Involvement of the Salicylic Acid Signaling Pathway in the Systemic Resistance Induced in Arabidopsis by Plant Growth-Promoting Fungus Fusarium equiseti GF19-1

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Abstract: Plant growth-promoting fungi (PGPF) are effective biocontrol agents for a number of soil-borne diseases and are known for their ability to trigger induced systemic resistance (ISR). In this study, we investigated the mechanisms triggered by PGPF Fusarium equiseti GF19-1, which is known to increase pathogen resistance in plants, by using GF19-1 spores and the culture filtrate (CF) to treat the roots of Arabidopsis thaliana. Subsequently, the leaves were challenged with Pseudomonas syringae pv tomato DC3000 (Pst) bacteria. Arabidopsis plants treated with GF19-1 spores or the CF elicited ISR against the Pst pathogen, resulting in a restriction of disease severity and suppression of pathogen proliferation. Examination of ISR in various signaling mutants and transgenic plants showed that GF19-1–induced protection was observed in the jasmonate response mutant jar1 and the ethylene response mutant etr1, whereas it was blocked in Arabidopsis plants expressing the NahG transgene or demonstrating a disruption of the NPR1 gene (npr1). Analysis of systemic gene expression revealed that GF19-1 modulates the expression of salicylic acid (SA)-responsive PR-1, PR-2, and PR-5 genes. Moreover, transient accumulation of SA was observed in GF19-1–treated plant, whereas the level was further enhanced after Pst infection of GF19-1–pretreated plants, indicating that accumulation of SA was potentiated when Arabidopsis plants were primed for disease resistance by GF19-1. In conclusion, these findings imply that the induced protective effect conferred by F. equiseti GF19-1 against the leaf pathogen Pst requires responsiveness to an SA-dependent pathway.

Key words: salicylic acid, induced systemic resistance, plant growth-promoting fungi, Fusarium equiseti GF19-1

1 INTRODUCTION

Plants have an ability to develop a state of enhanced defensive capacity, termed as induced resistance (IR), when they are properly stimulated[1]. IR enables plants to resist future attacks by virulent pathogens in local and distal parts, and it can be activated by different biotic and abiotic stresses. The best-characterized IR is systemic acquired resistance (SAR)[2]. SAR can be activated by many pathogens, especially those that cause tissue necrosis, or upon treatment with various chemical agents. SAR has been associated with the accumulation of salicylic acid (SA) and pathogenesis-related protein (PR)[3–5]. On the other hand, nonpathogenic rhizobacteria can activate a type of IR that is effective against necrotrophic pathogens; this resistance is known as induced systemic resistance (ISR). In Arabidopsis, the ISR response is mediated by jasmonic acid (JA) and ethylene (ET) hormones[1, 5, 6]. However, in some other cases, rhizobacteria-mediated ISR has led to the stimulation of SA-dependent defense responses[7, 8]. Although pathogen-induced SAR and rhizobacteria-mediated ISR follow distinct signaling pathways, both responses enhance broad-spectrum disease resistance throughout the plant[7–11].

Similar to rhizobacteria, several nonpathogenic fungi promoting plant growth are called plant growth-promoting fungi (PGPF). PGPF can suppress soil-borne diseases and also induce systemic resistance in plants[12–16]. However, from the viewpoint of molecular mechanisms, only a few PGPF have been characterized, and most of them employed Trichoderma species[17–20]. The findings of these studies indicate that the induction of resistance by PGPF triggers a wide range of responses; however, the underlying mechanisms of these inducible responses are highly dis-
2 MATERIALS AND METHODS

2.1 Origin of seeds, pathogen, and inducer

Arabidopsis wild-type Columbia (Col-0) was provided by K. S. Park (NIAST, Suwon, Korea). Mutants jrr1 (jasmonic acid insensitive), etr1 (ethylene insensitive), and npr1 (non-expression of PR gene) were collected from the Nottingham Arabidopsis Stock Center. The transgenic NahG line (bacterial nahG gene encoding salicylate hydroxylase) was a personal gift. All mutant and transgenic Arabidopsis plants were generated on the Col-0 background. The rifampicin-resistant virulent pathogen Pseudomonas syringae pv. tomato DC3000 (Pst) was provided by Y. Ichinose (Okayama University, Okayama, Japan). The PGPF isolate F. equiseti GF19-1 was obtained from the rhizospheres of zoysiagrass (Zoysia tenuifolia).

2.2 Preparation of spore suspensions and a culture filtrate of GF19-1

GF19-1 was cultured in potato dextrose broth (PDB) with shaking at 25°C. After 3 days of cultivation, GF19-1 spores were collected by centrifugation and resuspended in distilled water at a final concentration of 2.5 × 10^5 spores mL^-1. After 10 days of cultivation in PDB, the culture filtrate (CF) of GF19-1 was separated from the mycelia by double filtration through 3 layers of filter paper (Whatman No. 2) after which the CF was filter sterilized (0.22 μm, Milipore, Bedford, USA).

2.3 Cultivation of plants in soil for the spore suspension experiment

Arabidopsis plants were grown in a commercial potting medium, Star bed (soil-less, peat-based potting medium; Kyodohiryo Co. Ltd., Aichi, Japan), which contains humus, peat, rock phosphate and composted plant materials. After autoclaving twice at 24 h intervals at 121°C for 1 h, potting medium was placed in sterilized plastic pots (150 mL). Arabidopsis seeds were soaked in 0.5 mL of distilled water in microcentrifuge tubes and kept overnight in a refrigerator at 4°C to synchronize germination. Fifteen seeds were sown in each pot. Ten plants were grown in each pot, and the rest of the plants were thinned out at the seedling stage. The plants were grown for 2 weeks in a growth chamber with a 12-h day/night cycle (cool fluorescent lamps, 300 μEm^-2 s^-1) at 23°C, and they were watered on alternate days.

2.4 Hydroponic culture of plants for the culture filtrate experiment

All Arabidopsis genotypes were grown in a hydroponic culture following the procedure previously described by Hossain et al. (2007).

2.5 Induction of resistance by the spore suspension or culture filtrate of GF19-1

Arabidopsis roots were treated with the spore suspensions and the CF of GF19-1 inoculum. Arabidopsis plants grown in soil were pretreated with a 10 mL spore suspension of GF19-1 (2.5 × 10^5 spores mL^-1) by inoculating the potting medium just prior to sowing the seeds. Potting medium supplemented with an equal volume of sterilized distilled water was used as a control. The hydroponically grown plants were induced 1 day before the challenge inoculation, which was performed by dipping the roots of 2-week-old seedlings in the CF of GF19-1. Through a screening program, 5 hours of dipping the roots in the CF was selected as the optimum induction period. The control plants were treated with sterilized distilled water in a similar manner.

2.6 Colonization ability

The spore suspension experiment was carried out by determining the root colonization of Arabidopsis plants after growing them for 2 weeks in soil. Roots were harvested from 6 randomly selected plants, freed of soil with running tap water, rinsed 3 times in sterilized distilled water, and blotted dry. The roots were then cut into approximately 0.5 cm-long segments, plated on Komada’s Fusarium selective medium and incubated for 3 days at 25°C. After incubation, GF19-1 colonies growing from the root segments were counted, and the isolation frequency was determined as described by Meera et al. (1994). Root colonization ability was measured based on the isolation frequency.

The root colonization pattern in Arabidopsis roots was observed after growing the plants for 2 weeks on 1/2 Murashige & Skoog (MS) agar (0.8%) media containing 2% glucose in a growth chamber with a 12-h day/night cycle at 23°C. Each Arabidopsis root was inoculated with a 10 μL
drop of the spore suspension of GF19-1\((2.5 \times 10^5 \text{ spores mL}^{-1})\) and incubated for 1 week. Plant roots treated with spore suspensions of GF19-1 were collected, fixed in formyl-acetic-alcohol (FAA) for 24 h, cleaned in 10% KOH for 12 h, and stained with 0.01% chlorazol black E for 30 min at 100°C. All photomicrographs were recorded with an Olympus BX51 (Olympus Optical Co. Ltd., Tokyo, Japan).

2.7 Challenge inoculation and disease assessment

The soil-grown plants were challenge-inoculated at an age of 2 weeks whereas those grown in the hydroponic system were pathogen-inoculated 1 day after induction. Inoculation of the pathogen on *Arabidopsis* leaves was carried out by *Pst* cultured in liquid King’s medium B\(^{26}\) as described by Hossain et al. (2007).\(^{24\text{a}}\) Five days after the pathogen challenge, the disease severity for each plant was measured by recording the percentage of total leaf surface showing visible symptoms, where 0% = no symptoms and 100% = most severe with necrotic symptoms. Additionally, the number of *Pst* in inoculated leaves was assessed in 4 sets of 10 whole plants per treatment. The leaves were weighted, rinsed thoroughly in sterile water, and homogenized in sterilized distilled water. Further, appropriate dilutions were plated onto King’s B medium that had been supplemented with 50 mg L\(^{-1}\) rifampicin. After incubating for 48 h at 25°C, the number of rifampicin-resistant colony-forming units (CFUs)/per gram of infected leaf tissues was determined.

2.8 RNA extraction and reverse transcription-polymerase chain reaction analysis

For each treatment, leaves of 10 randomly selected plants were placed together in 1.5 mL eppendorf tubes and frozen in liquid nitrogen. RNA was extracted from these tissues and treated with RNase-free DNase, as described by Hossain et al. (2007).\(^{24\text{a}}\) Approximately 1 \(\mu\)g of total RNA was reverse transcribed into a single stranded cDNA. The obtained cDNA was amplified by reverse transcription polymerase chain reaction by using gene-specific primers. The expression of the SA-regulated gene *PR1* (encoding pathogenesis related protein), *PR2* (encoding \(\beta\)-1,3-glucanase), and *PR5* (encoding thaumatin-like protein), *JA* and/or ET-regulated gene *PDF1.2* (encoding small protein with antifungal activity), *CHIT-B* (encoding basic chitinase), *AtVsp* (encoding vegetative storage protein), and *Hel* (encoding hevein-like protein with antifungal activity) were investigated in this study. Expression of these genes and the housekeeping gene \(\beta\)-tubulin was analyzed using gene-specific primers (Table 1).

2.9 Measurement of SA

SA was measured by growing the plants in a hydroponic system for 2 weeks and then treating the roots for 5 h with the CF of GF19-1 or sterilized distilled water, as described earlier. Pathogen inoculation was carried out 1 day after induction; the leaf tissues (approximately 0.05 g) were harvested from 0–5 days after treatment and boiled in 2% (v/v) acetic acid (100 times their volume per weight) at pH 2.7 for 10 minutes. Boiling at an acidic pH hydrolyses conjugated SA to free SA and glucose. The extracts were filtered through a Millipore filter (pore size 0.22 \(\mu\)m; Millipore, Bedford, USA), and the concentration of SA in the extract was measured using high-performance liquid chromatography (HPLC). The extract was injected into an HPLC column (Shodex C18 5A; Showa denko, Tokyo, Japan) and was eluted with 25% methanol in 20 mM sodium acetate buffer at a flow rate of 1 mL min\(^{-1}\) and a temperature of 40°C. SA was detected with a fluorescence detector (exci-

| **Table 1** Gene specific primer sequences used in RT-PCR. |
|-----------------|-----------------|---|
| **Target gene** | **Primer sequences** | **bp** |
| **Pathogenesis related protein** | F 5'-GTAGGGTGTCTTCTGGTCTCC-3' | 421 |
| **PR2** | R 5'-TTCACAATTTCCACAGGAGG-3' | 376 |
| **\(\beta\)-1,3-glucanase** | F 5'-TCAGGAAAGGTTCAGGGATG-3' | 281 |
| **PR5** | R 5'-TCGGGATCTCCATTCTCCA-3' | 484 |
| **thraumatin like protein** | F 5'-ATGGGCAATATTCAGATATCCA-3' | 583 |
| **PDF1.2** | R 5'-ATGTCGGGCGCAAGCCGCTGAGG-3' | 281 |
| **plant defensin protein** | F 5'-AATGAGCTCTACTTGCTAGTTGCTTC-3' | 219 |
| **CHIT-B** | R 5'-ATCCCATGGAATACACAAGTGAT/AAAC-3' | 475 |
| **basic chitinase** | F 5'-CGGGCAGCCGAGTCGAGTCCCCG-3' | 351 |
| **AtVsp** | R 5'-ATCCCATGGAATACACAAGTGAT/AAAC-3' | 475 |
| **vegetative storage protein** | F 5'-CTGCTGACATCAGTGTC-3' | 516 |
| **Hel** | R 5'-TGGGATCAGTCCAGTCC-3' | 516 |
| **hevein like protein** | F 5'-CGGGCAGCCGAGTCGAGTCCCCG-3' | 281 |
| **\(\beta\)-tubulin** | R 5'-CTGCTGACATCAGTGTC-3' | 475 |
tation 290 nm, emission 405 nm), and the amount of SA determined indicated the total amount of free and conjugated SA in the tissues.

2.10 Statistical analysis

The experimental design was completely randomized, consisting of 3–4 replications for each treatment. The experiment was repeated at least twice, and significance treatment effects were determined by a t-test or Fisher’s LSD test (where appropriate) at p < 0.05. One representative trial of each experiment is reported in the results section.

3 RESULTS

3.1 A spore suspension of *F. equiseti* GF19-1 induces systemic resistance in *Arabidopsis*

The GF19-1-mediated ISR in *A. thaliana* was tested against the bacterial speck pathogen *Pst*, by growing the plants in soil amended with a spore suspension of GF19-1 for 2 weeks followed by inoculation with virulent *Pst* bacteria. Induced protection against the pathogen was quantified by determining the percentage area of leaves showing visible symptoms as well as by assessing the proliferation of the pathogen in the leaves. Five days after challenge inoculation with *Pst*, *Arabidopsis* plants grown in soil amended with a spore suspension of GF19-1 displayed a reduction in disease symptoms compared with the control plants (Fig. 1A). The reduction in disease severity was estimated to be, on average, 44%, in GF19-1–treated plants. Determination of the number of CFUs of *Pst* in challenged leaves showed that plants treated with GF19-1 showed a 2.0-fold decrease in the number of *Pst* population in challenged leaves (Fig. 2A).

3.2 A cell-free filtrate of *F. equiseti* GF19-1 induces systemic resistance in *Arabidopsis*

The CF of GF19-1 was also found to trigger an enhanced resistance against *Pst* in *A. thaliana*. An exposure time of 5 hours was selected based on a screening program, as the optimum time for dipping the roots in the CF after which a substantial suppression of disease was observed without visible symptoms of stress. The plants were grown in a hydroponic system for 2 weeks, after which the roots were dipped in the CF, followed by inoculation of the leaves with *Pst* 1 day after the induction. Application of the CF to the roots appeared to have a clear effect on symptom development (Fig. 3A), and *Pst* proliferation (Fig. 4A) showed a 47% lower proportion of disease symptoms on the leaf surface and a 2.3-fold decrease in the growth of *Pst* in challenged leaves. This observation confirms that the CF of GF19-1 was equally effective as live *Fusarium* in inducing systemic resistance against *Pst*.

3.3 Colonization ability of *F. equiseti* GF19-1 in *Arabidopsis*

The root colonization ability of GF19-1 was examined in *Arabidopsis* Col-0 plants. A high re-isolation frequency of GF19-1 was found from the root segments inoculated with spore suspensions of GF19-1 (data not shown). The average re-isolation frequency was found to be 65.5%. However, no *Fusarium* was detected in association with untreated roots.

A microscopic investigation revealed that GF19-1–treated roots exhibited abundant hyphal growth on the root surface and intimate contact of the hyphae with the host (Fig. 5A and 5B). Appressoria-like structures were also detected (Fig. 5C). Interestingly, the hyphae were mainly present in the intercellular space but were not seen inside the cell (Fig. 5D).

3.4 *F. equiseti* GF19-1 protects *Arabidopsis* mutants with impaired JA/ET-dependent signaling but not *Arabidopsis* plants defective in SA-dependent signaling

Transgenic *Arabidopsis* or mutants impaired in signal transduction pathways were tested for induced responses toward infection with *Pst* to elucidate the course of defense pathways induced by GF19-1 and its CF. The JA/ET signal transduction pathway was investigated using the ET-insensitive *etr1* mutant, and *jar1*, a mutant affected in the JA response pathway. Significantly, both *jar1* and *etr1* plants developed systemic resistance after pretreatment with GF19-1 and its CF, similar to wild-type Col-0, indicating that these responses are not involved with the plant’s ability to respond to these signals (Figs. 1C, 1D and 3C, 3D). Induction of the SA pathway was analyzed using NahG-expressing *Arabidopsis* specimens, which are unable to accumulate SA, and *npr1*, a mutant that is unresponsive to inducers of SAR. The blockage of SA accumulation in NahG plants and loss of NPR1 activity in the *npr1* mutant compromised GF19-1–mediated systemic resistance against *Pst*, demonstrating that GF19-1–induced resistance requires functional SA and NPR1-dependent pathways (Figs. 1B, 1E and 3B, 3E).

*Pst* populations were determined in challenged leaves of *Arabidopsis* mutants treated with GF19-1 and its CF. A significant decrease in the number of *Pst* was found in *jar1* and *etr1*, but not in NahG and *npr1* plants (Figs. 2B–2E and 4B–4E). These results reaffirmed that GF19-1–induced resistance requires SA and NPR1-dependent pathways.

3.5 *F. equiseti* GF19-1 treatment stimulates systemic expression of SA-inducible plant defense genes

The contribution of the SA-dependent pathway to GF19-1–mediated ISR was confirmed by characterizing the expression of a set of defense pathway-specific marker genes responsive to the SA pathway. These included genes...
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The expressions of these genes were systemically analyzed in the leaves of 2-week-old plants grown in soil with or without GF19-1. Inoculation of Arabidopsis roots with a spore suspension of GF19-1 resulted in an induced expression of SA-inducible PR-1, PR-2, and PR-5 genes in the leaves of treated plants compared with control plants, but not of JA and/or ET-inducible PDF1.2, CHIT-B, AtVsp, and Hel genes (Fig. 6). Similar increase in expression of these genes was also observe in the leaves of CF-treated plants compared with untreated plants (Fig. 7), which demonstrated that GF19-1 application triggers SA-dependent defense signaling in Arabidopsis.

3.6 *F. equiseti* GF19-1 treatment potentiates transient accumulation of SA in Arabidopsis

SA production was measured in plants at several specific

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**Fig. 1** Induced suppression of disease symptoms of *Pseudomonas syringae* pv. *tomato* in Arabidopsis thaliana wild-type Col-0, NahG, *jar1*, *etr1* and *npr1* plants in soil experiment. Error bars are SE from three replicates, each from eight plants that received the same treatment. Within each frame, values followed by asterisks are significantly different compared to untreated control (t-test) \( p < 0.05 \).
times after the induction. Although an increased SA level was observed in treated plants immediately upon induction, no significant difference was observed between GF19-1–treated and untreated plants at 1 day after the induction. However, challenging the plants with *Pst* produced a significant difference in the SA levels between leaves of CF-treated and untreated plants. The maximum level of SA was observed in *Pst*-infected and CF-treated plants at 4 days post *Pst* infection, compared with *Pst*-infected plants alone. This enhanced accumulation of SA in GF19-1–treated plants was thought to be due to the potentiation effect of the GF19-1 treatment (Fig. 8).

4 DISCUSSION

*F. equiseti* GF19-1 is a beneficial fungus that promotes plant growth after establishing a symbiotic relationship with the host plant. In our study, GF19-1 caused growth promotion of *Arabidopsis* when it colonized the roots but...
failed to induce growth as a cell-free CF (data not shown), suggesting that growth promotion is dependent on root colonization ability. Therefore, the mechanisms of growth promotion could involve the ability of the fungus to provide minerals to plants in a more available form rather than to its ability to produce growth-regulating substances.

This evidence suggests that colonization of roots to a certain extent by introduced beneficial microorganisms is necessary to exert beneficial effects on the plant. The root colonization assays showed that GF19-1 successfully colonized the Arabidopsis root, showing abundant hyphal growth on the root surface. Hyphal growth was mainly limited to the intercellular space; growth was not observed inside the cell, demonstrating an interaction between the fungus and the root without any undesirable interference. These observations are in agreement with findings for Trichoderma asperellum T-203, which colonizes intercellular spaces, mainly in the epidermis and outer cortex of cucumber roots, allowing the establishment of a beneficial fungus-plant association.

Fig. 3 Induced suppression of disease symptoms of Pseudomonas syringae pv. tomato in Arabidopsis thaliana wild-type Col-0, NahG, jar1, etr1 and npr1 plants in hydroponic experiment. Within each frame, values indicated with asterisks are significantly different compared to untreated control (t-test) \( p < 0.05 \).
Our previous observation showed that GF19-1 was capable of suppressing disease by activating the systemic resistance to several diseases in cucumber when applied as mycelia, spores, or barley grain inoculum to the soil. Therefore, this isolate has dual benefits for plants: activation of the plant’s defense responses and improvement of the plant’s nutritional level. In the present study, GF19-1 significantly decreased the disease symptoms caused by *P. syringe pv. tomato* DC3000 (*Pst*) in comparison to the control when applied as a soil amendment to plants. In this system, roots were treated with a spore suspension of GF19-1, and the pathogen was inoculated on leaves, thereby remaining spatially separated on the plant. Moreover, bacterial inhibition between GF19-1 and *Pst* was not found in vitro, suggesting that direct interactions such as competition and mycoparasitism did not occur between the 2 populations. Additionally, treatment of the roots of hydroponically grown *Arabidopsis* with a CF of GF19-1 also protected *Arabidopsis* against *Pst*. Thus, the protective effect by GF19-1 against *Pst* appeared to be systemic.

**Fig. 4** Induced suppression of number of *Pseudomonas syringae* pv. *tomato* in *Arabidopsis thaliana* wild type Col-0, NahG, *jar1*, *etr1* and *npr1* plants in hydroponic experiment. Within each frame, values indicated with asterisks are significantly different compared to untreated control (t-test) $p<0.05$. 
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Such a phenomenon in Arabidopsis has also been found in other studies with PGPF24, 36.

Plants are capable of differentially activating distinct defense pathways against different stimuli. In elucidating the signaling pathway(s) during GF19-1-mediated ISR in Arabidopsis against Pst, we observed that GF19-1 treatment induced similar a level of resistance in jar1 and etr1 plants, which are insensitive to JA and ET responses, respectively. However, transgenic NahG and mutant npr1, defective in SA and PR-1 gene accumulation, respectively, did not show induced protection against Pst, which indicates that GF19-1 mediates systemic resistance that is mainly dependent on SA and NPR1. These results also show that the induction of systemic resistance in Arabidopsis by GF19-1 does not follow the same pathways involved in ISR that are mediated by rhizobacteria P. fluorescens WCS417r, one of the most widely studied PGPF on Arabidopsis17, or the pathways mediated by T. asperellum (T203) in cucumber plants18. However, the role of SA in the induction of systemic resistance by certain other PGPF remains to be clarified24, 37. Thus, the signaling pathway elicited by GF19-1 closely resembles the pathway of SAR.

The SAR response correlates with the activation of SA-inducible PR genes encoding for PR proteins that have direct antimicrobial activities. It was shown that the SAR markers PR-1, PR-2, and PR-5 were accumulated in the leaves of Arabidopsis plants at the onset of resistance induced by GF19-1. Similar induction of SAR marker genes was also described for nonpathogenic F. oxyporum Fo47-mediated resistance against fusarium wilt in tomato38, indicating that nonpathogenic Fusarium isolates function as inducers of SAR39.

We measured SA concentrations to investigate whether SA was accumulated in leaf tissues of Arabidopsis plants with roots that were treated with the GF19-1 CF. SA tended to accumulate in Arabidopsis immediately after induction treatment by the CF, but the level was remarkably lowered to that of the control plants 1 day after induction.

Fig. 5 Colonization ability of GF19-1 in Arabidopsis thaliana wild-type Col-0 in MS agar test. A and B, Hyphae of GF19-1 colonizing Arabidopsis root surface; A, abundant hyphae of GF19-1 covering root surface; B, extensive colonization of the root surface and intimate contact of hyphae of GF19-1 with the host; C, appressoria-like structure; D, hyphal growth in the intercellular space. H: hyphae of GF19-1; AP: appressoria-like structure.

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The level of SA was again raised after infection of GF19-1–pretreated Arabidopsis with \textit{Pst}, compared with the level of SA in plants exclusively infected with \textit{Pst}. This finding suggests that GF19-1 treatment primes the Arabidopsis plant for potentiated production of SA, which becomes apparent only after \textit{Pst} infection, confirming that priming significantly contributes to the resistance mediated by GF19-1.

### 5 CONCLUSION

In conclusion, \textit{F. equiseti} GF19-1 is an effective inducer for the resistance and pathogen defense responses in \textit{Arabidopsis}. This fungus elicits resistance against the \textit{Pst} pathogen through a classic SAR-like mechanism that has been demonstrated in many plant species and is known to confer resistance against a broad spectrum of plant pathogens. Future research on the manipulation of GF19-1–mediated SAR could lead to novel and effective strategies for improvement of disease resistance in plants.

**Fig. 6** Expression of salicylic acid-, jasmonic acid-, and ethylene-regulated genes in \textit{Arabidopsis thaliana} wild-type Col-0 by spore suspensions of \textit{Fusarium equiseti} GF19-1 in the soil system.

**Fig. 7** Expression of salicylic acid-, jasmonic acid-, and ethylene-regulated genes in \textit{Arabidopsis thaliana} wild-type Col-0 by culture filtrate of \textit{Fusarium equiseti} GF19-1 in the hydroponic system.

**Fig. 8** Endogenous salicylic acid in leaf tissues in of \textit{Arabidopsis thaliana} plants with roots that were treated with the culture filtrate of GF19-1. Each value is the mean ± SE. Within each frame, different letters indicate statistically significant differences between treatments (LSD) $p < 0.05$. 

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REFERENCES


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