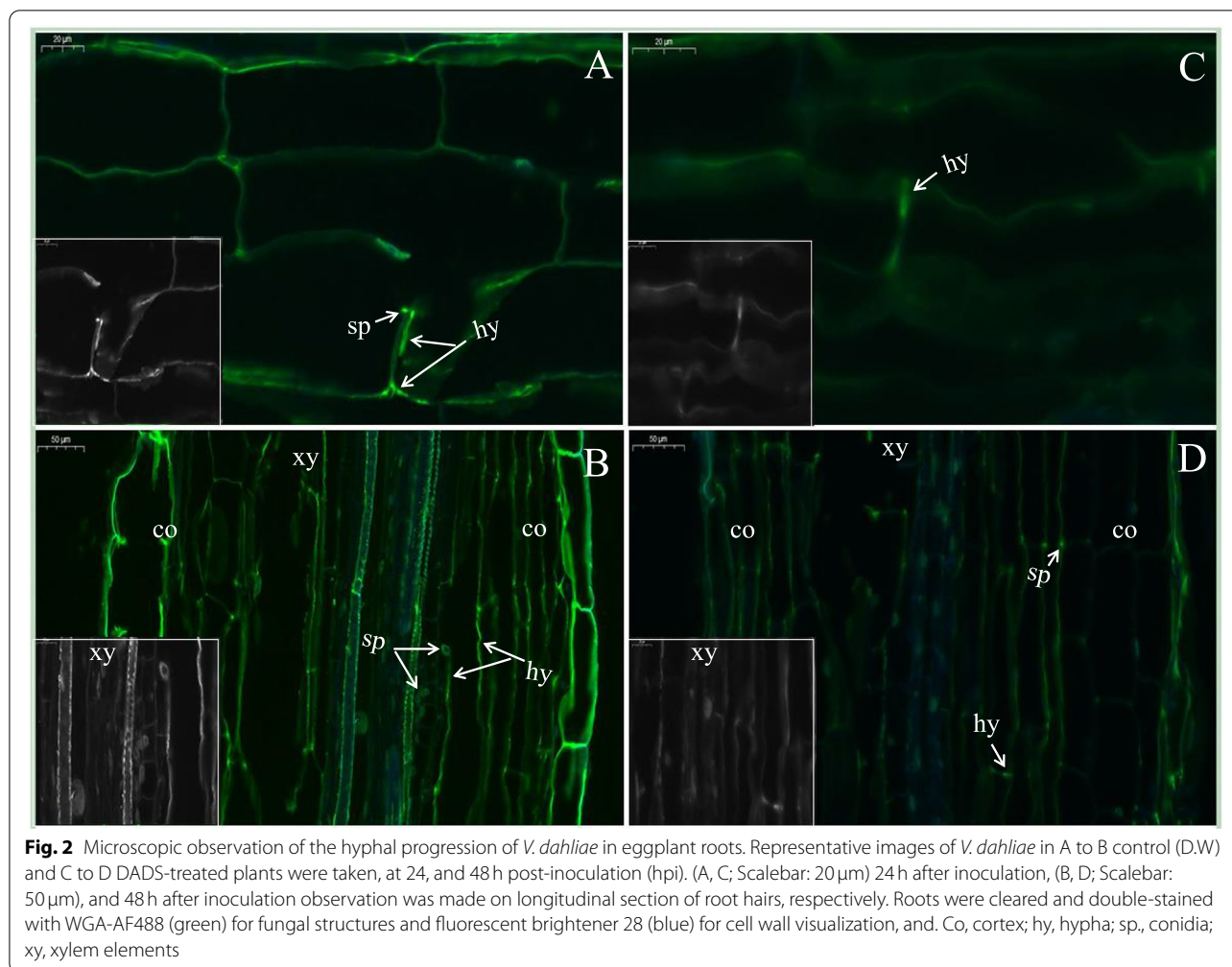
At 0 hpi before the pathogen inoculation, the levels of JA and IAA in both shoot and root were maximum in response to DADS-treatment when compared to Vd-/control plants. As the pathogen infection proceeded at 48 hpi in Vd+/control plants, the JA and IAA levels in the shoots were sharply increased by 5.74 and 4.44-fold, respectively. Whereas in DADS-treated inoculated plants, the JA and IAA levels in shoots were considerably lower at 48 hpi, resulting in 3.10 and 3.36-fold differences, respectively, as compared to the Vd-/control plants. Similar trends were observed in the JA and IAA levels of eggplant roots (Figs. 4B and 5A).

At 72 hpi, significant maximum ABA content was accumulated in Vd+/control plants by 4.98-fold in shoots and 4.09-fold in roots, respectively. Whereas in DADS-treated inoculated plants, the ABA levels were considerably lower by 1.31-fold in shoots and 2.22-fold in roots,

respectively, as compared to the Vd-/control plants (Fig. 5B).

Expression of defense-related genes

In our results, defense-related genes were induced in DADS-treated plants at 24 and 48 hpi of *V. dahliae* infection in comparison to Vd+/control plants. At 24 h of pathogen inoculation, *PR2*, *PR3*, *MPK1*, and *LOX* gene expression levels in the DADS-treated plants were significantly increased by 2.0, 3.4, 2.5, and 2.8-folds, respectively, compared to Vd+/control plants. In DADS-treated plants at 48 hpi, the expression level of genes increased significantly by 2.6, 3.6, 4.5, 11.8, and 4.3folds in *PR1*, *PR2*, *PR5*, *MPK1*, and *LOX*, respectively, when compared to the Vd+/control plants. The *PR3* gene expression level in control plants was upregulated by 2.3-folds as compared to the Vd+/DADS treated plants. The relative expression of the studied genes in Vd-/control and Vd-/DADS-treated plants showed no significant differences. At 24 and 48 h, *PR2* gene expression was significantly increased in DADS-treated plants by 8.3 and 7.2-folds, respectively, when compared to the Vd-/control plants (Fig. 6).



DADS treated plants have stronger physical barriers and altered phenolic metabolism to induce defense resistance

The phloroglucinol-HCl staining indicated that at 48 hpi, hypocotyl sections of DADS inoculated plants displayed deeper color in the xylem vessel wall and parenchyma cell walls than those of Vd+/control plants (Fig. 7A). Furthermore, the quantitative analysis of uninoculated plants showed that the levels of lignin in the shoot and root of DADS were higher than those of Vd-/control plants. At 48 hpi, DADS inoculated plants showed inclined levels of lignin in the shoot and root, respectively (Fig. 7B).

Root phenolic contents, i.e., caffeic acid, ferulic acid, and *p*-coumaric acid, showed differences under pathogen infection and DADS application. At 24 hpi, caffeic acid showed a significant increase in DADS treated inoculated plants in comparison to Vd+/control plants. At 48 hpi, caffeic acid, ferulic acid, and *p*-coumaric acid were significantly increased in the DADS treated inoculated plants up to 1.8, 1.8, and 1.3-folds, respectively, then in

those of Vd+/control plants. No significant changes were observed between Vd-/control and Vd-/DADS-treated plants, respectively (Fig. 7. C, D, and E).

Discussion

Verticillium dahliae is a soilborne pathogen that causes vascular wilt in eggplant. In our work, the DADS treated plants showed improved physiological parameters, which can be strongly corroborated with our previous work regarding the growth-promoting effect of garlic allelochemicals on eggplant [61], tomatoes [42, 62] and cucumber plants [43]. Previous research revealed that DADS has a dose-dependent effect on cucumber and tomato seedling growth, with lower concentrations (0.01–0.62 mM) promoting growth and higher concentrations (6.20–20.67 mM) inhibiting growth [42, 43]. The allelopathic potential of garlic is based on its biochemical compositions, which include allicin, diallyl disulfides, diallyl trisulfides, etc., and are the major organosulfur compounds in garlic



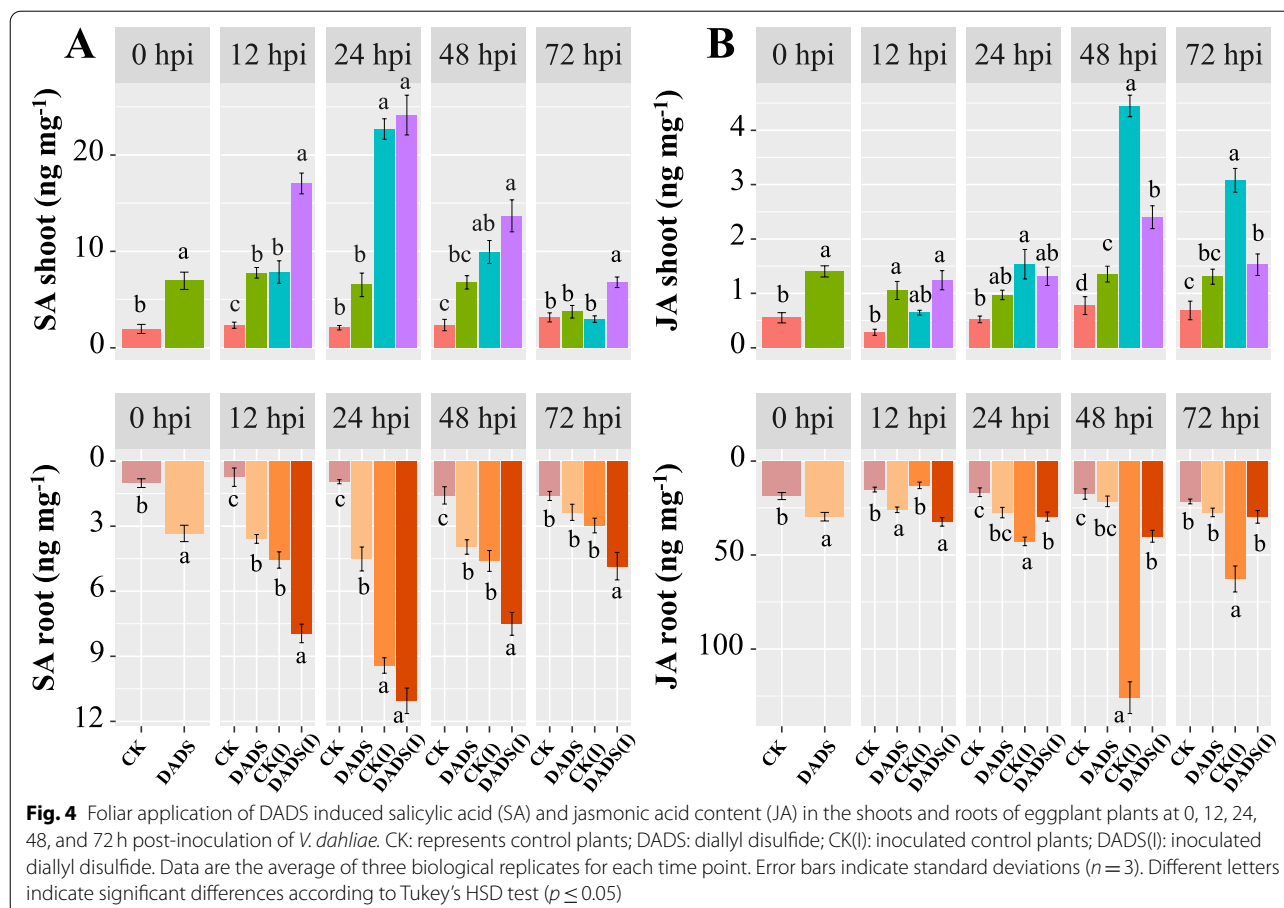
[34, 42, 63]. In our study, the DADS-treated inoculated plants remained comparatively healthier, affirming that the DADS chemical structure is stable and does not denature quickly, which suggests its usage in a long-term agricultural application [64]. Its peculiar and pure form of organosulfur characteristics from garlic extracts aid in decomposing cell wall degrading enzymes of fungus, thereby limiting infection spread in a host cell [65, 66].

ROS levels mark metabolic disparities within the living system, and plants respond to incompatible reactions by expressing resistance genes, stress-responsive proteins, the generation of ROS, the release of phytoalexins and toxins, and localized hypersensitive reactions [24, 67]. Normally, a plant's ROS activities are maintained in equilibrium due to plant antioxidant and hormonal balance in the cell's cytoplasm. However, a slight imbalance may trigger a defense system to scavenge the overproduction of ROS. Antioxidant enzymes balance hydrogen peroxide by disintegrating it into water and oxygen molecules, which is a primary

defense mechanism against any foreign pathogenic attack. Therefore, any variation in their activity indicates the level of stress or damage [68, 69].

In fungal inoculated plants, the oxidative free radicals must have been produced due to fungal infection that raised POD activity (Fig. 3B). H₂O₂ is an oxidative burst marker during plant-pathogen interactions and has been found to slightly shoot up in the roots of DADS treated plants and conversely in control ones, enhancing signaling during hypersensitive reactions and promoting systemic acquired resistance [70, 71]. In our work, the Vd+/control plants revealed an increase in ROS levels. It must be contributing towards growth as oxidative stress hampers plant growth and promotes the appearance of diseased characteristics. Furthermore, any imbalance in ROS production can cause oxidative damage to major macromolecules, i.e., DNA, lipids, and proteins [72, 73].

PAL is involved in phenylpropanoid metabolism, which is a primary step in the metabolism of several phenylpropanoid plant compounds. The DADS-treated plants showed maximum activity at 12 hpi and then

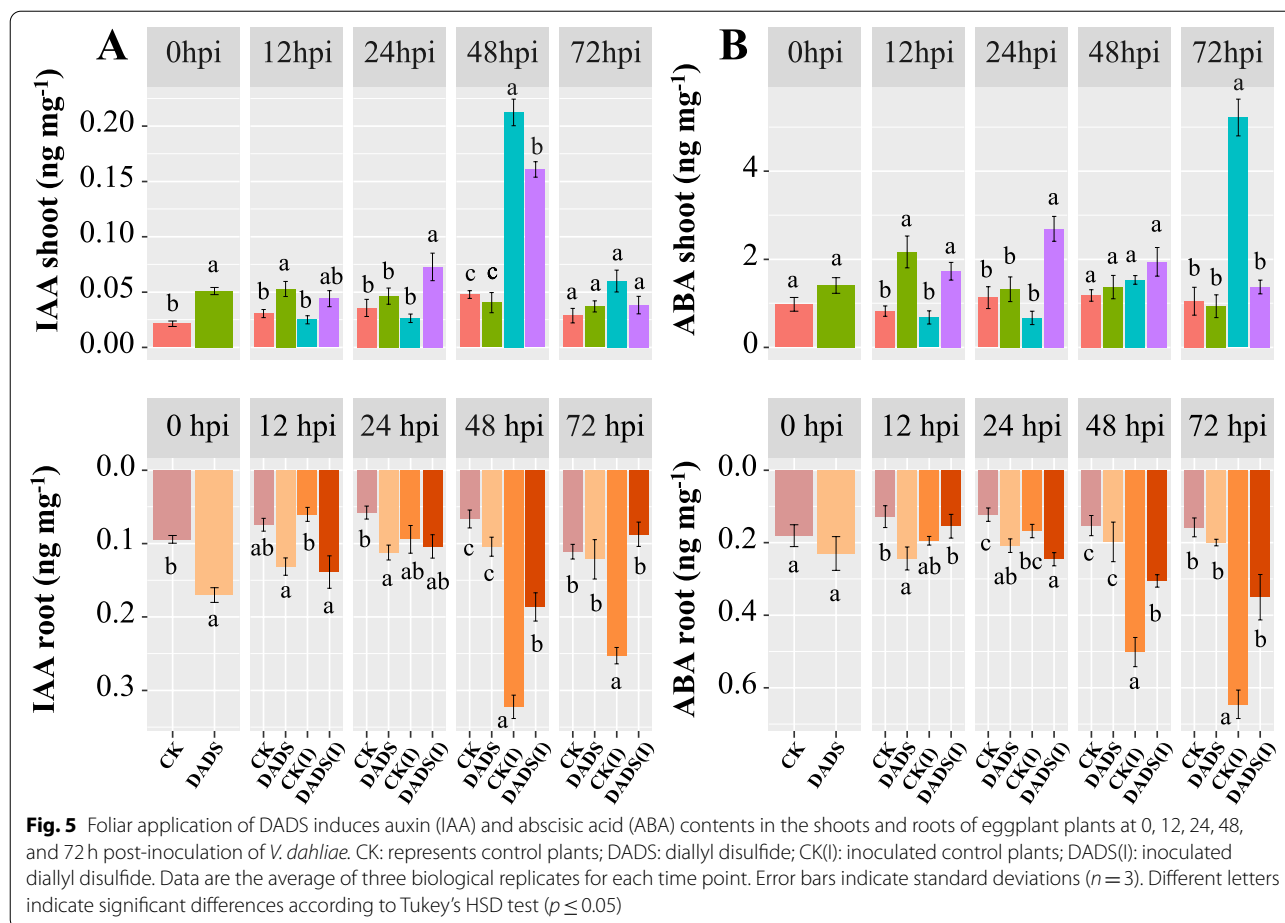


gradually declined, while the Vd+/control plants had a gradual increase and declined after 144 hpi. It can be related to elevated H₂O₂ levels at 24 hpi. The increased PAL activity in alignment with elevated H₂O₂ levels has been demonstrated in resistant tomato plants, while susceptible plants show a delayed rise in PAL activity [24].

Plant hormones are naturally occurring signaling molecules that regulate the growth and development of plants. Pathogen attack activates a hormone signaling network, which limits pathogen invasion [74, 75]. In our results, DADS treatment altered the levels of SA, JA, IAA, and ABA in the shoots and roots of eggplant during *V. dahliae* infection. At 12 to 24 hpi, the rate of SA increase in shoots and roots was significantly lower in the DADS treatment as compared to the Vd+/control plants. The greater fungal biomass accumulation as the disease progresses further and activates additional defense response pathways might be the reason for the rapid increase in SA levels of Vd+/control plants. SA-mediated signaling pathways are predominantly involved in the activation of defense responses against biotrophic and hemibiotrophic pathogens and trigger systemic acquired resistance [22, 76]. In *Arabidopsis*, eggplant,

and tomatoes, SA pathways are essential for resistance to *Verticillium* wilt [52, 77, 78].

Higher levels of JA were detected in the roots of DADS-treated inoculated plants at 12 hpi. However, as the infection progressed, lower JA levels were detected in DADS-treated plants, which could activate SA-mediated responses by prolonging *V. dahliae*'s biotrophic phase. Our results are in accordance with the study of Guerreiro et al. (2016) [79] that reported that the JA pathway is activated during the first hours of interaction between grapevine and *Plasmopara viticola*, a biotrophic pathogen. The JA-SA antagonistic interaction between defense signaling pathways provides focused resistance against pathogens at later inoculation time-points [80]. Similarly, IAA levels in both shoots and roots were significantly higher in Vd+/control plants. The higher auxin signaling in Vd+/control plants might be a useful strategy by the pathogen to suppress the SA-dependent defense response during the initial biotrophic phase of *V. dahliae*. As reported by [81], IAA levels were significantly increased in plant leaves with a higher peak at 3 to 4 days after infection with either *Pseudomonas syringae* pv. tomato or *Arabidopsis-Xanthomonas campestris* pv. *campestris*. In



addition, IAA and SA signaling pathways also work in an antagonistic way, thereby enhancing susceptibility to biotrophic pathogens [82]. In our results, at 0 hpi, a significant increase in the IAA of DADS treated plants were observed. It might be due to the involvement of DADS in improving cellular mitotic division and root growth expansion in cucumber and tomato seedlings via the regulation of the plant hormone auxin [42, 43]. Furthermore, the higher ABA levels in Vd+/control plants and lower in the DADS treated plants suggest an impaired infection progression. This observation is consistent with the findings of [83], who reported that ABA concentrations increased in *Arabidopsis* leaves at 2 dpi in response to *V. longisporum*. Furthermore, at 6 and 8 dpi, ABA levels were higher in *Arabidopsis* roots infected with *V. longisporum* [84]. In addition, ABA and SA have antagonistic interactions [85], and this antagonism is thought to be responsible for ABA's increased disease susceptibility against the soilborne fungus *V. longisporum* [86], and *Fusarium oxysporum* [87].

Our results show that the relative expression levels of pathogenesis-related (PR) genes and mitogen-activated

protein kinase (MAPK1) levels were up-regulated in DADS-treated inoculated plants. MAPK cascades are important for plant development and response to biotic and abiotic stresses [88]. The up-regulation of the PR genes and MAPK1 levels indicates the biological function of garlic allelochemicals in conferring resistance, thereby SA-mediated activation of SAR, which prevents the spread of infection to healthy tissues. These results are consistent with the previous reports that PR genes, PR1, PR2, and PR5 are the marker genes induced by SA [89]. The increased expression of the chitinase (PR3) gene in Vd+/control plants is consistent with previous findings that JA signaling activation increased the expression of JA marker genes (PR3, PR4, and PR12) during necrotrophic pathogens [90]. Auxin influx triggers the expression of LAX genes [91]. They promote shoot apical meristem and root primordia by reinforcing the auxin-dependent induction of a distinct set of cell-wall-remodeling enzymes [92]. The garlic allelochemical (DADS) function as signaling molecules by regulating plant developmental progression and root growth. DADS up-regulates the expression of LAX5, and IAA2 in tomatoes [42,

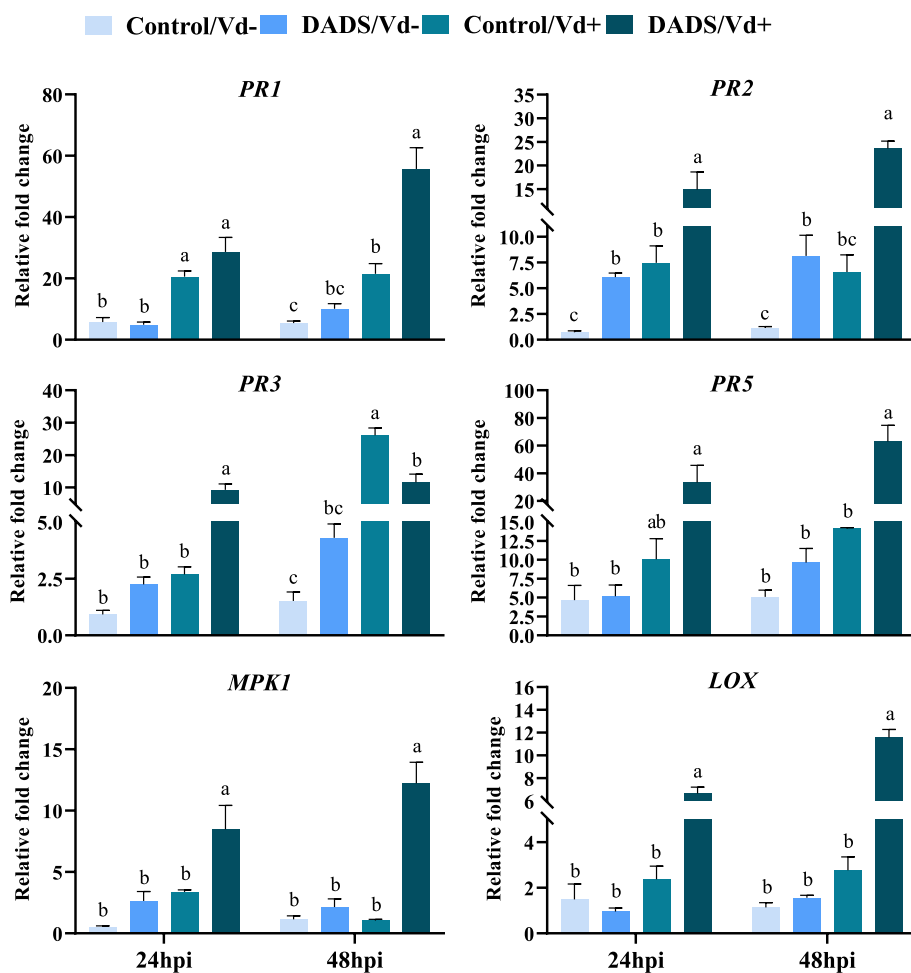
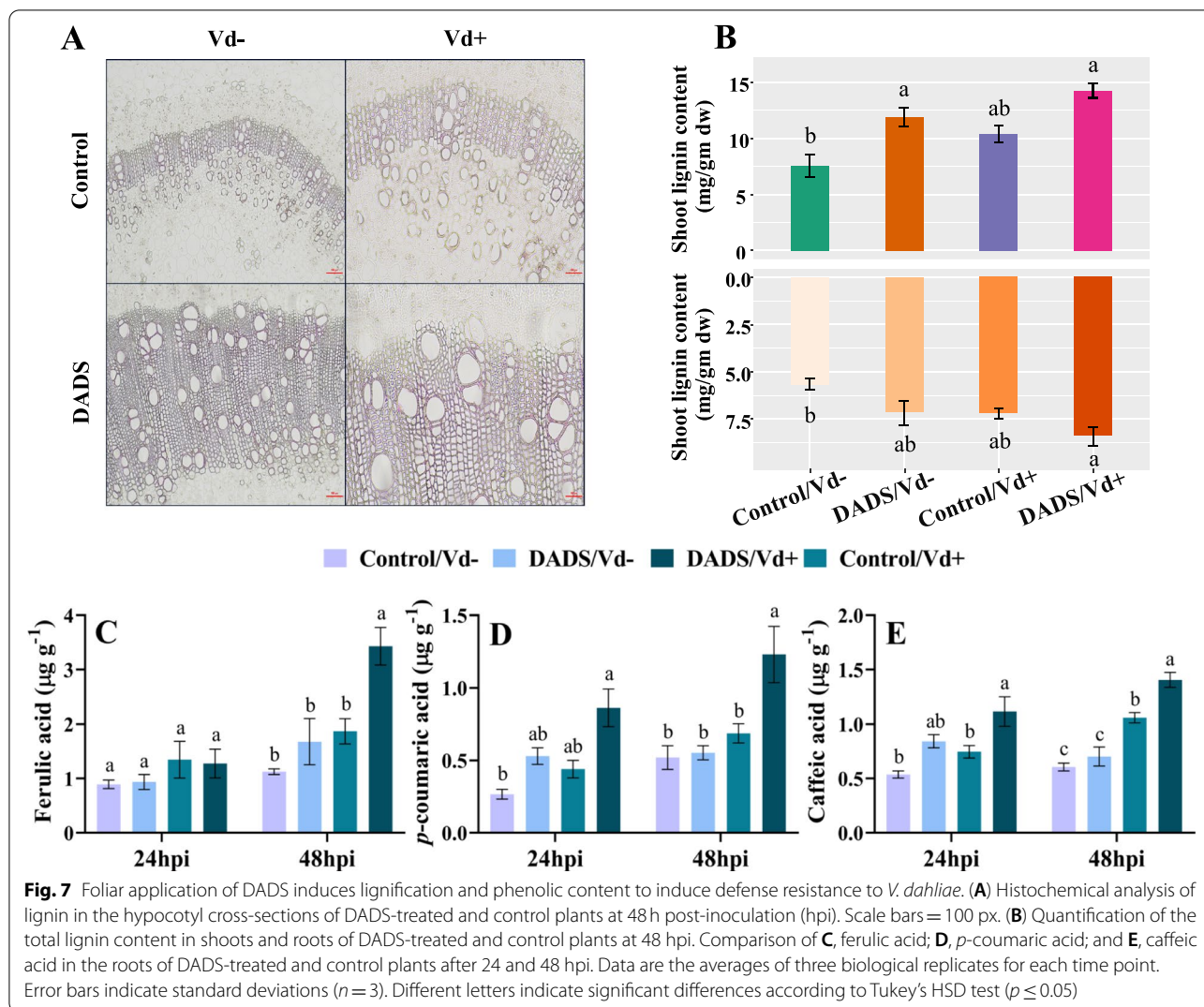


Fig. 6 Foliar application of DADS induces relative expression of defense-related genes in eggplant roots at 24- and 48-h post-inoculation of *V. dahliae*. Data are the averages of three biological replicates, and three technical replicates for each timepoint (standard deviation: SD, $n = 3$). Different letters indicate significant differences according to Tukey's HSD test ($p \leq 0.05$)

93], mitotic-related genes such as *CDKA* (cyclin-dependent kinase A), and *CDKB* (cyclin-dependent kinase B) in cucumber plants [43], which supports the role of DADS at a molecular level.

The rapid advancement of confocal laser scanning microscopy (CLSM) has greatly aided our understanding of *V. dahliae* colonization of various plant roots [94]. Histological analysis of the roots revealed that the progression of *V. dahliae* is restricted in DADS-treated plants. The microscopic observation indicates the resistive nature of DADS in plants, which enables them to restrict fungal invasion. Accumulation of lignin, phenolics, etc. plays a key role in resistance to pathogens invading vascular tissues [95–97]. The increased lignin content in DADS treated plants might be due to the activation of successful resistance to fungal invasion in response to *V. dahliae* attack. A cell wall consisting of higher lignin content is more water-resistant, making it less accessible

to enzymes that degrade fungal cell walls [98]. Inoculation with *V. dahliae* caused a significant increase in the lignin content of pepper varieties with varying degrees of resistance response, according to [58]. The abundance of caffeic acid, ferulic acid, and *p*-coumaric acid was significantly increased in the roots of DADS-treated plants with fungal inoculation. The accumulation of these phenolics might also be responsible for the increased resistance against *V. dahliae*. Consistent with other reports, these phenolics are linked to cell wall strength, digestibility, and resistance to pathogen infections [99]. The cross-linking of lignin-polysaccharide interactions in the secondary cell walls of gramineous plants is mediated by *p*-Coumaric acid [100]. Caffeic acid and ferulic acid inhibit the hyphal growth of *V. dahliae* [26]. The fungus *Fusarium oxysporum* encouraged the biosynthesis of caffeic acids and ferulic acid in pepper roots, which strengthened the cell wall to prevent pathogen penetration [101].



Conclusions

Current research findings are vital in understanding the bioactivity of diallyl-disulfide (DADS) in the defense of eggplant against *V. dahliae* inoculation. Application of DADS induces different defensive responses, resulting in the activation of plant enzymes (POD and PAL), accumulation of phenolic compounds (caffeic acid, ferulic acid, and *p*-coumaric acid), and the reinforcement of cell walls. Here, the intrinsic defense against fungal colonization was significantly higher in DADS-treated plants. By up-regulation of PR genes associated with the hormonal signaling pathways, which indicates that garlic allelochemicals act as a signaling molecule in plant defense responses. Furthermore, these findings lay the foundation that the organosulfur compounds of garlic can act as plant biostimulants, resulting in induced resistance to *Verticillium* infection, particularly in plastic tunnels or glasshouse conditions.

Abbreviations

DADS: Diallyl disulfide; POD: Peroxidase; PAL: Phenylalanine-ammonia lyase; SA: Salicylic acid; JA: Jasmonic acid; IAA: Indole acetic acid; ABA: Abscisic acid; PR: Pathogenesis-related; MPK1: Mitogen-activated protein kinase; LOX: Lipoxigenase; ROS: Reactive oxygen species; H₂O₂: Hydrogen peroxide; qPCR: Quantitative polymerase chain reaction; WGA-AF488: Wheat Germ Agglutinin-Alexa Fluor 488; FB28: Fluorescence brightener 28; hpi: Hours post-inoculation; DI: Disease index.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-022-03527-7>.

Additional file 1.

Acknowledgements

The authors are grateful to Dr. Yupeng Pan, Siyu Zhang and Xi'ao Wang (NWSUAF) for their help in lab techniques.

Authors' contributions

Conceptualization, M.A., and Z.C.; Data curation, H.A., B.A., and M.J.A.; Formal analysis, M.A.; Funding acquisition, Z.C.; Investigation, M.A.; Methodology, M.A.;

Project administrator, Z.C.; Resources, Z.C.; Software, M.A., H.A.; Supervision, Z.C.; Validation, M.A.; Z.C.; Writing-original draft, M.A.; Writing review and editing, M.A. All authors read and approved the final manuscript.

Funding

This research was supported by grants from the “National Natural Science Foundation of China” (Grant No. 31772293).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The present study involving the plant materials complies with Northwest A&F University guidelines.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no conflicts of interest.

Received: 16 August 2021 Accepted: 8 March 2022

Published online: 05 April 2022

References

- O'Brien MJ. Evaluation of eggplant accessions and cultivars for resistance to verticillium wilt. *Plant Dis.* 1983;67:763. <https://doi.org/10.1094/pd-67-763>.
- Klosterman SJ, Atallah ZK, Vallad GE, Subbarao KV. Diversity, pathogenicity, and management of verticillium species. *Annu Rev Phytopathol.* 2009;47:39–62.
- Wilhelm S. Longevity of the Verticillium wilt fungus in the laboratory and field. *Phytopathology.* 1955;45:180–1.
- Bhat RG, Subbarao KV. Host range specificity in *Verticillium dahliae*. *Phytopathology.* 1999;89:1218–25.
- Rampersad SN. A rapid colorimetric microtiter bioassay to evaluate fungicide sensitivity among *Verticillium dahliae* isolates. *Plant Dis.* 2011;95:248–55.
- Stefani A, D'Arc Felício J, de Andréa MM. Comparative assessment of the effect of synthetic and natural fungicides on soil respiration. *Sensors.* 2012;12:3243–52. <https://doi.org/10.3390/s120303243>.
- Nile AS, Kwon YD, Nile SH. Horticultural oils: possible alternatives to chemical pesticides and insecticides. *Environ Sci Pollut Res.* 2019;26:21127–39. <https://doi.org/10.1007/s11356-019-05509-z>.
- Daunay MC, Lester RN, Laterrot H. The use of wild species for the genetic improvement of Brinjal eggplant (*Solanum melongena*) and tomato (*Lycopersicon esculentum*). *Solanaceae III Taxon Chem Evol.* 1991;27:389–413.
- Van Eck J, Snyder A. Eggplant (*Solanum melongena* L.). In: *Agrobacterium Protocols: Humana Press*; 2006. p. 439–48. <https://doi.org/10.1385/1-59745-130-4.439>.
- Mileša C, Wimer J, Inglis D. Grafting eggplant and tomato for verticillium wilt resistance. *Acta Hortic.* 2015;1086:113–8.
- Garibaldi A, Minuto A, Gullino ML. Verticillium wilt incited by *Verticillium dahliae* in eggplant grafted on *Solanum torvum* in Italy. *Plant Dis.* 2005;89:777.
- Mu XY, Liu XR, Cai JH, Zhu WJ, Wang Z, Yang Q, et al. MiR395 overexpression increases eggplant sensibility to *Verticillium dahliae* infection. *Russ J Plant Physiol.* 2018;65:203–10.
- Yakhin OI, Lubyantsov AA, Yakhin IA, Brown PH. Biostimulants in plant science: a global perspective. *Front Plant Sci.* 2017;7.
- Yoon MY, Cha B, Kim JC. Recent trends in studies on botanical fungicides in agriculture. *Plant Pathol J.* 2013;29:1–9.
- Ali M, Cheng Z, Hayat S, Ahmad H, Ghani MI, Liu T. Foliar spraying of aqueous garlic bulb extract stimulates growth and antioxidant enzyme activity in eggplant (*Solanum melongena* L.). *J Integr Agric.* 2019;18:1001–13.
- Abd El-Mageed TA, Semida WM, Rady MM. Moringa leaf extract as biostimulant improves water use efficiency, physio-biochemical attributes of squash plants under deficit irrigation. *Agric Water Manag.* 2017;193:46–54.
- Hussein N, Hussein M, Gadel Hak S, Shaalan H, Hammad M. Effect of two plant extracts and four aromatic oils on *Tuta absoluta* population and productivity of tomato cultivar gold stone. *J Plant Prot Pathol.* 2015;6:969–85.
- Rayirath P, Benkel B, Mark Hodges D, Allan-Wojtas P, MacKinnon S, Critchley AT, et al. Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in *Arabidopsis thaliana*. *Planta.* 2009;230:135–47.
- Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. *Trends Plant Sci.* 2004;9:490–8.
- Foyer CH, Noctor G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell.* 2005;17:1866–75.
- Smaili A, Laachir A, Rifai LA, Koussa T, Bentiss F, Guesmi S, et al. Effect of altering the steric hindrance of the nitrogen atom in 2,5-bis (pyridin-yl)-1,3,4-thiadiazole isomers on their ability to elicit tomato defense responses against Verticillium wilt and crown gall diseases. *Eur J Plant Pathol.* 2018;151:1035–48.
- Glazebrook J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol.* 2005;43:205–27.
- Ellinger D, Naumann M, Falter C, Zwickowicz C, Jamrow T, Manisseri C, et al. Elevated early callose deposition results in complete penetration resistance to powdery mildew in Arabidopsis. *Plant Physiol.* 2013;161:1433–44.
- Gayoso C, Pomar F, Novo-Uzal E, Merino F, de Ilárduya OM. The Ve-mediated resistance response of the tomato to *Verticillium dahliae* involves H₂O₂, peroxidase and lignins and drives PAL gene expression. *BMC Plant Biol.* 2010;10:232.
- García T, Veloso J, Díaz J. Vanillyl nonanoate induces systemic resistance and lignification in pepper plants. *J Plant Physiol.* 2018;231:251–60.
- Xiong XP, Sun SC, Zhu QH, Zhang XY, Li YJ, Liu F, et al. The cotton lignin biosynthetic gene GH4CL30 regulates lignification and phenolic content and contributes to verticillium wilt resistance. *Mol Plant-Microbe Interact.* 2021;34:240–54.
- Bacete L, Mérida H, Miedes E, Molina A. Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. *Plant J.* 2018;93:614–36.
- Sanchez-Rodriguez C, Rubio-Somoza I, Sibout R, Persson S. Phytohormones and the cell wall in Arabidopsis during seedling growth. *Trends Plant Sci.* 2010;15:291–301.
- Mielke S, Gasperini D. Interplay between plant cell walls and jasmonate production. *Plant Cell Physiol.* 2019;60:2629–37.
- Verhage A, van Wees SCM, Pieterse CMJ. Plant immunity: It's the hormones talking, but what do they say? *Plant Physiol.* 2010;154:536–40.
- Durrant WE, Dong X. Systemic acquired resistance. *Annu Rev Phytopathol.* 2004;42:185–209.
- Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. *Avicenna J phytomedicine.* 2014;4:1–14.
- Capasso A. Antioxidant action and therapeutic efficacy of *Allium sativum* L. *Molecules.* 2013;18:690–700.
- Martins N, Petropoulos S, Ferreira ICFR. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: a review. *Food Chem.* 2016;211:41–50 d.
- Hayat S, Cheng Z, Ahmad H, Ali M, Chen X, Wang M. Garlic, from remedy to stimulant: evaluation of antifungal potential reveals diversity in phytoalexin alliin content among garlic cultivars; alliin containing aqueous garlic extracts trigger antioxidants in cucumber. *Front Plant Sci.* 2016;7:1235.
- Cavallito CJ, Bailey JH. Alliin, The antibacterial principle of *Allium sativum*. L. Isolation, physical properties and antibacterial action. *J Am Chem Soc.* 1944;66:1950–1951.
- Leontiev R, Hohaus N, Jacob C, Gruhlke MCH, Slusarenko AJ. A Comparison of the antibacterial and antifungal activities of thiosulfinate analogues of alliin. *Sci Report* 2018 8:1–19.

38. Wang M, Wu C, Cheng Z, Meng H. Growth and physiological changes in continuously cropped eggplant (*Solanum melongena* L.) upon relay intercropping with garlic (*Allium sativum* L.). *front. Plant Sci.* 2015;6.
39. Ding H, Cheng Z, Liu M, Hayat S, Feng H. Garlic exerts allelopathic effects on pepper physiology in a hydroponic co-culture system. *Biol Open.* 2016;5:631–7.
40. Du L, Huang B, Du N, Guo S, Shu S, Sun J. Effects of garlic/cucumber relay intercropping on soil enzyme activities and the microbial environment in continuous cropping. *HortScience.* 2017;52:78–84.
41. Gong B, Blossies S, Li X, Wei M, Yang F, Shi Q, et al. Efficacy of garlic straw application against root-knot nematodes on tomato. *Sci Hortic (Amsterdam).* 2013;161:49–57.
42. Cheng F, Cheng Z, Meng H, Tang X. The garlic allelochemical diallyl disulfide affects tomato root growth by influencing cell division, phytohormone balance and Expansin gene expression. *Front. Plant Sci.* 2016;7.
43. Ren K, Hayat S, Qi X, Liu T, Cheng Z. The garlic allelochemical DADS influences cucumber root growth involved in regulating hormone levels and modulating cell cycling. *J Plant Physiol.* 2018;230:51–60. <https://doi.org/10.1016/j.jplph.2018.08.007>.
44. Ali M, Ahmad H, Hayat S, Ghani MI, Amin B, Atif MJ, et al. Application of garlic allelochemicals improves growth and induces defense responses in eggplant (*Solanum melongena*) against *Verticillium dahliae*. *Ecotoxicol Environ Saf.* 2021;215:112132.
45. Yang F, Wang H, Zhi C, Chen B, Zheng Y, Qiao L, et al. Garlic volatile diallyl disulfide induced cucumber resistance to downy mildew. *Int J Mol Sci.* 2021;22.
46. Hu D, Wang C, Tao F, Cui Q, Xu X, Shang W, et al. Whole genome wide expression profiles on germination of *Verticillium dahliae* microsclerotia. *PLoS One.* 2014;9:100046.
47. Polle A, Otter T, Seifert F. Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). *Plant Physiol.* 1994;106:53–60.
48. El Ghaouth A, Wilson CL, Callahan AM. Induction of chitinase, β -1,3-glucanase, and phenylalanine ammonia lyase in peach fruit by UV-C treatment. *Phytopathology.* 2003;93:349–55.
49. Ahmad H, Li J. Impact of water deficit on the development and senescence of tomato roots grown under various soil textures of Shaanxi. *China BMC Plant Biol.* 2021;21:1–16.
50. Atif MJ, Amin B, Ghani MI, Ali M, Zhang S, Cheng Z. Effect of photoperiod and temperature on garlic (*Allium sativum* L.) bulb and selected endogenous chemical factors. *Environ Exp Bot.* 2020;180:104250.
51. Tani E, Kizis D, Markellou E, Papadakis I, Tsamadia D, Leventis G, et al. Cultivar-dependent responses of eggplant (*Solanum melongena* L.) to simultaneous *Verticillium dahliae* infection and drought. *Front. Plant Sci.* 2018;9:1181. <https://doi.org/10.3389/fpls.2018.01181>.
52. Mahesh HM, Murali M, Anup Chandra Pal M, Melvin P, Sharada MS. Salicylic acid seed priming instigates defense mechanism by inducing PR-proteins in *Solanum melongena* L. upon infection with *Verticillium dahliae* Kleb. *Plant Physiol Biochem.* 2017;117:12–23.
53. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods.* 2001;25:402–8.
54. Redkar A, Jaeger E, Doehlemann G. Visualization of growth and morphology of fungal hyphae in planta using WGA-AF488 and propidium iodide co-staining. *Bio.* 101:e2942. <https://doi.org/10.21769/BioProtoc.2942>.
55. Zuo W, Depotter JRL, Gupta DK, Thines M, Doehlemann G. Cross-species analysis between the maize smut fungi *Ustilago maydis* and *Sporisorium reilianum* highlights the role of transcriptional change of effector orthologs for virulence and disease. *New Phytol.* 2021;232:719–33.
56. Uzal EN, Gómez Ros LV, Pomar F, Bernal MA, Paradelo A, Albar JP, et al. The presence of sinapyl lignin in *Ginkgo biloba* cell cultures changes our views of the evolution of lignin biosynthesis. *Physiol Plant.* 2009;135:196–213.
57. Iiyama K, Wallis AFA. An improved acetyl bromide procedure for determining lignin in woods and wood pulps. *Wood Sci Technol.* 1988;280:271–80.
58. Pomar F, Novo M, Bernal MA, Merino F, Barceló AR. Changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during *Verticillium* wilt in *Capsicum annuum*. *New Phytol.* 2004;163:111–23.
59. Zhang B, Yang Y, Chen T, Yu W, Liu T, Li H, et al. Island cotton Gbve1 gene encoding a receptor-like protein confers resistance to both defoliating and non-defoliating isolates of *Verticillium dahliae*. *PLoS One.* 2012;7.
60. Veronese P, Narasimhan ML, Stevenson RA, Zhu JK, Weller SC, Subbarao KV, et al. Identification of a locus controlling *Verticillium* disease symptom response in *Arabidopsis thaliana*. *Plant J.* 2003;35:574–87.
61. Ali M, Hayat S, Ahmad H, Ghani MI, Amin B, Atif MJ, et al. Priming of *Solanum melongena* L. seeds enhances germination, alters antioxidant enzymes, modulates ROS, and improves early seedling growth: indicating aqueous garlic extract as seed-priming bio-stimulant for eggplant production. *Appl Sci.* 2019;9.
62. Cheng F, Ali M, Liu C, Deng R, Cheng Z. Garlic allelochemical diallyl disulfide alleviates autotoxicity in the root exudates caused by long-term continuous cropping of tomato. *J Agric Food Chem.* 2020;68:11684–93.
63. Ramirez DA, Locatelli DA, Torres-Palazzo CA, Altamirano JC, Camargo AB. Development of garlic bioactive compounds analytical methodology based on liquid phase microextraction using response surface design. Implications for dual analysis: cooked and biological fluids samples. *Food Chem.* 2017;215:493–500.
64. Yun HM, Ban JO, Park KR, Lee CK, Jeong HS, Han SB, et al. Potential therapeutic effects of functionally active compounds isolated from garlic. *Pharmacol Ther.* 2014;142:183–95.
65. Wang Y, Wei K, Han X, Zhao D, Zheng Y, Chao J. The antifungal effect of garlic essential oil on *Phytophthora nicotianae* and the inhibitory component involved. *Biomolecules.* 2019;1–12.
66. Gruhlke MCH, Hemmis B, Noll U, Wagner R, Lühring H, Slusarenko AJ. The defense substance allicin from garlic permeabilizes membranes of *Beta vulgaris*, *Rhoeo discolor*, *Chara corallina* and artificial lipid bilayers. *Biochim Biophys Acta, Gen Subj.* 2015;1850:602–11. <https://doi.org/10.1016/j.bbagen.2014.11.020>.
67. Ali M, Cheng Z, Ahmad H, Hayat S. Reactive oxygen species (ROS) as defenses against a broad range of plant fungal infections and case study on ROS employed by crops against *Verticillium dahliae* wilts. *J Plant Interact.* 2018;13:353–63.
68. Baxter A, Mittler R, Suzuki N. ROS as key players in plant stress signalling. *J Exp Bot.* 2014;65:1229–40.
69. Wan R, Hou X, Wang X, Qu J, Singer SD, Wang Y, et al. Resistance evaluation of Chinese wild vitis genotypes against *Botrytis cinerea* and different responses of resistant and susceptible hosts to the infection. *Front. Plant Sci.* 2015;6.
70. Segonzac C, Zipfel C. Activation of plant pattern-recognition receptors by bacteria. *Curr Opin Microbiol.* 2011;14:54–61.
71. Kadota Y, Shirasu K, Zipfel C. Regulation of the NADPH oxidase RBOHD during plant immunity. *Plant Cell Physiol.* 2015;56:1472–80.
72. Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci.* 2014;53(1-13).
73. Apel K, Hirt H. REACTIVE OXYGEN SPECIES: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* 2004;55:373–99.
74. Dhar N, Chen JY, Subbarao KV, Klosterman SJ. Hormone signaling and its interplay with development and defense responses in *verticillium*-interactions. *Front Plant Sci.* 2020;11:1700.
75. López MA, Bannenber G, Castresana C. Controlling hormone signaling is a plant and pathogen challenge for growth and survival. *Curr Opin Plant Biol.* 2008;11:420–7.
76. Corina Vlot A, Dempsey DA, Klessig DF. Salicylic acid, a multifaceted hormone to combat disease. *Annu Rev Phytopathol.* 2009;47:177–206.
77. Dhar N, Short DPG, Mamo BE, Corrión AJ, Wai CM, Anchieta A, et al. *Arabidopsis* defense mutant ndr1-1 displays accelerated development and early flowering mediated by the hormone gibberellic acid. *Plant Sci.* 2019;285:200–13.
78. Fradin EF, Zhang Z, Ayala JCY, Castroverde CDM, Nazar RN, Robb J, et al. Genetic dissection of *verticillium* wilt resistance mediated by tomato Ve1. *Plant Physiol.* 2009;150:320–32.
79. Guerreiro A, Figueiredo J, Sousa Silva M, Figueiredo A. Linking jasmonic acid to grapevine resistance against the biotrophic oomycete *Plasmopara viticola*. *Front Plant Sci.* 2016;7:565.
80. Shim JS, Jung C, Lee S, Min K, Lee YW, Choi Y, et al. AtMYB44 regulates WRKY70 expression and modulates antagonistic interaction between salicylic acid and jasmonic acid signaling. *Plant J.* 2013;73:483–95.
81. O'Donnell PJ, Schmelz EA, Moussatche P, Lund ST, Jones JB, Klee HJ. Susceptible to intolerance - a range of hormonal actions in a susceptible *Arabidopsis* pathogen response. *Plant J.* 2003;33:245–57.

82. Kazan K, Manners JM. Linking development to defense: auxin in plant-pathogen interactions. *Trends Plant Sci.* 2009;14:373–82.
83. Roos J, Bejai S, Oide S, Dixelius C. RabGAP22 is required for defense to the vascular pathogen *Verticillium longisporum* and contributes to stomata immunity. *PLoS One.* 2014;9:e88187.
84. Iven T, König S, Singh S, Braus-Stromeyer SA, Bischoff M, Tietze LF, et al. Transcriptional activation and production of tryptophan-derived secondary metabolites in Arabidopsis roots contributes to the defense against the fungal vascular pathogen *Verticillium longisporum*. *Mol Plant.* 2012;5:1389–402.
85. Yasuda M, Ishikawa A, Jikumaru Y, Seki M, Umezawa T, Asami T, et al. Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. *Plant Cell.* 2008;20:1678–92.
86. Häffner E, Karlovsky P, Splivallo R, Traczewska A, Diederichsen E. ERECTA, salicylic acid, abscisic acid, and jasmonic acid modulate quantitative disease resistance of Arabidopsis thaliana to *Verticillium longisporum*. *BMC Plant Biol.* 2014;14. <https://doi.org/10.1186/1471-2229-14-85>.
87. Anderson JP, Badruzaufari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C, et al. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *Plant Cell.* 2004;16:3460–79.
88. Xu J, Zhang S. Mitogen-activated protein kinase cascades in signaling plant growth and development. *Trends Plant Sci.* 2015;20:56–64.
89. Zhang J, Du X, Wang Q, Chen X, Lv D, Xu K, et al. Expression of pathogenesis related genes in response to salicylic acid, methyl jasmonate and 1-aminocyclopropane-1-carboxylic acid in Malus hupehensis (Pamp.) Rehd. *BMC Res Notes.* 2010;3:1–6.
90. Ali S, Ahmad Z, Anshika M, Javaid T, Chandrashekar N, Kumar P, et al. Identification and comparative analysis of Brassica juncea pathogenesis - related genes in response to hormonal, biotic and abiotic stresses. *Acta Physiol Plant.* 2017;39:1–15.
91. Swarup K, Benková E, Swarup R, Casimiro I, Péret B, Yang Y, et al. The auxin influx carrier *LAX3* promotes lateral root emergence. *Nat Cell Biol.* 2008;10:946–54.
92. Bhalerao RP, Eklöf J, Ljung K, Marchant A, Bennett M, Sandberg G. Shoot-derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. *Plant J.* 2002;29:325–32.
93. Hayat S, Ahmad H, Nasir M, Khan MN, Ali M, Hayat K, et al. Some physiological and biochemical mechanisms during seed-to-seedling transition in tomato as influenced by garlic allelochemicals. *Antioxidants.* 2020.
94. Zhao P, Zhao YL, Jin Y, Zhang T, Guo HS. Colonization process of *Arabidopsis thaliana* roots by a green fluorescent protein-tagged isolate of *Verticillium dahliae*. *Protein Cell.* 2014;5:94–8.
95. Bhuiyan NH, Selvaraj G, Wei Y, King J. Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *J Exp Bot.* 2009;60:509–21.
96. Eynck C, Koopmann B, Karlovsky P, Von Tiedemann A. Internal resistance in winter oilseed rape inhibits systemic spread of the vascular pathogen *Verticillium longisporum*. *Phytopathology.* 2009;99:802–11.
97. Xu L, Zhu L, Tu L, Liu L, Yuan D, Jin L, et al. Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahliae* as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. *J Exp Bot.* 2011;62:5607–21.
98. Hüchelhoven R. Cell wall-associated mechanisms of disease resistance and susceptibility. *Annu Rev Phytopathol.* 2008;45:101–27.
99. Mnich E, Bjarnholt N, Eudes A, Harholt J, Holland C, Jørgensen B, et al. Phenolic cross-links: building and de-constructing the plant cell wall. *Nat Prod Rep.* 2020;37:919–61.
100. Pan GX, Bolton JL, Leary GJ. Determination of ferulic and *p-Coumaric* acids in wheat straw and the amounts released by mild acid and alkaline peroxide treatment. *J Agric Food Chem.* 1998;46:5283–8.
101. Veloso J, Alabouvette C, Olivain C, Flors V, Pastor V, García T, et al. Modes of action of the protective strain Fo47 in controlling verticillium wilt of pepper. *Plant Pathol.* 2016;65:997–1007.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

