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PGPR control *Phytophthora capsici* in tomato through induced systemic resistance, early hypersensitive response and direct antagonism in a cultivar-specific manner

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Abstract Plant pathogens cause significant crop losses worldwide and present significant challenges to reliable food systems. The hemibiotroph *Phytophthora capsici* is ranked within the top 10 most problematic oomycete plant pathogens. *P. capsici* induces significant damage to plants by causing root rot, stem blight, and fruit rot, leading to decreased crop yields, economic losses, and increased plant susceptibility to secondary infections. The current study tested the hypothesis that plant growth promoting rhizobacteria (PGPR), namely *Bacillus velezensis* UQ9000N and *Pseudomonas azotoformans* UQ4510An, have inhibitory effects against various fungi and oomycetes. This study also hypothesised that the plant genotype affects the capability of UQ4510An to control *P. capsici* infection. Our results revealed that *B. velezensis* UQ9000N and *P. azotoformans* UQ4510An inhibited the growth of several plant pathogens by 50% or higher. These bacteria also induced abnormal mycelial morphology of *P. capsici* and *Fusarium oxysporum* f. sp. *lycopersici*. Moreover, *P. azotoformans* UQ4510An exhibited anti-oomycete activity *in vitro* and *in planta,* by reducing symptoms of *P. capsici* infection in various tomato plant cultivars in a genotype-dependent manner. Some tomato cultivars which presented less improvements of phenotypic parameters from the UQ4510An inoculation in the absence of the pathogen had a more pronounced reduction in symptoms upon *P. capsici* infection. Furthermore, our results indicated that the main biocontrol mechanism of *P. azotoformans* UQ4510An against *P. capsici* is a combination of direct antagonism and induction of induced systemic resistance (ISR) involving a hypersensitive response (HR) in the plant host at early stages of infection.

Keywords Phytopathogens · Antagonism · Plant defence gene expression · Biocontrol agent · Plant variety · Biopesticide · *Solanum lycopersicum*

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Introduction

The global impact of plant pathogens on agriculture is substantial, leading to challenges to food production due to crop losses. This is further exacerbated

by worsening impacts of climate change (Agrios, 2005; Lamour, 2013; Ristaino et al., 2021; Savary et al., 2017; Singh et al., 2023). These substantial losses are linked to plant diseases affecting every component of food security, including production, distribution, availability, quality and nutritive value (Agrios, 2005; Savary et al., 2017). One of the most devastating plant pathogens is *Phytophthora*, suitably named "plant destroyer" (derived from Greek), which affects crop production in temperate and tropical regions (Guha Roy, 2015; Lamour, 2013). Currently, there are over 120 described *Phytophthora* species (Érsek & Ribeiro, 2010; Lamour et al., 2013), of which the most destructive have been ranked within the top 10 most problematic oomycete plant pathogens, including *P. infestans, P. ramorum, P. sojae, P. capsici*, *P. cinammomi* and *P. parasitica* (Kamoun et al., 2015).

Phytophthora capsici causes *Phytophthora* blight, which is a polycyclic disease that is spread through contaminated soil and farming equipment, diseased plant debris, and flowing water (Agrios, 2005; Granke et al., 2012; Kamoun et al., 2015). *P. capsici* infects approximately 50 host species including solanaceous (tomato, potato, pepper), legume (lima bean and snap bean) and most cucurbit plants (cucumber, melon, pumpkin, zucchini) (Granke et al., 2012; Kamoun et al., 2015; Pegg et al., 2015). Different plant parts can be affected, leading to seedling death, crown and stem lesions, root rot, leaf blight and fruit rot, which cause damage to vegetable production valued at over 1 billion USD annually (Granke et al., 2012; Kamoun et al., 2015; Lamour et al., 2012; Pegg et al., 2015). *P. capsici* is a hemibiotrophic phytopathogen that during initial phases of infection relies on biotrophy. After 24–48 h it then becomes necrotrophic and begins to produce sporangia on the surface of infected tissues (Granke et al., 2012; Kamoun et al., 2015; Lamour et al., 2012). The control of *Phytophthora* blight has varying degrees of success (Kamoun et al., 2015; Lamour et al., 2012). The development of resistant strains of *P. capsici* reduces the effectiveness of chemical control measures (Barchenger et al., 2018; Bi et al., 2014; Miao et al., 2016). Furthermore, breeding cultivars with durable disease resistance is often a difficult and costly process, given the genetic diversity of *P. capsici* and hence its ability to overcome host plant resistance mechanisms (Lamour et al., 2012). Eradication efforts of *Phytophthora* blight are further complicated by the ability of *Phytophthora capsici* to infect multiple plant species, and to live in the soil, irrigation water and plant debris (Quesada-Ocampo et al., 2011; Babadoost & Pavon, 2013; Moreira-Morrillo et al., 2023; and Quesada-Ocampo et al., 2023).

Resistance to *Phytophthora capsici* within the Solanaceae family encompasses a wide range of components, including broad-spectrum protection, isolate-specific defence mechanisms, and tissue-specific responses (Quesada-Ocampo et al., 2023). Mechanisms of disease resistance in plants involve complex, multicomponent and deeply interconnected immune defence systems (Andersen et al., 2018). There are two main inducible defence responses, namely systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Pieterse et al., 2012; Ramirez-Prado et al., 2018). SAR is elicited in response to a wide range of phytopathogens (mostly biotrophic) and is mediated by SA signalling and occurs after hypersensitive response (HR; Pieterse et al., 2012; Ramirez-Prado et al., 2018). Alternatively, ISR is elicited in response to mainly necrotrophic and hemibiotrophic pathogens and plant-growth-promoting rhizobacteria (PGPR), being mediated primarily by jasmonic acid (JA) and ethylene (ET) signalling pathways and does not involve a hypersensitive response (HR) (Pieterse et al., 2012; Ramirez-Prado et al., 2018).

The use of beneficial microorganisms as biofertilisers and biopesticides has emerged as important sustainable alternatives to the use of harmful chemical fertilisers and pesticides in modern industrialised food systems (Rodriguez et al, 2019; Saritha & Tollamadugu, 2019). Some PGPR control phytopathogenic organisms through production of various antimicrobial compounds and/or ISR in plants, being generally referred to as biopesticides (Beneduzi et al., 2012; Balog et al., 2017; Gouda et al., 2018; Rodriguez et al., 2019; Saritha and Tollamadugu, 2019). PGPR isolates belonging to *Bacillus* and *Pseudomonas* genera have been reported as biocontrol agents, particularly within the *Bacillus subtilis* species complex and the *Pseudomonas fluorescens* species complex (Garrido-Sanz et al., 2016; Rabbee et al., 2019; Sang et al., 2014; Shafi et al., 2017). Bacterial isolates belonging to these genera produce a large variety of bioactive compounds, including antimicrobials, hormones and volatile organic

compounds with wide-ranging applications (Hazarika et al., 2019; Minaxi and Saxena, 2010; Syed-Ab-Rahman et al., 2018; Wang et al., 2021a, 2021b). Various *Bacillus velezensis* and *Pseudomonas azotoformans* have been reported to be involved in plant growth promotion, abiotic stress alleviation and biocontrol via ISR in cucumber ant tomato plants (Ansari et al., 2021; Fang et al., 2016; Fan et al., 2018; Rabbee et al., 2019; Sang et al., 2014; Stoll et al., 2021; Syed-Ab-Rahman et al., 2019).

Advantages of biopesticides over chemical pesticides include relatively low or no impact on beneficial soil microorganisms, non-target insects, livestock and humans, and minimal impacts on ecological processes and biodiversity (Balog et al., 2017; ICP-SW, 2018; Saritha and Tollamadugu, 2019). Major challenges hinder the use of PGPR-based biopesticides, such as inconsistencies between studies claiming their efficiency or between trials conducted in greenhouses and in the field (Pieterse et al., 2016; Trivedi et al., 2017; Wallenstein, 2017; Wintermans et al., 2016). Contrasting results may be caused by differences in climate, soil conditions, competition with the native microbiome, and plant genotypes (Pieterse et al., 2016; Trivedi et al., 2017; Wallenstein, 2017; Wintermans et al., 2016).

The current study evaluated the use of *B. velezensis* and *P. azotoformans* as biocontrol agents and assessed the effect of the plant genotype on disease suppression by testing the following hypotheses: 1) *B. velezensis* UQ9000N and *P. azotoformans* UQ4510An inhibit a range of fungal and oomycete pathogens and change the hyphal structure of pathogen mycelia; 2) the plant genotype affects the capability of UQ4510An to control *P. capsici* infection. We also aimed to elucidate the underlying mechanisms associated with the alleviation of *P. capsici* symptoms in tomato (*Solanum lycopersicum*) by bacterial inoculations. We used tomato plants for the pot experiments given the worldwide economic relevance of this horticultural crop. Tomato is ranked seventh in global production, after maize, rice, wheat, potato, soybean and cassava, and it is the second most consumed horticultural product, coming just after potato (Adhikari et al., 2017; Gerszberg et al., 2015). In 2020, worldwide tomato production was around 187 million metric tons with a value of more than $60 billion USD, cultivated on an area of 5 million hectares (FAOSTAT, 2020; Gerszberg et al., 2015).

Materials and Methods

Isolation and cultivation of PGPR isolates

Bacillus velezensis UQ9000N (GenBank access number OM281413.1) and *Pseudomonas azotoformans* UQ4510An (GenBank access number OM281426.1) were originally isolated from a clay soil collected in Brisbane, Australia (27°31′37.0"S 152°59′51.7"E). The isolates were pre-cultured from -80 °C glycerol stocks and grown in yeast extract peptone (YEP) broth (10 g/L bactopeptone, 10 g/L yeast extract, 5 g NaCl) overnight on a flat shaker incubator at 28 °C in 50 mL conical tubes with 25 mL of medium in each tube (100 rpm). Then each of the two bacterial cultures were diluted in phosphate-buffered saline (PBS) to an OD 600 nm of 0.1, which contained 1×10^6 colony forming units (CFU) mL^{-1} for *B. velezensis* and 10^7 CFU mL^{-1} for *P. azotoformans*. These diluted UQ4510An and UQ9000N cultures served as the inoculum for all experiments. Water and YEP broth were used as controls.

Bacterial 16S rDNA gene amplicon sequencing

Bacterial isolates were streaked on YEP plates and grown for three days at 25 °C in the dark. Subsequently, a single colony was picked with a sterile loop and transferred into 20 µL of nuclease-free water and heated at 95 °C for 10 min to rupture the bacterial cells. After that, the solution was centrifuged at 11,000 x*g* for 3 min. Then, PCR was performed in a 25 µL reaction mixture containing: 2 µL of the bacterial lysate, 12.5 µL of PCR Mango Master Mix, 9.5 µL of Nuclease-free water and 1 µL of the primer mix (universal 16S primer set consisting of 27F 5'-AGAGTTTGATCMTGG CTCAG-3' and 1492R 5'-TACGGYTACCTTGTT ACGACT-3'). The PCR thermocycler conditions were set as follows: one step of 98 °C for 30 s, 40 cycles of 98 °C for 15 s, 56 °C for 30 s and 72 °C for 45 s, followed by one step of 72 °C for 7 min. The PCR amplification was visually confirmed for the presence of a single band by 1% (w/v) agarose gel electrophoresis. The unpurified amplified PCR products were then submitted to Australian Genome Research Facility Ltd for two-directional Sanger sequencing.

In vitro biocontrol analyses

Dual-culture screening assay of bacterial isolates and pathogenic fungi and oomycetes

The bacterial isolates *Pseudomonas azotoformans* (UQ4510An) and *B. velezensis* (UQ9000N) were tested against 20 fungal and oomycete isolates for their inhibitory capability using dual-culture assays (Kumar et al., 2012; Syed-Ab-Rahman et al., 2018). These included seven *Phytophthora* spp., namely *P. capsici, P. cinnamomi, P. citricola*, *P. cactorum*, *P. medicaginis*, *P. nicotianae,* and *P. palmivora*, five *F. oxysporum* isolates including four formae speciales (f. sp.): *F. oxysporum* f. sp. *cubense* subtropical race 4*, F. oxysporum* f. sp. *lycopersici* (*Fol*), *F. oxysporum* f. sp. *medicaginis*, *F. oxysporum* f. sp. *zingiberi* and *F. oxysporum*, two *Alternaria* spp.: *A. brassicicola* and *A. solani*, as well as one isolate of *Althelia rolfsii*, *Aspergillus* sp., *Gliocladiopsis* sp., *Macrophomina phaseolina*, *Pyricularia oryzae* and *Pythium sulcatum* (Table S1). The assay consisted of preparing dual-cultures of the pathogens and bacterial isolates on potato dextrose agar (PDA, Oxoid) plates (39 g of PDA powder per 1 L of deionised water). An *E. coli* strain (DH5α) which does not markedly inhibit plant pathogens was used as an additional negative control. Standardised bacterial suspensions at OD 600 nm of 0.1 were prepared from overnight cultures in YEP media all three bacterial isolates. An agar plug with mycelium of each fungal/oomycete isolate previously grown on PDA plates for 7 days at 25 °C under dark conditions was placed in the centre of a PDA plate, followed by inoculation of 10 µL of the bacterial culture three cm away from the pathogen at two opposite sides of the plate. Negative control plates contained the agar plug with mycelium placed on PDA plates but received 10 µL of sterile liquid YEP medium instead of bacterial cultures. Plates were then incubated at 25 °C for 7 days. Subsequently, the percent inhibition (PI) was calculated as: $\mathrm{PI} = \left(\mathrm{C} - \frac{\mathrm{P}}{\mathrm{C}}\right) \times 100$, where the C is diameter of mycelial growth (mm) of pathogen from control plates and P is diameter of mycelial growth (mm) along the axis where PGPR cultures were inoculated. Each experiment had three technical replicates per sample and was repeated three times.

Fluorescence microscopy analysis of pathogen mycelia morphology after treatment with bacterial isolates

To elucidate mechanisms of *P. capsici* antagonism by PGPR, effects of bacterial treatments on mycelial morphology of the inhibited plant pathogens were evaluated using fluorescence microscopy. Firstly, a small piece of the mycelial mat at the leading edge of each pathogen was excised using a scalpel blade and then transferred onto a glass microscope slide. After that, the mycelial sample was treated with calcofluor white stain (Sigma-Aldrich) according to the manufacturer's instructions. A volume of 10 µL of calcofluor white stain and 10 µL of 10% potassium hydroxide were added onto the mycelial sample, a cover slip was placed over the specimen and left to stand for 1 min. The slides were then observed by confocal fluorescence microscopy (ZEISS LSM700) using a 405 nm laser with blue filter setting and wavelength as described by the Calcofluor white stain manufacturer. The images were acquired using the ZEN black edition software (ZEISS) and then analysed using ZEN 2.6 blue lite edition software (ZEISS).

Characterisation of *in vitro* biocontrol traits of *B. velezensis* UQ9000N and *P. azotoformans* UQ4510An

To run the following assays bacterial cultures were grown overnight in liquid YEP broth in the dark at 25 °C and diluted in PBS to 0.1 at OD 600 nm. Production of siderophores by the bacterial isolates was assessed using the universal chrome azurol S (CAS) assay (Louden et al., 2011; Schwyn & Neilands, 1987). Protease biosynthesis was conducted according to Adinarayana et al. (2003) with a modification that 10 µL from each overnight bacterial culture was transferred onto the skim milk agar and incubated in the dark at 25 °C for 2 days. Cellulase production was evaluated using carboxymethyl cellulose (CMC) agar medium as described in Syed-Ab-Rahman et al. (2018).

Pot trial for biocontrol evaluation

Plant cultivation and bacterial treatment

Given the inhibitory effect that *P. azotoformans* UQ4510An had on *P. capsici*, we assessed whether

the plant genotype influences the control of pathogen infection by UQ4510An. Six tomato cultivars were used for pot experiments, namely Money Maker (MM), Grosse Lisse (GL), Tommy Toe (TT), Red Cherry (RC), Oxheart (OX) and Rouge de Marmande (RdM). Initially, seeds were surface sterilised with 70% ethanol for 5 min, followed by soaking in 1% sodium hypochlorite for 5 min, and then rinsed with sterilised distilled water five times. Seeds were then placed onto sterilised filter paper moistened with sterile water and left for 5 days. Thirty germinated seeds were then transferred into 30-cell trays (64 × 76 mm) filled with moist UQ23 potting mix composed of composted pine bark (up to 5 mm; 70%), cocoa peat (30%) and mineral fertiliser. Deionised water was used to water the plants by pouring it into the tray every 3–4 days. Seedlings were kept in a growth cabinet at 12 h of light, 25 °C during the day/19 °C at night and 70% humidity. Tomato plants were inoculated twice (at week 2 and 4 after sowing) with *P. azotoformans* UQ4510An by adding 5 mL of the bacterial suspension (1×10^7 CFU/mL of PBS) per plant to the soil around the stem base.

P. capsici inoculation

Tomato plants were grown for 4 weeks as previously described and then inoculated with *P. capsici* using the following modified method (Bostock et al., 2014; Syed-Ab-Rahman et al., 2018). *P. capsici* was grown on 20% V8 agar plates for 7 days at 25 °C under dark conditions. A soil extract was prepared by mixing 1 g of non-sterile soil in 100 mL water and leaving it to settle for 2 days. Ten small cuttings (approximately 5×5 mm^2) of aerial mycelium were removed from the surface of 1-week-old *P. capsici* cultures and placed in 10 mL of the soil extract solution in a Petri dish and incubated for 2 days at 25 °C under constant fluorescent light (40 W) to promote sporangia production. Plates were then placed into a cold room at 4 °C for 30 min, and, subsequently, left at room temperature for 60 min to stimulate the release of zoospores. Zoospore concentration was then measured using a hemocytometer under a light microscope and the concentration adjusted to 1×10^6 zoospores/mL by adding sterile water. The *P. capsici* inoculum was prepared by mixing 50 g of organic wheat seeds and 25 mL of distilled water in a 100 mL glass laboratory bottle. The bottles were autoclaved twice for 15 min at 121 °C. The wheat seeds were inoculated with five small cuttings (approximately 5×5 mm^2) of aerial mycelium from the surface of 1-week-old *P. capsici* cultures. The *P. capsici* inoculum was incubated at 24 °C in the dark for 4 weeks. Then the plants were infected with both the zoospore solution and mycelia. Firstly, the trays were filled with distilled water containing zoospores (10^6 mL^{-1}), subsequently each plant was treated with 10 mL of zoospore solution and 20 g of *P. capsici*-infected wheat seeds. In the negative control non-infected wheat seeds and water were used. The trays were covered with plastic wrap for three days to maintain high humidity. After 2-, 3- and 7-days post *P. capsici* inoculation, the tomato plants were harvested, the root and shoot tissues were snap frozen in liquid nitrogen and then stored at -80 °C prior to RNA extraction and quantitative real-time RT-PCR. The disease assessment was conducted at 4 weeks after *P. capsici* inoculation. Disease progression was recorded for each plant using the following scale: **0**—no symptoms, **1**—1 to 30% leaf wilting, dropping, and curling or roots with increasing levels of root rot, **2**—31 to 50% symptoms, **3**—51 to 70% symptoms, **4**—71 to 90% symptoms, and **5**—> 90% wilting or dead plant (Quesada-Ocampo & Hausbeck, 2010). After symptom scoring, root and shoot lengths were measured along with fresh and dry biomass (root and shoot combined). Furthermore, the photosynthetic and transpiration rates, net assimilation rate of CO_2, and stomatal conductance were measured between 11:00am and 1:00 pm with the LI-6800 Portable Photosynthesis System (LI-COR, NE, United States) from individual leaflets (n = 3) of each plant.

Quantitative real-time RT-PCR to assess defence gene modulation by bacterial isolates in *P. capsici*-infected tomato plants

Total RNA isolations were performed using frozen tomato (cv. Money Maker) shoot and root samples (2-, 3- and 7-days post *P. capsici* inoculation) as starting material with a Maxwell RSC Plant RNA Kit (PROMEGA) following the manufacturer's instructions. The concentration and purity of the obtained RNA samples was measured using Nanodrop Spectrophotometer (Thermo Scientific). The cDNA was generated by reverse transcription using the Tetro cDNA synthesis kit (BIOLINE) following the manufacturer's instructions. The reactions included 12 µL of RNA samples at a concentration of 10 ng µL^{-1}

(total amount ~ 120 ng) in a 20 µL reaction using both random hexamers and oligo dT primers. Real-time qRT-PCR was performed on the CFX Opus 384 Real-Time PCR System (Bio-Rad Laboratories). Nine tomato genes involved in plant defence pathways were targeted. The targeted genes and primers used for this experiment are shown in Table 1.

Each reaction had a volume of 10 µL and contained 4 µL of sample DNA (~10 ng μL^{-1}), 5 µL of SYBR green master mix, and 1 µL of mixed forward and reverse primers (3 µM). *SlACTIN* was used as the housekeeping gene for normalisation. Thermal cycling conditions were set as follows: (1) heat activation step with 1 cycle of 95 °C for 2 min, then (2) amplification step with 40 cycles of 95 °C for 10 s, and 60 °C for 20 s, followed by (3) melt curve analysis step with 1 cycle of 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. Relative expression of each target gene was investigated using three biological replicates (five plants each) with three technical replicates. Data analysis was performed with QuantStudio™ Real-Time PCR Software v1.1 (Applied Biosystems). Relative expression (n-fold) of the normalised target genes in both treatments was determined as proposed by Pfaffl (2001).

Statistical analysis

For comparisons between treatments and control, significant differences were determined based on a Student's t-test for pairwise unequal variance comparisons or ANOVA F test followed by a Tukey's HSD test using JMP software at the 95% confidence level ($p < 0.05$).

Results

In vitro inhibition assessments of PGPR isolates

P. azotoformans UQ4510An and *B. velezensis* UQ9000N were tested for inhibitory activity against 20 various fungi and oomycetes using the dual-culture assay method (Fig. 1). The percentage threshold of growth inhibition considered significant was 50% or higher. The results revealed that *P. azotoformans* UQ4510An significantly inhibited three oomycete species, namely *P. cactorum*, *P. sulcatum*, *P. capsici*, with 68%, 66%, and 63% inhibition, respectively. Similarly, the *B. velezensis* UQ9000N isolate was able to efficiently inhibit seven fungi and one

Table 1 Primers used for qRT-PCR

Code	Target Gene	Sequence (5'-3')	Reference
SlRBOHD-F	*Respiratory Burst Oxidase Homolog Protein D*	TCAGGTCAAGCATCAAAGCCGTT	Dixit et al. (2018)
SlRBOHD-R		TGGTGAAACCGCAGCACAGT	
SlCAT-F	*Catalase*	TGGAAGCCAACTTGTGGTGT	Zhang et al. (2015)
SlCAT-R		ACTGGGATCAACGGCAAGAG	
SlSOD-F	*Superoxide Dismutase*	CAAGATGATGATGGTCCAAC	Khanna et al. (2019)
SlSOD-R		CTCCATGTGTCAATTTATTCGG	
SlLOXD-F	*Lipoxygenase D*	CCATCCTCACCACCCTCATC	Beris et al. (2018)
SlLOXD-R		TACTCGGGATCGTTCTCGTC	
SlPAL1-F	*Phenylalanine Ammonia-Lyase 1*	CATTGTACAGGTTGGTGAGAG	Abbasi et al. (2019)
SlPAL1-R		CATCTCTTGAGACACTCCA	
SlNPR1-F	*Nonexpressor of Pathogenesis-Related 1*	TGTGGGAAAGATAGCAGCACG	Beris et al. (2018)
SlNPR1-R		GTCCACACAAACACACACATC	
SlPR1-F	*Pathogenesis-Related Protein 1*	GGTAACTGGAGAGGACAA	Abbasi et al. (2019)
SlPR1-R		GACAATCGATCACTTTATTC	
SlJAZ1-F	*Jasmonate-Zim-Domain Protein 1*	TTCCCTCAAGGTGGAATGAAGGCT	Chini et al. (2017)
SlJAZ1-R		TCCGAAACTCGGAACCACCAAATC	
SlERF1-F	*Ethylene Response Factor 1*	AGACTTGGGAGTTGAATTA	Abbasi et al. (2019)
SlERF1-R		TACATTGCGATCTTGATTA	
SlACTIN-F	*Actin*	AGGCAGGATTTGCTGGTGATGATGCT	Mascia et al. (2010)
SlACTIN-R		ATACGCATCCTTCTGTCCCATTCCGA	

oomycete, including *M. phaseolina* with 56% inhibition, *F. oxysporum* f. sp. *medicaginis* with 55% inhibition, *P. cactorum* and *F. oxysporum* f. sp. *lycopersici* (*Fol*) with 52% inhibition, and *F. oxysporum* f. sp. *cubense*, *A. rolfsii*, *A. solani* and *A. brassicicola* with 51% inhibition rate. The *E. coli* isolate, which was used as negative control, did not inhibit the growth of any of the 20 fungal and oomycete pathogens above 50%.

Abnormal mycelial morphology of *P. capsici* and *Fol* induced by UQ4510An and UQ9000N, respectively, was observed using fluorescence microscopy along the pronounced inhibition zone (Fig. 2). Morphological abnormalities included excessive branching and irregular hyphal shapes for both pathogens.

Bioactive compounds produced by PGPR

Two PGPR isolates were analysed for their ability to produce siderophores and cell wall-degrading enzymes. *P. azotoformans* UQ4510An produced proteases and siderophores, while *B. velezensis* UQ9000N produced proteases and cellulases (Supplementary Table S2 and Fig. S1).

Biocontrol of UQ4510An on *P. capsici*-infected tomato plants and differential responses by distinct plant genotypes

Based on its pronounced biocontrol activity over *P. capsici*, *P. azotoformans* UQ4510An was chosen to be tested in a pot trial using six tomato cultivars, namely Money Maker (MM), Grosse Lisse (GL), Tommy Toe (TT), Red Cherry (RC), Oxheart (OX) and Rouge de Marmande (RdM). UQ4510An treatments reduced *P. capsici* infection symptoms in TT, GL and RC tomato cultivars by 42.5%, 41.3% and 40.1%, respectively (Fig. 3). These three cultivars also had the highest disease symptom scores in the untreated-infected group, namely 4.25, 4.46 and 3.83, respectively. Symptom alleviation of 22.2%

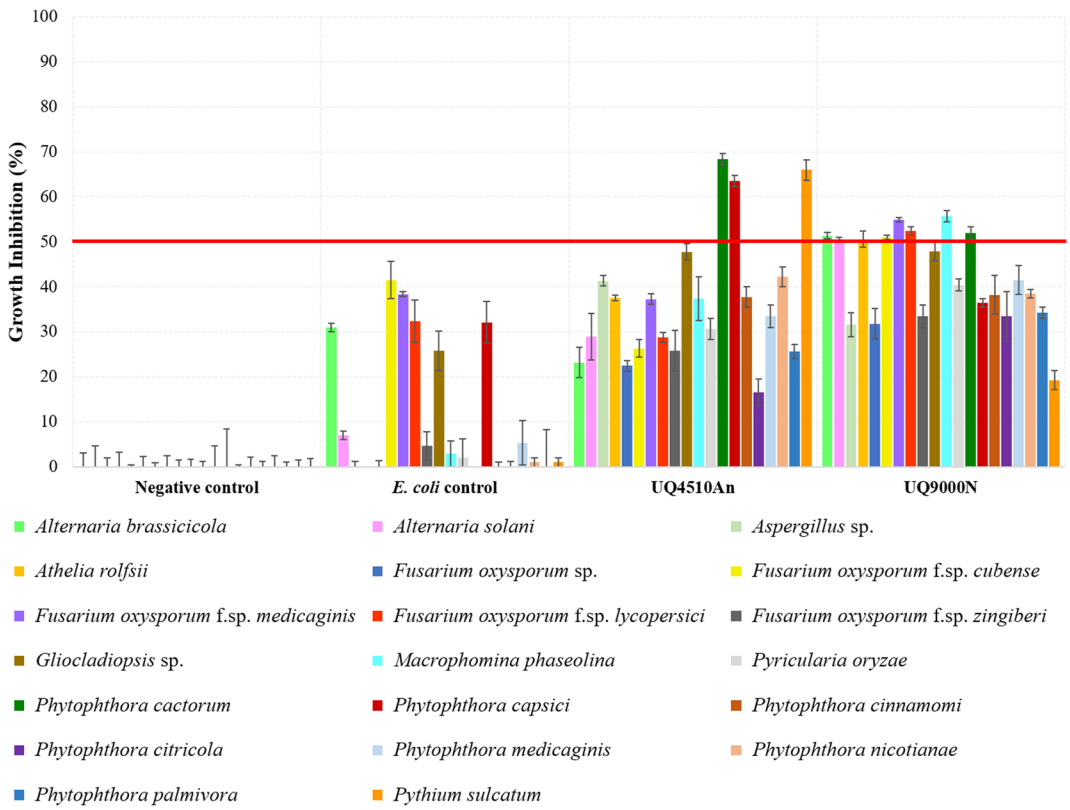


Fig. 1 Inhibition rates of *P. azotoformans* UQ4510An and *B. velezensis* UQ9000N against 20 different species of fungal and oomycete phytopathogens. The red line indicates the IC50, the inhibition values above the 50% threshold which were considered substantial. Shown are mean values ± SEs ($n = 9$ replicates)

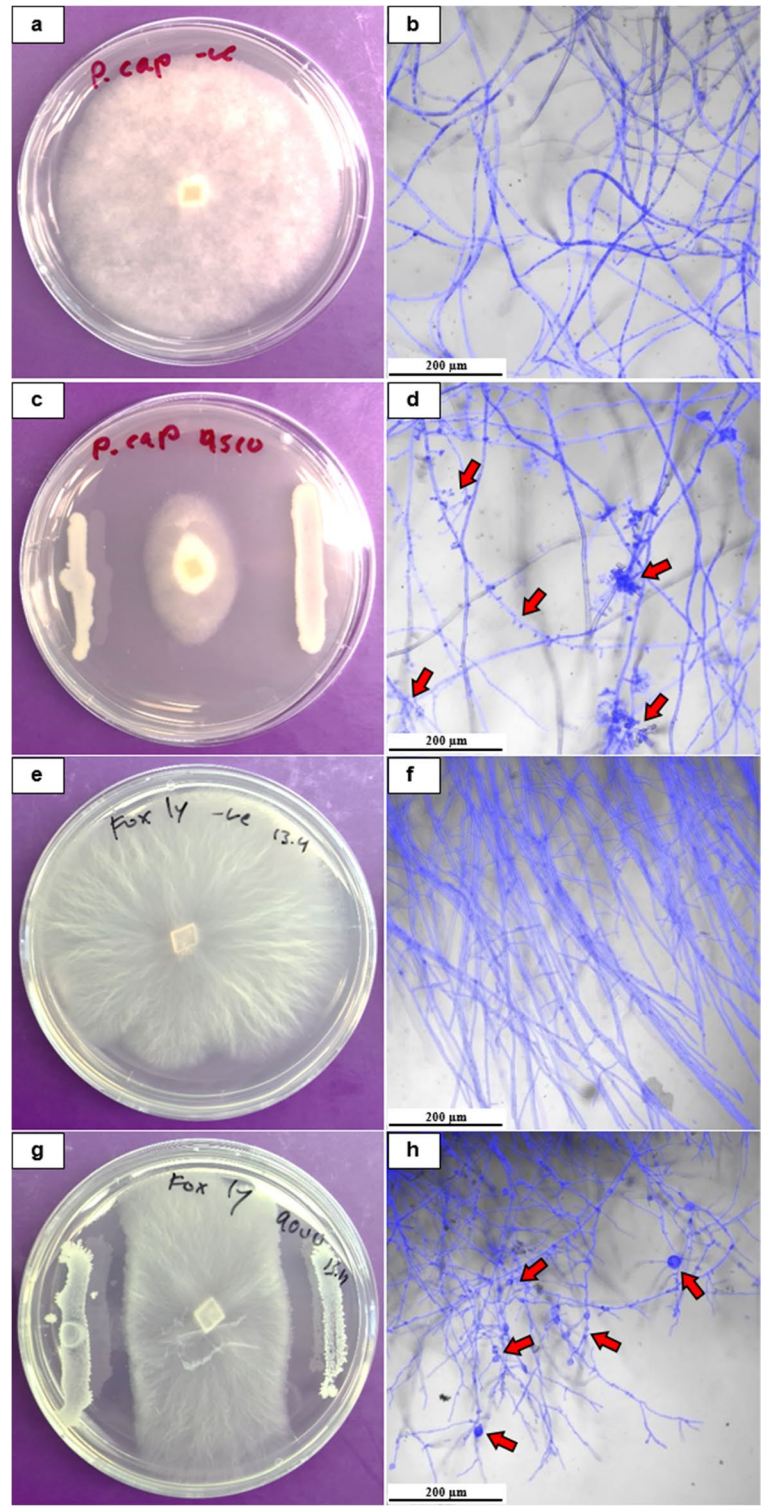


Fig. 2 Inhibitory effect and changes in hyphal structures induced by PGPR on phytopathogens. PDA plate images and fluorescent micrographs of *P. capsici* (**a** & **b**) single culture control and (**c** & **d**) co-cultured with *P. azotoformans* UQ4510An and *Fol* (**e** & **f**) single culture control and (**g** & **h**) co-cultured with *B. velezensis* UQ9000N. The bar indicated 200 µm on fluorescent micrographs. Mycelial abnormalities are indicated by red arrows

and 20.3% was observed for MM and RdM, respectively, which exhibited disease scores of 3.64 and 3.7 in untreated-infected group. No significant reduction of symptoms was observed for the OX cultivar, which also had the lowest disease symptom score of 3.4 (untreated-infected group) of all cultivars tested. Mock- and UQ4510An-treated plants, which were not treated with *P. capsici*, had disease symptom scores of 0 (data not shown).

Symptoms of the cultivar Money Maker in different treatment groups are shown in Fig. 4, including (1) mock-treated, *P. capsici*-uninfected control, (2) UQ4510An-treated, *P. capsici*-infected and (3) mock-treated, *P. capsici*-infected control groups. It was noticeable that UQ4510An alleviated disease symptoms including less yellowing and necrotic leaves, stem lesions and necrotic roots. As expected, the treatment group (4) UQ4510-treated plants that were not infected with *P. capsici* showed no disease symptoms (data not shown).

Seven phenotypic parameters (fresh and dry weight, shoot and root length, leaf transpiration rate, photosynthesis, CO_2 assimilation rate and stomatal conductance rate) were measured comparing: (1) negative control mock-treated, *P. capsici*-uninfected, (2) UQ4510An-treated *P. capsici*-infected, (3) positive control mock-treated *P. capsici*-infected and (4) UQ4510An-treated *P. capsici*-uninfected (not shown in Fig. 4) plants to evaluate the effects of this PGPR isolate on cultivar-specific responses to the pathogen.

Variable responses to the PGPR and the pathogen were observed (Fig. 5). Three tomato cultivars, namely MM, OX and RC responded positively to the UQ4510An treatment in the absence of pathogen, with a significant increase in fresh (Fig. 5a) and dry weight (Fig. 5b). Root length was only increased for MM cultivar (Fig. 5d), while there was no significant shoot length increase in the absence of the pathogen (Fig. 5c). Alternatively, after the UQ4510An treatments there was a significant decrease in dry weight of TT and RdM (Fig. 5b). Leaf transpiration rate was only increased for MM (Fig. 5e). Furthermore, photosynthetic CO_2 assimilation rates were significantly increased for three cultivars, namely TT, OX and RdM (Fig. 5f). Finally, stomatal conductance was increased for RdM and decreased for RC (Fig. 5g). Similarly, the photosynthetic CO_2 assimilation rate was significantly increased in the presence of *P. capsici* for cultivars GL, TT and RdM (Fig. 5f). In addition, stomatal conductance was significantly increased only RdM when infected plants were inoculated with UQ4510An (Fig. 5g). Upon *P. capsici* infection, no significant

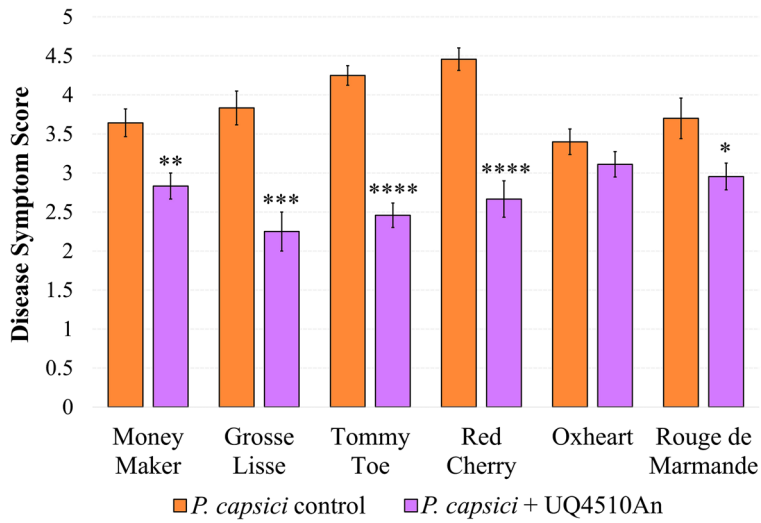


Fig. 3 Disease symptom scores of untreated (orange) and *Pseudomonas azotoformans* UQ4510An-treated (purple) tomato cultivars, namely Money Maker (MM), Grosse Lisse (GL), Timmy Toe (TT), Red Cherry (RC), Oxheart (OX) and Rogue de Marmande (RdM), infected with *P. capsici*. Shown are mean values ± SEs (n = 12 plants per treatment). The statistical significance was determined by Student's t-test, asterisks show significant differences to the untreated *P.capsici*-infected control plants (*P. capsici* control) with * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$

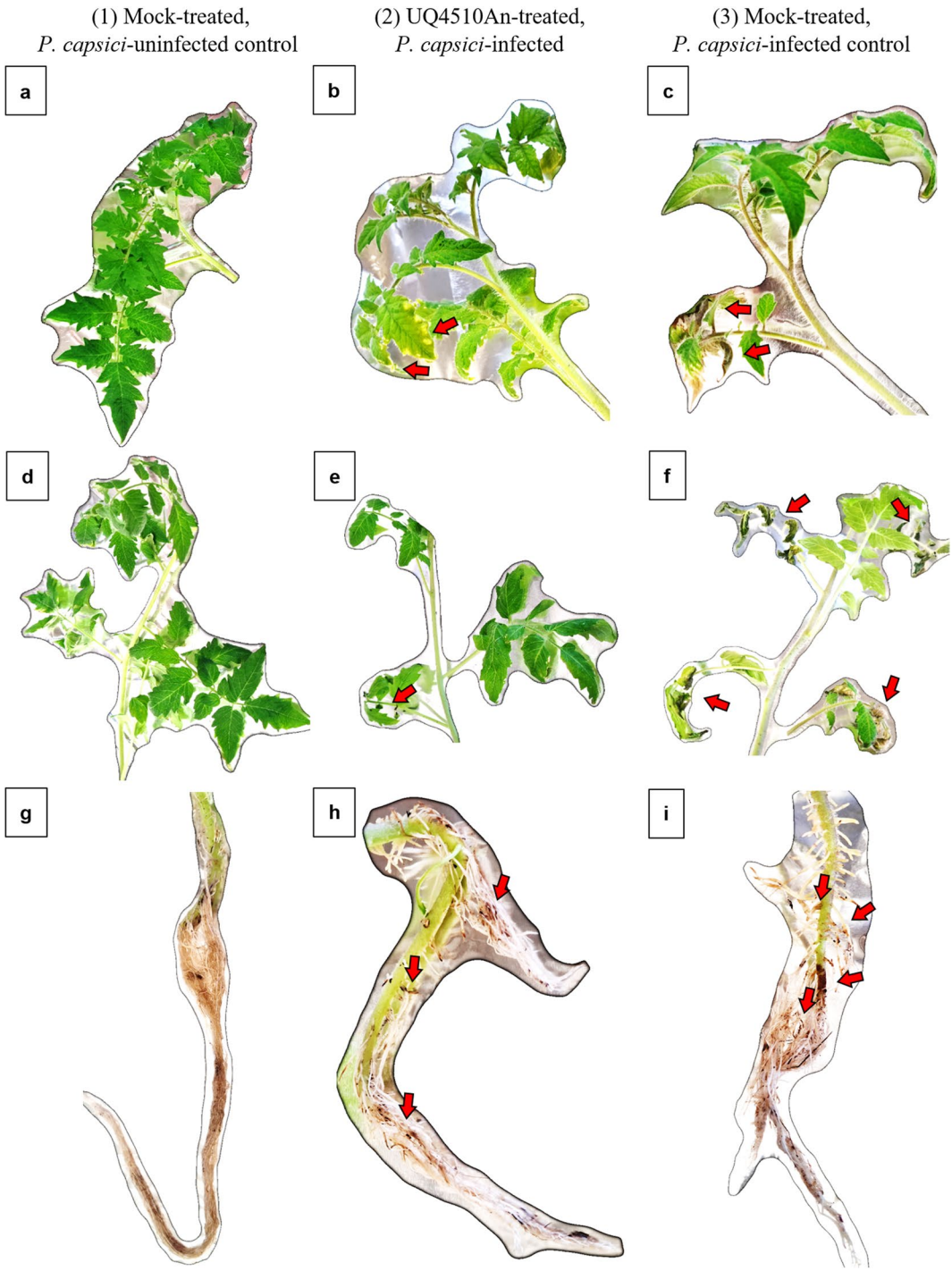


Fig. 4 Symptom alleviation in tomato plants (*S. lycopersicum*; cv. Money Maker) infected with *P. capsici* and treated with *P. azotoformans* UQ4510An. Shown are (**a**-**c**) top and (**d**-**f**) middle sections of the plant, (**g**-**i**) stem and roots. Red arrows indicate symptoms including discoloration, wilting and curling of leaf, stem lesions and root rot

differences were observed for shoot (Fig. 5c) and root (Fig. 5d) length, and leaf transpiration rate (Fig. 5e), except for a significant increase of RdM shoot length (Fig. 5c) and stomatal conductance (Fig. 5g) between the (3) infected, mock-treated and (4) infected, UQ4510An-treated plants for all tomato cultivars.

Evaluation of defence gene expression in tomato upon UQ4510An and *P. capsici* inoculation

Tomato gene expression after treatment with UQ4510An in absence and presence of *P. capsici* was significantly different between the different treatment groups at time points, 2-, 3- and 7-days post *P. capsici* inoculation (dpi, Fig. 6). Four marker genes involved in ROS signalling were examined, including *RBOHD*, *SOD*, *CAT* and *LOX1*. Following UQ4510An treatment in *P. capsici*-uninoculated plants, *RHOHD* expression was significantly downregulated by 5.5-fold at 3 dpi; and at 7 dpi it was significantly upregulated by 1.3-fold compared to mock-treated uninoculated control plants (Fig. 6a). In the untreated *P. capsici*-infected plants, *RHOHD* expression was significantly downregulated by 2.8-fold at 2 dpi, and at 3 dpi it was significantly upregulated by 1.7-fold compared to control plants. In contrast, in the UQ4510An-treated *P. capsici*-infected plants, *RHOHD* expression was initially significantly upregulated by twofold at 2 dpi, and then at 3 and 7 dpi it was downregulated by 2.1- and 3.8-fold, respectively, compared to control plants.

A similar trend was observed following UQ4510An treatment in uninfected plants for *CAT* expression, as it was significantly downregulated by 6.8- and 9.1-fold at 2 and 3 dpi, respectively, and then at 7 dpi it was significantly upregulated by 2.5-fold compared to control plants (Fig. 6b). In the untreated *P. capsici*-infected plants, the *CAT* expression was significantly downregulated by 14.9-fold at 2 dpi, then upregulated by 2.2-fold at 3 dpi, and then at 7 dpi again downregulated by 5.9-fold compared to control plants. Conversely, in UQ4510An-treated *P. capsici*-infected plants, the *CAT* expression was significantly upregulated by 2.6-fold at 2 dpi, and at 7 dpi downregulated by 19.6-fold compared to control plants.

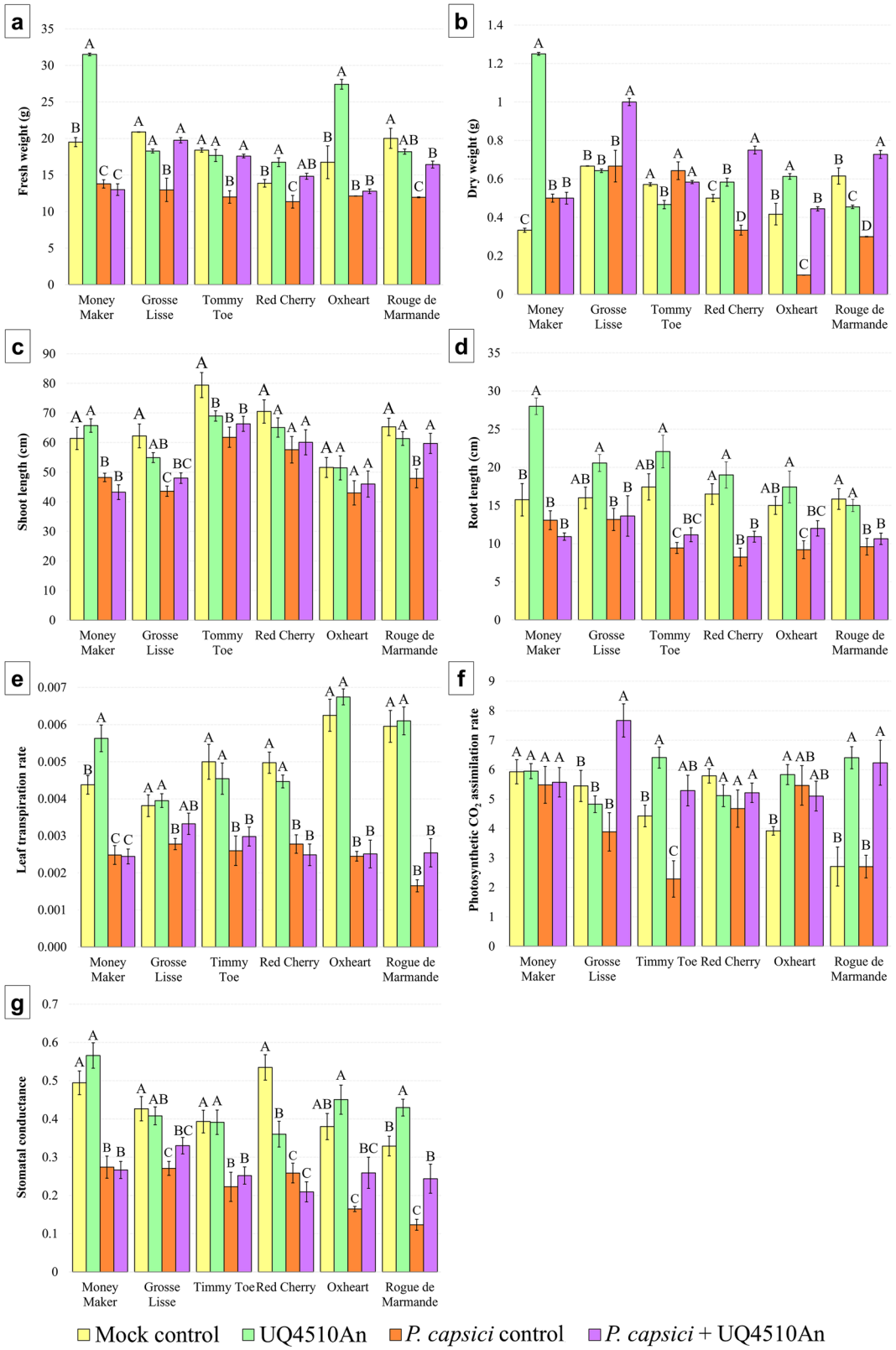
SOD expression was significantly downregulated by 15.9-fold following the UQ4510An treatment in uninfected plants at 2 dpi, and then at 7 dpi it was significantly upregulated by 1.5-fold compared to control plants (Fig. 6c). In the untreated *P. capsici*-infected plants, *SOD* expression was significantly downregulated at 2, 3 and 7 dpi by 62.5-, 1.8- and 15-fold, respectively. Alternatively, in the UQ4510An-treated *P. capsici*-infected plants, *SOD* expression was significantly downregulated by 5- and 38.9-fold at 3 and 7 dpi, respectively.

Following UQ4510An treatment in uninfected plants, *LOX1* expression was significantly downregulated by 7.2- and 3.8-fold at 2 and 3 dpi, respectively, and then at 7 dpi it was significantly upregulated by 2.1-fold compared to mock-treated uninfected control plants (Fig. 6d). In the untreated *P. capsici*-infected plants, *LOX1* expression was significantly downregulated by 13- and 1.2-fold at 2 and 7 dpi, respectively, compared to control plants. In contrast, in UQ4510An-treated *P. capsici*-infected plants, *LOX1* expression was initially significantly upregulated by twofold at 2 dpi, and then at 3 and 7 dpi it was significantly downregulated by 1.8- and 7.4-fold, respectively.

The marker genes involved in SA signalling (*PAL1*, *NPR1* and *PR1*) were not significantly affected by UQ4510An treatment in uninfected plants at any of the examined three time points compared to mock-treated uninfected control plants (Fig. 6e). Meanwhile, in untreated *P. capsici*-infected plants, *PAL1* expression was significantly downregulated by 4.7- and 1.5-fold at 2 dpi and 3 dpi, respectively, and then at 7 dpi it was significantly upregulated by 4.3-fold. Alternatively, in UQ4510An-treated *P. capsici*-infected plants, *PAL1* expression was initially significantly upregulated by 1.7-fold at 2 dpi, and then at 3 dpi it was significantly downregulated by 3.5-fold.

NPR1 was not significantly affected by UQ4510An treatment in uninfected plants at any of the examined three time points compared to control plants (Fig. 6f). In contrast, in the untreated *P. capsici*-infected plants, *NPR1* expression was significantly upregulated by 2.7-, 4.3- and 6.7-fold at 2, 3 and 7 dpi, respectively. Similarly, in UQ4510An-treated *P. capsici*-infected plants, *NPR1* expression was significantly upregulated by 2.5-fold at 2 and 3 dpi compared to control plants.

PR1 was not significantly affected by the UQ4510An treatment in uninfected plants at any of the examined three time points compared to control plants (Fig. 6g). In contrast, in the untreated *P. capsici*-infected plants, the *PR1* expression was significantly upregulated by 31.7-, 101- and 143-fold at 2, 3 and 7 dpi, respectively, compared to control plants. Similarly, in the UQ4510An-treated *P. capsici*-infected plants, *PR1* expression was significantly upregulated by 18- and 25-fold at 2 and 3 dpi, respectively, compared to control plants.


a
Fresh weight (g)
b
Dry weight (g)
c
Shoot length (cm)
d
Root length (cm)
e
Leaf transpiration rate
f
Photosynthetic CO2 assimilation rate
g
Stomatal conductance
Money Maker
Grosse Lisse
Tommy Toe
Timmy Toe
Red Cherry
Oxheart
Rouge de Marmande
Rogue de Marmande
Mock control
UQ4510An
P. capsici control
P. capsici + UQ4510An

◂**Fig. 5** Plant growth parameters of *P. capsici*-infected tomato plants (*S. lycopersicum*) 4 weeks after treatment with *P. azotoformans* UQ4510An. Shown are mean values ± SEs (n = 12 plants per treatment) of (**a**) Fresh and (**b**) Dry Weight, (**c**) Shoot and (**d**) Root Length, (**e**) Leaf Transpiration Rate (mol m^{-2} s^{-1}), (**f**) Photosynthetic CO_2 Assimilation Rate (µmol m^{-2} s^{-1}), and (**g**) Stomatal Conductance (mol m^{-2} $s.^{-1}$) of 8-week-old tomato plants, namely cv. Money Maker (MM), Grosse Lisse (GL), Timmy Toe (TT), Red Cherry (RC), Oxheart (OX) and Rogue de Marmande (RdM) treated with either PBS (mock) or UQ4510An and infected with *P. capsici*. The statistical significance was determined by ANOVA and Tukey's HSD; if the letters A-C are not shared between the different treatments, this indicates statistically significant difference ($P < 0.05$)

Two marker genes involved in JA and ET signalling were examined, *JAZ1* and *ERF1*. Following UQ4510An treatment in uninfected plants, *JAZ1* expression was significantly downregulated by 2- and 5.3-fold at 2 and 3 dpi, respectively, compared to mock-treated uninfected control plants (Fig. 6h). In the untreated *P. capsici*-infected plants, *JAZ1* expression was initially significantly downregulated by 4.2-fold, and then at 3 and 7 dpi it was significantly upregulated by 1.9- and 2.2-fold, respectively, compared to control plants. In UQ4510An-treated *P. capsici*-infected plants, *JAZ1* expression was initially significantly upregulated by 3.2-fold at 2 dpi, and then at 3 and 7 dpi it was significantly downregulated by 2.8- and 5.2-fold, respectively, compared to control plants.

ERF1 was not significantly affected by UQ4510An treatment in uninfected plants at any of the examined time points compared to mock-treated uninfected control plants (Fig. 6i). Conversely, in untreated *P. capsici*-infected plants, *ERF1* expression was only significantly upregulated by 2.5-fold at 7 dpi. In UQ4510An-treated *P. capsici*-infected plants, *ERF1* expression was significantly downregulated by 1.6-fold at 3 dpi.

Discussion

This study revealed the potential of *P. azotoformans* UQ4510An and *B. velezensis* UQ9000N as biopesticides against various fungal and oomycete pathogens, particularly *P. capsici* infecting tomato plants. This study also provided evidence that the plant genotype affects the capability of UQ4510An to control *P. capsici* infection.

In vitro biocontrol activity

In vitro co-culture inhibition assays showed that *P. azotoformans* UQ4510An inhibited three oomycete out of the 20 isolates tested (eight oomycetes and twelve fungi), while *B. velezensis* UQ9000N inhibited one oomycete and seven fungal isolates. Fluorescence microscopy revealed that both PGPR isolates induced mycelial morphological abnormalities of the inhibited phytopathogens (excessive branching, swellings and other irregular hyphae shapes). Both strains also produced bioactive compounds, including siderophores (UQ4510An only) and enzymes, namely proteases (both isolates) and cellulases (UQ9000N only). This data is consistent with numerous studies which showed the potential of PGPR isolates belonging to *Bacillus* and *Pseudomonas* genera as biocontrol agents, particularly those of the *B. subtilis* species complex (including *B. velezensis*) and the *P. fluorescens* group (including *P. azotoformans*) (Garrido-Sanz et al., 2016; Rabbee et al., 2019; Sang et al., 2014; Shafi et al., 2017). Bacterial isolates belonging to these genera produce a large variety of bioactive compounds (e.g. antimicrobials, hormones, volatile organic compounds, etc.) with wide-ranging applications, some of which have been shown to cause mycelial morphological abnormalities of pathogenic fungi and oomycetes (Hazarika et al., 2019; Minaxi and Saxena, 2010; Syed-Ab-Rahman et al., 2018; Wang et al., 2021a, 2021b). For example, Wang et al. (2021a) reported that the volatile organic compounds (VOCs) emitted by *P. fluorescens* ZX isolate caused serious damage and morphological abnormalities of *Penicillium digitatum* (causing citrus postharvest green mold) *in vitro* and *in vivo*, including deformation, irregular shrinkage, and multiple holes. Similarly, Wang et al. (2021b) found that a fengycin compound (designated as BVAP) produced by the *B. velezensis* strains HNAH 17806 induced abnormal swelling of hyphal tips of *Fusarium solani*, accumulation of chitin and nucleic acids at these swollen sites and increased cell membrane permeability. The focus of the present study was then restricted to the novel *P. azotoformans* isolate UQ4510An, as other *P. azotoformans* isolates had been reported as PGPR with growth promotion and biocontrol capabilities (Ansari et al., 2021; Fang et al., 2016; Sang et al., 2014).

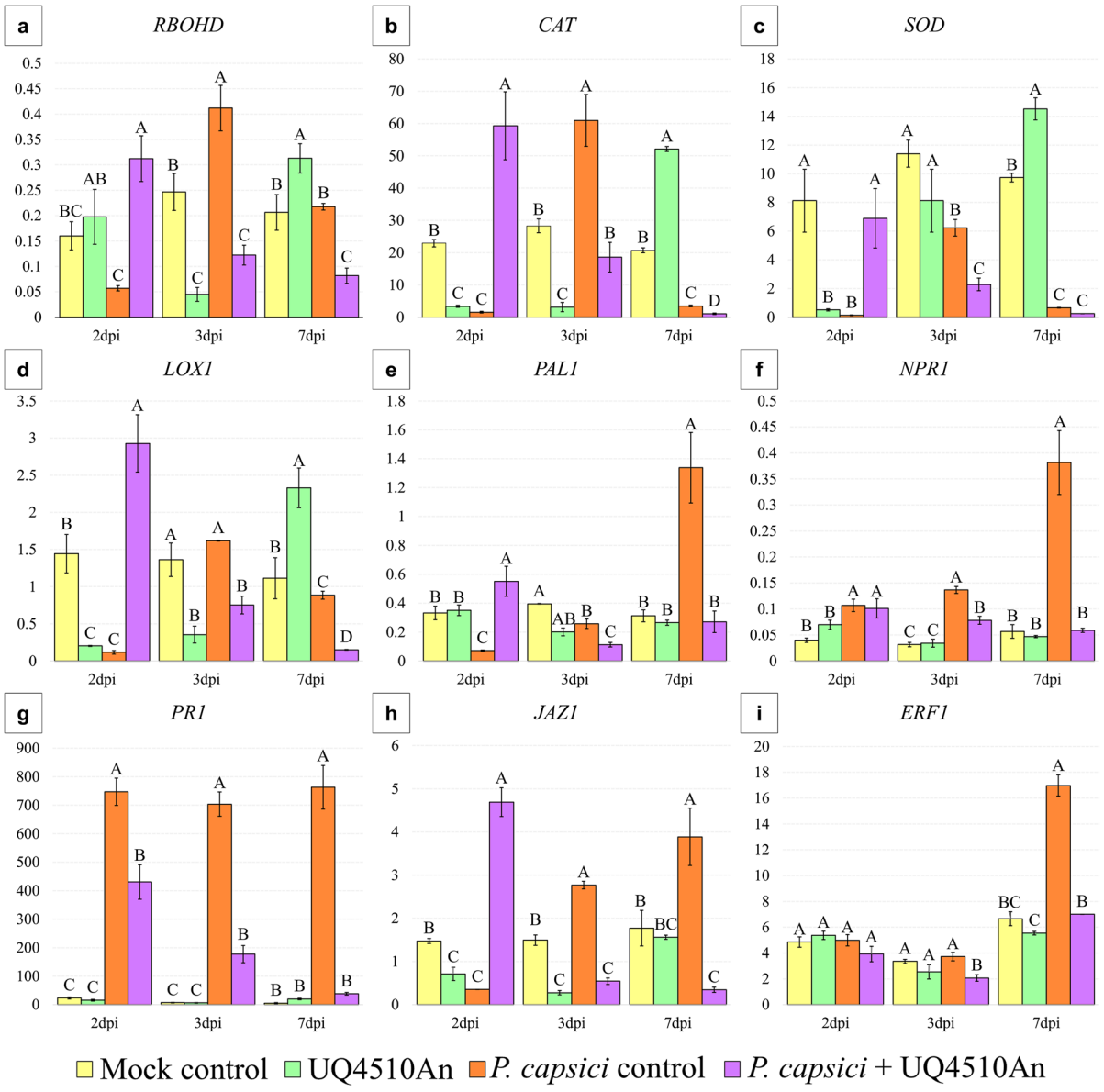


Fig. 6 Relative expression of defence genes in shoot tissue samples of tomato plants (*S. lycopersicum*; cv. Money Maker) treated with *P. azotoformans* UQ4510An infected and non-infected with *P. capsici* compared to mock-treated, uninfected control plants at 2, 3 and 7 days post inoculation (dpi) measured by quantitative real-time RT-PCR. Expression values were normalised by the transcript levels of *SlACTIN*. Shown are mean values ± SEs (n = 3 biological replicates with 5 plants each) of 9 genes of 5-week-old plants (**a-i**). The statistical significance was determined by ANOVA; if the letters A-D are not shared between the different treatments, this indicates statistically significant differences at the 95% confidence level ($P < 0.05$)

In planta genotype-specific biocontrol response

UQ4510An significantly reduced symptoms of *P. capsici* infection for five out of six tomato cultivars, particularly in the more susceptible cultivars Tommy Toe, Grosse Lisse and Red Cherry. Variable responses from the tomato cultivars to this PGPR in the presence and absence of *P. capsici* were observed. In line with our findings, several studies have reported genotypic and phenotypic

variations in different cultivars of crops, including wheat, maize, rice and tomato in response to plant–microbe interactions with PGPR from various genera, such as *Azospirillum* sp., *Bacillus* sp., and *Pseudomonas* sp., and some specific species, such as *Klebsiella pneumoniae* and *Citrobacter freundii* (Delfin et al., 2015; Khalid et al., 2003; Rozier et al., 2019; Sasaki et al., 2010; Uribe et al., 2010; Wallenstein, 2017; Wintermans et al., 2016). For example, Delfin et al. (2015) tested the responses of ten tomato cultivars under field conditions with the commercial PGPR product BioGro™ composed of *P. fluorescens/putida*, *K. pneumoniae* and *C. freundii* (Uribe et al., 2010). Half of the cultivars responded positively, while the other half responded in a negative manner, such as reduced shoot dry weight (Delfin et al., 2015). It has been suggested that the root exudates produced by the plant host holobiont have an important role in the plant–microbe and microbe-microbe interactions with PGPR and phytopathogens (Khatoon et al., 2020; Naamala & Smith, 2021; Philippot et al., 2013; Rodriguez et al., 2019).

Mounting evidence provided in this study and in the literature indicates that there is no universal PGPR isolate that can improve plant growth and increase disease resistance in all plant species, cultivars and environments. A successful selection of an effective PGPR isolate or consortia as a biofertiliser and/or biopesticide depends on many factors (Bhardwaj et al., 2014; Tabassum et al., 2017). These factors include compatibility of beneficial plant–microbe interactions between the PGPR isolate or consortia with the crop plant cultivar and its native microbiome, as well as the ability to survive/adapt to the climate, temperature and edaphic factors (Saritha and Tollamadugu, 2019; Tabassum et al., 2017).

Role of host defence genes during biocontrol activity of *P. azotoformans* UQ4510An

An indirect mechanism of phytopathogen biocontrol by PGPR is the stimulation of ISR in plants via the JA pathway, which has an advantage over SAR in terms of fitness and energy costs to the plant host (Martinez-Medina et al., 2016; Meena et al., 2020). Gene expression profiling was used to analyse the plant host defence gene modulation capability of *P. azotoformans* UQ4510An for biocontrol of *P. capsici* infection.

ROS signalling

ROS molecules act as secondary messengers during plant growth and development, plant–microbe interactions and responses to abiotic and biotic stresses (Huang et al., 2019; Nath et al., 2016; Zeng et al., 2017). In the present study, four marker genes involved in ROS signalling were examined, namely *RBOHD*, *CAT, SOD* and *LOX1*. *RBOHD* encodes a membrane-dependent NADPH oxidase, which is involved in the production of ROS molecules (e.g. O_2^- and H_2O_2) in plants during morphogenesis and development, and has a primary role during stress response (innate immunity) (Lee et al., 2020; Wang et al., 2018). Meanwhile, both *SOD* and *CAT* genes encode ROS scavenging enzymes which provide defence for plants from ROS damage, particularly under abiotic and biotic stresses (Choudhary et al., 2020; Tyagi et al., 2019). *LOX1* gene encodes a lipoxygenase from the 9-LOX gene family that is required for plant defence through lipid peroxidation, hypersensitive response (HR) and programmed cell death (PCD) (Hwang & Hwang, 2010; Wasternack & Song, 2017). In addition, other enzymes in the 13-LOX gene family are involved in the synthesis of JA and methyl jasmonate (MeJA) (Hwang & Hwang, 2010; Wasternack & Song, 2017). Hence, *LOX1* should be considered to be involved in ROS signalling. Both *RBOHD* and *CAT* were induced during *P. capsici* infection; however, in untreated infected plants this occurred at 3 dpi, while in UQ4510An pretreated plants the upregulation occurred earlier, at 2 dpi. This could indicate that UQ4510An induces ROS production earlier during *P. capsici* infection, which could help plants to mount defences earlier against this hemibiotrophic pathogen. *P. capsici* exhibits antimicrobial activity and suppressed ROS production at 1 dpi, which presumably is still in its biotrophic phase and the action of UQ4510An seems to restore this ROS production. Once *P. capsici* switches to its necrotrophic phase in later infection stages, it leads to oxidative stress and subsequent expression of SA signalling-related defence genes resulting in HR and PCD (Ali et al., 2012; Mhamdi & Van Breusegem, 2018; Saleem et al., 2021; Waszczak et al., 2018). An earlier activation of this oxidative burst when *P. capsici* is still in its biotrophic phase (e.g. 2 dpi) would lead to PCD and starvation of *P. capsici* and hence could explain the increased disease resistance in the presence of UQ4510An. Interestingly, *LOX1* was also

strongly repressed by *P. capsici* at 2 dpi, but then its expression was restored and even further induced by UQ4510An-treated *P. capsici*-infected plants at 2 dpi, which coincided with the earlier ROS signalling induction observed. Similar to the other ROS genes, *LOX1* was then suppressed at the later stages when *P. capsici* presumably enters the necrotrophic lifestyle.

SA signalling

SA is involved in several physiological processes of the plant, particularly the regulation of plant responses under biotic or abiotic stress conditions and it plays a role during growth and development (Ding & Ding, 2020). In the present study, three marker genes involved in SA signalling were examined, namely *PAL1*, *NPR1* and *PR1*. *PAL1* encodes a phenylalanine lyase which catalyses the first step in the phenylpropanoid pathway, producing hundreds of phenolic compounds (some with defensive functions), including SA, and is induced by various pathogens, including *Verticillium dahliae* on a resistant tomato plant cultivar (Gayoso et al., 2010; Kim & Hwang, 2014; Saleem et al., 2021). *NPR1* gene encodes an SA receptor and is the main regulator of the SA signalling pathway, being also involved in induction of SAR (usually against biotrophic and hemibiotrophic pathogens) through expression of pathogenesis-related proteins, including PR1 (Backer et al., 2019; Breen et al., 2017; Maier et al., 2011). *PAL1* was significantly repressed in the untreated infected plants at 2 dpi, while this gene was significantly induced following the UQ4510An treatment of *P. capsici*-infected plants at 2 dpi. This suggests that similarly to when ROS production is taking place, UQ4510An was able to restore *PAL1* expression and even further induced this gene (compared to the uninfected control plants). This may have led to increased SA biosynthesis and biosynthesis of multiple phytoalexins that could be anti-oomycete or help plants fortify their cell walls through lignin biosynthesis, providing another possible explanation for the UQ4510An-mediated resistance. SA signalling genes *NPR1* and *PR1* were both induced during the *P. capsici* infection (treated or untreated with UQ4510An). In the untreated infected plants this occurred from 2 to 7 dpi, with both genes remaining strongly induced (up to 6.7- and 143-fold, respectively). The induced SA pathway and oxidative bursts and the resulting HR and PCD are required to prevent or limit the *P. capsici* pathogen at the earlier stage of infection, while it is in the biotrophic stage (Beneduzi et al., 2012; Yang et al., 2015). However, at later stages of infection, *P. capsici* will probably switch to a necrotrophic lifestyle and the HR and PCD may become detrimental to the host (Jupe et al., 2013; Balint-Kurti, 2019). Indeed, in later time points (3 and 7 dpi) a suppression of genes involved in ROS production and ROS-scavenging genes occurred, which may help the plant prevent further damage when *P. capsici* enters its necrotrophic phase. These results indicate that UQ4510An has the capability to manipulate the plant ROS and SA signalling at the earlier and later stages of infection so that it can counteract its initial biotrophic and later necrotrophic lifestyles. Further studies may focus on whether the timing of these lifestyle switches in *P. capsici* indeed occurs between 2 and 3 dpi.

JA and ET signalling

JA is a key component of plant development and responses to abiotic and biotic stresses, in particular necrotrophic pathogens, as well as in beneficial plant–microbe interactions (including priming/ISR) (Carvalhais et al., 2015; Jang et al., 2020). Its crosstalk with other phytohormones (e.g., SA) is essential during the modulation of plant growth and development (He et al., 2017; Jang et al., 2020; Yang et al., 2019). In the present study, two marker genes involved in JA and ET signalling were examined, namely *JAZ1* and *ERF1*. *JAZ1* encodes a nuclear-localised protein involved in JA signalling which is degraded in response to JA stimulus, and is involved in activation of ISR, while *ERF1* is a transcription factor regulated by both, ET and JA, signalling pathways to regulate other pathogen response genes involved in both of these signalling pathways (Cheng et al., 2013; Chung et al., 2008; Major et al., 2017; Mao et al., 2016; Thines et al., 2007). As indicated by the strong induction of *JAZ1* at 2 dpi (up to 3.2-fold) during the *P. capsici* infection, the pre-treatment with UQ4510An likely induced ISR in the tomato plants, while in the untreated *P. capsici*-infected plant this gene was first suppressed and only induced at 3 dpi.

Similar results were observed in potato plants during *Phytophthora infestans* infection by exogenous application of JA (Arévalo-Marín et al, 2021). Arévalo-Marín et al. (2021) concluded that application of JA had a priming effect in potato plants, evidenced by a decrease in the number of necrotic lesions, reduction in lesion area, and an enhanced transcriptional induction of defence genes related to ROS and HR. Furthermore, Betsuyaku et al.

(2018) reported that during HR resulting from effector-triggered immunity (ETI) in *Arabidopsis thaliana* plants, SA and JA were activated simultaneously in spatially different domains around the infection site of *Pseudomonas syringae* pv. tomato DC3000, which is also a hemibiotrophic pathogen. Several studies also reported that the priming mechanism in *Solanaceous* plants involves the phenylpropanoid pathway (Mhlongo et al., 2014, 2021), which corresponds to the induction of *PAL1* at 2 dpi in this study. Sang et al (2014) reported that *P. azotoformans* GC-B19 induced ISR in cucumber plants (*Cucumis sativus* L. cv. Baeknokdadagi) against the pathogenic fungus *Colletotrichum orbiculare*. Moreover, Mhlongo et al. (2021) reported that that four PGPR isolates, including *Pseudomonas fluorescens* N04, induced priming in tomato (cv. Money Maker) plants.

In the future, systems biology approaches should be applied to study the complexity of the plant–microbe and microbe-microbe interactions, with a combination of multiple omics approaches and quantitative modelling to utilise the PGPR as biofertilisers and biocontrol agents to their full potential (Rodriguez et al, 2019; Sharma et al., 2020; Van Dijk et al, 2021).

The present study examined the biocontrol potential of the two PGPR isolates against various fungal and oomycete pathogens with previously confirmed plant growth promotion capabilities. The later focus of the study was on evaluating *P. azotoformans* UQ4510An as a biopesticide candidate against *P. capsici*-infected tomato plants. *P. azotoformans* UQ4510An exhibited anti-oomycete activity *in vitro*, and *in planta* it reduced symptoms of *P. capsici* infection in various tomato plant cultivars in a genotype-dependent manner. Some tomato cultivars, which showed less improvements of phenotypic parameters from the UQ4510An inoculation in the absence of the pathogen, had a more pronounced alleviation of symptoms during the *P. capsici* infection. Furthermore, our results suggest that the main method of biocontrol of *P. capsici* by the *P. azotoformans* UQ4510An is a combined effect of direct antagonism of the pathogen and induction of ISR involving HR responses in the plant host at early stages of infection against *P. capsici*.

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Author contribution A.A. is the principal researcher of this study. A.A., L.C.C. and P.M.S. designed the work. A.A. performed the assays and analysed the results. A.A. wrote the first version of the manuscript. L.C.C. and P.M.S. contributed to the manuscript writing, editing and critical reading. All authors have read and agreed to the published version of the manuscript.

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Data availability The 16S sequences produced in this study have been deposited into NCBI GenBank database and can be accessed using the accession numbers as specified in the Materials and Methods subsection 'Isolation and cultivation of PGPR isolates'.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no conflict of interest.

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