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Antifungal activity and resistance induction against *Sclerotium cepivorum* by plant growth-promoting fungi in onion plants

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Abstract

The influence of the inoculation with plant growth-promoting fungi (PGPF) isolates on growth enhancement of onion plants and progress of white rot disease in onions, caused by *Sclerotium cepivorum*, was evaluated. The tested PGPF isolates were *Phoma* sp. GS8-1, *Phoma* sp. GS 8-3, *Trichoderma asperellum* SKT-1, *Fusarium equiseti* GF18-3 and *Penicillium simplicissmum* GP17-2. Abnormal shape and lysis in the mycelia of the pathogen were reported in dual cultures of PGPF and *S. cepivorum* using scanning electron microscopy. The germination of sclerotia was reduced after soaking in culture filtrates of PGPF. The highest reduction was recorded in *P. simplicissmum* GP17-2 treatment (70.85%). The results demonstrated that the treatments with PGPF isolates significantly enhanced the plant height, root length, bulb perimeter and plant dry weight. Additionally, PGPF clearly reduced disease severity of white rot disease of onions. Among PGPF isolates, *T. asperellum* SKT-1 and *P. simplicissmum* GP17-2 showed the best effects in reducing the incidence and severity of white rot and enhancing onion growth. On the other hand, the levels of peroxidase and polyphenol oxidase were improved in the treated onion plants than in the untreated plants at 7 days after induction treatments. Similarly, early and strong expressions of defence genes, *PR1* and *PR2*, were reported in plants treated with PGPF. Overall, this research suggested that PGPF treatments generated favourable effects on the suppression of white rot disease of onions.

Keywords: Antifungal activity, Resistance, Induced systemic resistance, Plant growth-promoting fungi (PGPF), *Sclerotium cepivorum*, Onion, White rot

Background

Onion plants are subjected to infection with many diseases that affect the yield quantity and quality. *Sclerotium cepivorum* Berk. is a dangerous and widespread pathogen affecting onion production all over the world. Onion white rot is a severe disease not only causing losses in the yield of bulbs, but also in soil infestation with the pathogen, which makes it unsuitable for onion production for more than 15 years. Sclerotia of the pathogen stay in inactive conditions with fungistasis in the soil (Hyakumachi et al. 2014). Aqueous extracts and exudates of onions enhance the germination of sclerotia (Reddy 1992).

Approaches for the control of white rot disease still depend on the chemical fungicides. However, the intensive use of these compounds for controlling plant diseases causes serious problems not only to the environment but also to human health. Additionally, some pesticides have shown phytotoxicity and also inconsistent control of *S. cepivorum* (Ryan and Kavanagh 1976). These reasons emphasize the need for new methods to control plant diseases. One of the main alternatives to fungicides is biological control. Many beneficial microorganisms are being used as biocontrol agents (BCAs). Plant growth-promoting fungi (PGPF) are non-pathogenic soil-living fungi (Hyakumachi 1994) reported to improve plant growth and health after treatments in several plants (Elsharkawy et al. 2012; Hassan et al. 2014). PGPF are usually useful for controlling soil-borne fungi (Saldajeno and Hyakumachi 2011). Payghami (2001) successfully used *Trichoderma harzianum* and *Trichoderma viride* to prevent the infection with *S. cepivorum*. He also found

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that both isolates (obtained from the mycoflora of onion rhizosphere) significantly increased the growth of onion plants in pots. Additionally, Clarkson et al. (2002) studied the ability of certain bioagents against the white rot of onions. Two of the potential bioagents were both recognized as *T. viride* (L4 and S17 A). They added that L4 and S17A were used for onion bulbs treatment which resulted in significantly reduced the white rot symptoms under field conditions.

Biocontrol agents (BCAs) restrict plant pathogens by a series of mechanisms such as antibiosis, fungistasis, induced systemic resistance, hyperparasitism and/or modification of the rhizosphere (Elsharkawy et al. 2012). Defence of plants against pathogens is regulated through a complex of peroxidase and polyphenol oxidase activities (Hassan et al. 2014). Some reports stated that peroxidase and polyphenol oxidase are important enzymes associated with the stimulated systemic resistance (Rasmussen et al. 1995). Activities of peroxidase and polyphenol oxidase were increased in cucumber plants treated with PGPF against the infection of anthracnose disease (Hassan et al. 2014). On the other hand, upregulation of pathogenesis-related (PR) genes is a common indicator of defence response and highly related to the improved resistance against different pathogens (Elsharkawy et al. 2012).

The aim of this study was to investigate the effect of the PGPF isolates on crop parameters and induced resistance against the white rot of onions.

Materials and methods

Isolation and identification of the causal pathogen

Infected samples exhibiting standard symptoms of the white rot disease were collected from different locations in Egypt such as Kafr El-Sheikh (KS), Qutour (QT), Mahalet Rough (MR) and Negrig (NG). Isolation of sclerotia of the causal pathogen was carried out as described by Utkhede and Rahe (1979) to get pure cultures on potato dextrose agar (PDA). The isolated fungus was identified as *Sclerotium cepivorum* in Kafrelsheikh University, Egypt, based on Gilman (1957). Pathogenicity test was carried out in plastic pots using four isolates of *S. cepivorum* on onion seedlings (Giza-20) under greenhouse conditions following the method of El-Sheshtawi et al. (2009). Inocula of *S. cepivorum* isolates grown on barley grains were prepared following the method reported by Abd El-Moity (1976). Well-mixed inoculum from each isolate (at a concentration of 2% w/w) with sterilized clay soil (with 5% formalin) was placed in sterilized pots (25 cm in diameter). All pots were irrigated every other day starting from 14 days before planting to guarantee the development of the pathogen. PGPF isolates, *Phoma* sp. GS8-1, *Phoma* sp. GS 8-3, *T. asperellum* SKT-1, *F. equiseti* GF18-3 and *P. simplicissmum* GP17-2, were grown on PDA (Hyakumachi 1994).

Evaluation of the antagonistic effect of PGPF isolates against *S. cepivorum*

The antagonism of PGPF isolates toward *S. cepivorum* (NG) was tested according to the dual culture protocols described by Bell et al. (1982). Mycelial discs (5 mm) obtained from the active PGPF cultures (grown on PDA medium) were inoculated on the side of a medium, and on the opposite side, a disc of *S. cepivorum* was inoculated then the plates were incubated at 17 °C for 2 weeks. The growth area, average of inhibition zone and number of sclerotia of *S. cepivorum* (NG) were measured. Five Petri dishes were used for each PGPF isolate.

Microscopic examination

Scanning electron microscopy (SEM) was utilized to evaluate the effects of *P. simplicissmum* GP17-2 and *T. asperellum* SKT-1 (the most effective biological control agents compared with other bioagents) on the growth of *S. cepivorum*. The samples were prepared by fixation and dehydration methods. The samples were immersed in 3% glutaraldehyde and phosphate buffer (0.1 M) for 3.5 h at room temperature. Samples were washed three times by sterilized distilled water (SDW). Samples were post-fixed in osmium tetroxide at room temperature for 2 h and washed again. The fixed specimens were dehydrated in stepwise ethanol concentrations and dried at critical point (CPD). The specimens were covered by a metal coating and sputter coating. The samples were examined with a field emission electron microscope (TESLA BS-300) in the Faculty of Agriculture, Mansoura University, Egypt.

Evaluation of the antagonistic effect of cultural filtrates of PGPF

The effects of cultural filtrates (CFs) of PGPF isolates on sclerotial germination were evaluated. CFs of PGPF isolates were prepared by inoculating potato dextrose broth medium (250 ml PDB) with 20 mycelial discs (5 mm) from the edge of the PGPF cultures and incubated for 11 days at 23 °C (Elsharkawy et al. 2012). CFs were filtered through a sterile Whatman™ filter paper grade 2 then a sterile Millipore filter (0.22 mm) was used. CFs were transferred to Petri dishes, and then the plates were inoculated in the centre with a disc from *S. cepivorum* and incubated for 2 weeks at 17 °C. The diameter of *S. cepivorum* colony was measured. The percentage of germinated sclerotia was recorded. Non-inoculated PDB was served as control. Six Petri dishes were used for each PGPF isolate.

Barley grain inoculum of PGPF

Autoclaved barley grains (100 g in 100 ml distilled water) were inoculated by 12–18 mycelial discs from active cultures of PGPF isolates grown on PDA and incubated at

Table 1 Sequence of primers used in qRT-PCR analysis

Prime	Direction	Sequence 5′-3′
PR1	Forward	TTCTTCCCTCGAAAGCTCAA
	Reverse	CGCTACCCCAGGCTAAGTTT
PR2	Forward	TCCGGGGTATGTTATGGAAGA
	Reverse	GGCCATCCACTCTCAGACACA
EF1α	Forward	ATTGGAAACGGATATGCTCCA
	Reverse	TCCTTACCTGAACGCCTGTCA

22 °C for 12–14 days with shaking at 4 days intervals. The inoculum was dried then ground to 1 mm and kept in a refrigerator until use.

Pot experiments

Two pot experiments were performed separately and repeated three times under greenhouse conditions.

Measuring growth enhancement

Pots (25 cm in diameter) were filled with clay soil blended with each of the PGPF isolates (at inocula rates 2% w/w), giving a total of seven treatments for the experiment: (a) *Phoma* sp. GS8-1, (b) *Phoma* sp. GS 8-3, (c) *T. asperellum* SKT-1, (d) *F. equiseti* GF18-3, (e) *P. simplicissmum* GP17-2, (f) the fungicide Folicur® 250 EC (25 ml/l) and (g) uninoculated control (control). Three onion transplants (60 days old) from the cultivar Giza-20 were transplanted per pot. After 100 days of growth, the plants were uprooted and the shoots were separated from the roots and washed with running tap water. Plant height, root length, bulb perimeter and the shoot dry weight (36 h at 80 °C) were measured.

Suppressive effects of PGPF isolates

The barley grain inoculum (BGI) of each PGPF isolate (2% w/w) was hand-mixed thoroughly with the potting medium. The fungicide Folicur® 250 EC (tebuconazole) was used as a positive control (25 ml/l). Plastic pots were filled with the inoculated soil pre-infested with the sclerotia of *S. cepivorum* (NG) (100 sclerotia/kg soil) as reported by Metcalf et al. (2004). Three transplants were then grown in each plastic pot of the aforementioned treatments. The plants were kept for symptom development. Three pots were used per replicate. To evaluate the disease severity, plants were uprooted after 100 days from transplanting and graded for disease severity according to the 0–100 arbitrary disease severity scale based on the described scale by Abd El-Moity (1976) and modified as follows: 0 = no symptoms, 25 = slight severe (small roots and yellow leaves), 50 = moderate-severe (rotting of the roots and dieback of the leaves), 75 = severe (yellowing of the whole plant and soft rot of the roots) and 100 = highly severe (dead plants, completely rotten roots).

Disease incidence = No. of infected plants/No. of total plants × 100 (Brix and Zinkernagel 1992).

Field experiment

A field experiment was carried out at Negrig, Gharbiah, Egypt, in a plot of clay soil naturally infected with *S. cepivorum* (Negrig isolate). The onion seedlings (Giza 20) were grown in sterilized pots filled with clay soil and treated with BGI of each PGPF isolate for 60 days. BGI of each isolate was added at a rate of 400 g/m length of the row. Each row was 55 cm wide and 5 m long. Each treatment was repeated three times. Eighty seedlings were transplanted in each row. The fungicide Folicur® 250 EC, as a dipping treatment, was used for comparison with PGPF treatments. Disease incidence was reported 140 days post-transplanting.

Antioxidant enzymes

Leaf materials (0.5 g) were collected from different treatments of the pot experiment. The activities of peroxidase and polyphenol oxidase enzymes were determined in onion plants, 7 days after transplanting (Anand et al. 2007). Peroxidase (POX) activity was determined according to the typical procedure described by Thimmaiah (1999). Peroxidase activity was measured spectrophotometrically (Beckman Spectrophotometer Du®7400) every 30 s up to 3 min. Polyphenol oxidase (PPO) activity was measured according to a typical method proposed by

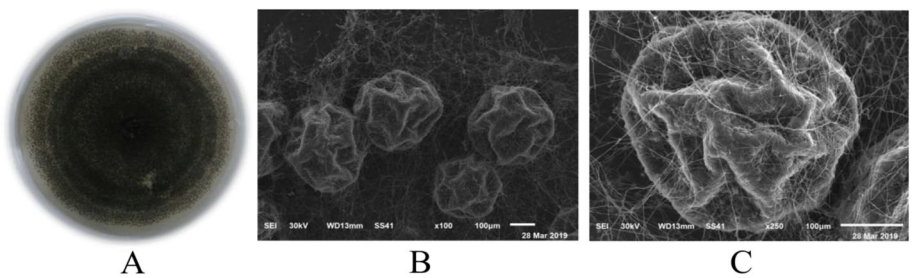

Fig. 1 Morphology of *Sclerotium cepivorum* (NG isolate) on PDA medium (**a**). Scanning electron microscopy (SEM) of *Sclerotium cepivorum* (NG isolate) sclerotia (**b**, **c**)

Table 2 Interaction between PGPF isolates and *Sclerotium cepivorum* in dual cultures at 14 days after inoculation

Treatments	Average growth area (cm^2)	Average inhibition zone (cm^2)	Average no. of sclerotia (cm^2)
Control	68.33a	0.00e	82.38a
GS8-1	41.99c	2.47b	43.98c
GS8-3	42.38c	2.13c	44.79c
SKT-1	27.87e	0.00e	41.13d
GF18-3	47.31b	1.77d	49.79b
GP17-2	32.92d	3.98a	39.24e

Different letters indicate statistically significant differences between treatments by Fisher's LSD test

Sadasivam and Manickam (1991). The activity was calculated as absorbance min^{-1} g^{-1} fresh weight.

Total RNA extraction and real-time PCR relative comparative analysis

Collections of leaf samples from treated and control plants were carried out 1 week after transplanting (Elsharkawy et al. 2012). RNeasy Mini Kit was used to extract total RNA from treated and non-treated plants following the manufacturer's protocol (QIAGEN, Germany). The first-strand cDNA was synthesized as described by El-Morsi et al. (2015), and RT-PCR amplification was carried out. The housekeeping gene elongation factor 1-α (EF1-α) and PR genes (*PR1* and *PR2*) (Table 1) were utilized for this investigation. QRT-PCR was conducted using a SYBR Green PCR Master Mix (Fermentas, USA). Accurate quantification of relative expression ratio was calculated following typical methods described by Livak and Schmittgen (2001). Automated threshold analysis on ABI system was used to calculate the C_T (threshold of the cycle) value of each detected gene.

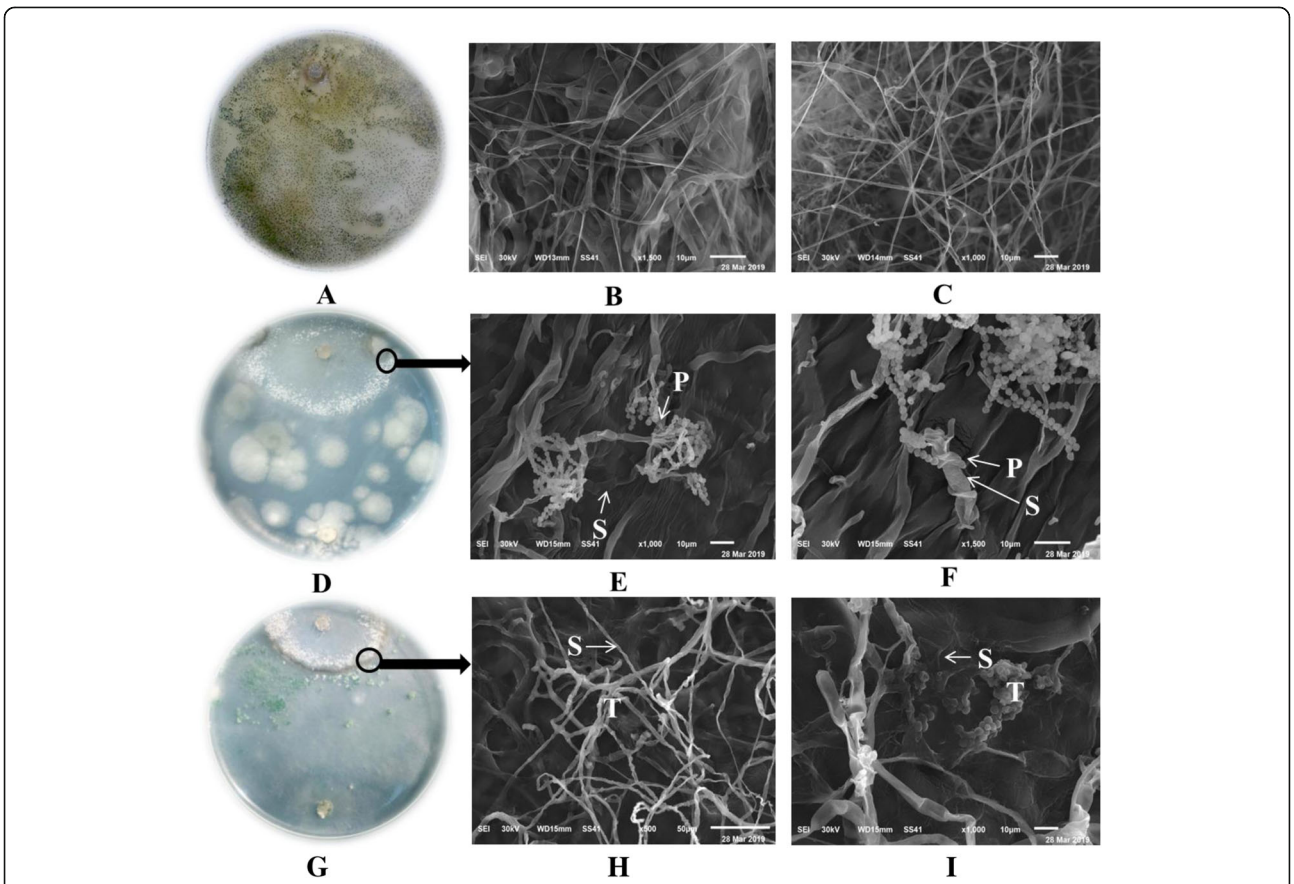

Fig. 2 Scanning electron microscopy (SEM) of *S. cepivorum*. The growth characters of white rot pathogen in the control (**a**–**c**), dual culture with *P. simplicissmum* GP17-2 (**d**–**f**) and dual culture with *T. asperellum* SKT-1 (**g**–**i**)

Table 3 Effect of soaking sclerotia of *Sclerotium cepivorum* in cultural filtrates of PGPF isolates

Treatments	Germination of sclerotia	Reduction (%)
Control	97.31a	–
GS8-1	46.19d	52. 53
GS8-3	49.97c	48. 64
SKT-1	32.72e	66.38
GF18-3	54.48b	44.01
GP17-2	28.37f	70.85

Different letters indicate statistically significant differences between treatments by Fisher's LSD test

Statistical analysis

Analysis of variance (ANOVA) was carried out for the data by E KUSERU- T OUKEI 2010 (SSRI Co., Ltd., Japan). Fisher's least significant difference (LSD) test was used to separate treatment means. All comparisons were determined at $P \leq 0.05$.

Results and discussion

Isolates of *S. cepivorum*

Isolates of the pathogen were collected from severely infested soil locations in Kafr El-Sheikh, Qutour, Mahalet Rough and Negrig, identified as four isolates of *S. cepivorum*. Based on the pathogenicity test, the isolates showed different degrees of severity on onion transplants cv. Giza-20. The Negrig isolate proved to be the most aggressive (Fig. 1), while the Kafr El-Sheikh isolate exhibited the lowest severity. Due to its severity on onion plants, the NG isolate was chosen as the main isolate in this study.

Antagonistic activity of PGPF isolates against *S. cepivorum*

The ability of PGPF isolates to inhibit the growth and germination of the sclerotia of *S. cepivorum* was evaluated. The interaction of PGPF isolates and *S. cepivorum* is presented in Table 2. *T. asperellum* SKT-1 hindered the development of the pathogen by growing over the colony of *S. cepivorum*. *Phoma* sp. GS 8-1 and GS 8-3 isolates exhibited inhibition zones of 2.47 and 2.13 cm^2, respectively. Growth inhibition and the average number of formed sclerotia by the pathogen caused by *F. equiseti* GF18-3 and *P. simplicissmum* GP17-2 were 30.76 and 49.79%, and 51.82 and 39.24%, respectively. *P. simplicissmum* GP17-2 was the most effective one, followed by *T. asperellum* SKT-1 and *Phoma* sp. GS8-1.

PGPF isolates *P. simplicissmum* GP17-2 and *T. asperellum* SKT-1 were potentially employed against several plant pathogens (Hyakumachi 1994; Elsharkawy et al. 2012, 2014). Additionally, *F. equiseti* proved to be effective bioagents in suppressing the infection of soil-borne fungi (Saldajeno and Hyakumachi 2011).

Microscopic examination by scanning electron microscopy

SEM examination showed typical morphological characteristics and complete fungal growth of *S. cepivorum* of the control (Fig. 2a). The pathogen developed sclerotia in their right form. However, *P. simplicissmum* GP17-2 (Fig. 2b) and *T. asperellum* SKT-1 (Fig. 2c) inhibited the growth of *S. cepivorum* causing morphological anomaly such as coiling, atrophy and lysis of the fungal mycelia. Secretion of antifungal compounds was found to prevent the development of different plant pathogens (Hassan et al. 2014; Elsharkawy et al. 2014). Antifungal substances produced by *Trichoderma* sp. were considered the key players in controlling the infection with *Podosphaera xanthii* (El-Kot and Derbalah 2011).

Effect of cultural filtrates of PGPF on germination of sclerotia

Data in Table 3 showed that all the culture filtrate (CF) treatments of PGPF isolates significantly decreased the germination of sclerotia. Filtrate of *P. simplicissmum* GP17-2 exhibited the best results in this respect, followed by *T. asperellum* SKT-1, *Phoma* sp. GS8-1, *Phoma* sp. GS8-3 and *F. equiseti* GF18-3. The reduction in the germination of sclerotia was 70.85% due to the CF

Table 4 Effect of plant growth-promoting fungi (PGPF) colonization on the growth of onion plants with or without the infection of *Sclerotium cepivorum* under greenhouse conditions

Treatments	Plant height (cm)		Root length (cm)		Bulb perimeter (cm)		Dry weight (g)	
	No pathogen	Pathogen	No pathogen	Pathogen	No pathogen	Pathogen	No pathogen	Pathogen
Control	46.81e	5.27d	11.69e	4.76f	8.52e	5.00f	14.50d	5.08f
Folicur	48.54d	10.36b	12.61d	7.29c	8.60e	12.03c	14.56d	12.03c
GS8-1	54.64b	12.12a	15.17b	8.17a	9.75c	14.36a	17.59b	14.36a
GS8-3	50.48c	8.43c	13.92c	6.01d	9.45cd	10.23d	16.19c	10.13d
SKT-1	57.29a	11.76a	16.35a	7.85b	10.65a	13.09b	18.76a	13.09b
GF18-3	48.81d	7.95c	13.01d	5.64e	9.04d	9.13e	15.75c	9.23e
GP17-2	56.15ab	10.86b	15.14b	7.35c	10.13b	12.57bc	17.92b	12.57bc

Different letters indicate statistically significant differences between treatments by Fisher's LSD test

Table 5 Effect of plant growth-promoting fungi (PGPF) colonization on the growth of onion plants infected with *Sclerotium cepivorum* under field conditions

Treatments	Plant height (cm)		Root length (cm)		Bulb perimeter (cm)		Dry weight (g)	Yield of bulb (kg/m^2)		
	2017–2018	2018–2019	2017–2018	2018–2019	2017–2018	2018–2019	2017–2018	2018–2019	2017–2018	2018–2019
Control	21.00f	21.84e	6.27d	8.44f	5.38d	6.08e	9.24f	10.45e	0.81g	0.92f
Folicur	54.75a	58.92a	13.64a	14.54a	13.57a	14.91a	22.37a	23.66a	2.77a	3.05a
GS8-1	43.72d	49.87c	10.87c	12.59cd	10.84b	12.58c	18.28cd	19.96c	2.17d	2.38d
GS8-3	42.94de	45.96d	10.54c	12.02de	10.39bc	11.29d	17.15de	19.13cd	2.06e	2.19e
SKT-1	48.42c	51.69c	12.58b	13.32bc	12.90a	13.56b	19.43bc	21.97b	2.38c	2.57c
GF18-3	41.23e	43.97d	10.15c	11.31e	9.89c	11.51d	16.24e	18.63d	1.88f	2.11e
GP17-2	52.34b	55.30b	12.97ab	13.98ab	13.41a	14.41a	20.69b	22.89ab	2.50b	2.76b

Different letters indicate statistically significant differences between treatments by Fisher's LSD test

of *P. simplicissmum* GP17-2, while it was 44.01% in the CF treatment of *F. equiseti* GF18-3.

Excretion of chitinase, cutinase and other cell wall-degrading enzymes by bioagents was advocated to perform an important function against fungal pathogens (Tweddell et al. 1994). CFs of PGPF triggered the expression of genes related to defence enzymes such as β-1, 3 glucanases and acidic and basic chitinase (Elsharkawy et al. 2012).

Impact of PGPF on some crop parameters

Four crop parameters, i.e. plant height, root length, bulb perimeter and plant dry weight, were evaluated at the end of the experiment (100 days post-infection) to determine the effect of PGPF isolates on onion growth. Data presented in Table 4 revealed that crop parameters of plants treated with PGPF isolates were significantly increased than the control plants. Similarly, the PGPF-treated onion plants under the infection of *S. cepivorum* exhibited significantly increased crop parameters in comparison with the infected control under greenhouse and field conditions (Tables 4 and 5). Additionally, the fungicide Folicur significantly increased the crop parameters than at the control treatment. Several studies have reported the utility of PGPF isolates for promoting plant growth (Saldajeno and Hyakumachi 2011).

Impact of PGPF on disease severity

Onion seedlings pre-treated with PGPF isolates prior to pathogen inoculation could significantly suppress the white rot symptom improvement than in the control (Fig. 3). Both disease severity and incidence were decreased in response to PGPF treatments in greenhouse and field trials (Tables 6 and 7, respectively). This result indicated that PGPF protected onion plants against *S. cepivorum* and reconfirmed previous studies which have shown that PGPF could efficiently reduce the incidence of root diseases (Elsharkawy et al. 2014). *P. simplicissmum* GP17-2 showed the best results in this respect, while *F. equiseti* GF18-3 showed the lowest activity against *S. cepivorum*. Successful biological control of the onion white rot has been reported by a number of

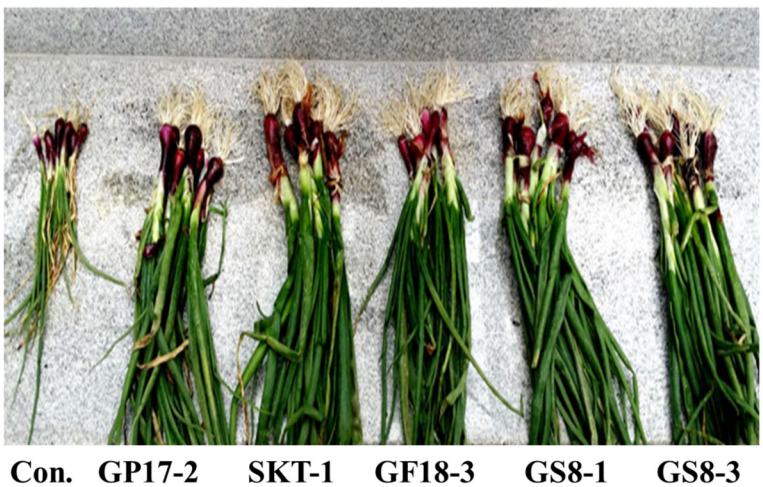

Fig. 3 Effect of PGPF isolates compared with the control treatment on the disease symptoms of onion seedlings infected with white rot (*Sclerotium cepivorum*) under field conditions

Table 6 Disease incidence and severity of white rot-infected onion plants treated with barley grain inoculum of plant growth-promoting fungi or with the fungicide (Folicur) relative to non-treated control plants under greenhouse conditions

Treatments	Disease incidence (%)	Disease severity (%)
Control	95.83a	92.71a
Folicur	12.50g	8.37e
GS8-1	29.83d	27.78b
GS8-3	33.33c	29.17b
SKT-1	20.83e	18.75c
GF18-3	37.50b	30.21b
GP17-2	16.67f	13.55d

Different letters indicate significant differences by Fisher's LSD test ($P \leq 0.05$)

Table 7 Disease incidence and severity of white rot-infected onion plants treated with barley grain inoculum of plant growth promoting fungi or with the fungicide (Folicur) relative to non-treated control plants under field conditions

Treatments	Disease incidence (%)		Disease severity (%)	
	2017–2018	2018–2019	2017–2018	2018–2019
Control	75.55a	72.22a	80.55a	77.77a
Folicur	11.11f	7.78f	13.89f	11.13e
GS8-1	21.01cd	17.78c	30.55c	22.02c
GS8-3	22.22bc	20.00bc	36.11b	27.78b
SKT-1	17.78de	14.43d	25.00d	19.84c
GF18-3	25.56b	22.22b	38.89b	30.15b
GP17-2	15.56e	11.12e	19.45e	13.89d

Different letters indicate significant differences by Fisher's LSD test ($P \leq 0.05$)

researchers in glasshouse and field trials, using fungal and bacterial isolates (Abd El-Moity 1976; Clarkson et al. 2002).

Effect of the PGPF on stimulation of peroxidase and polyphenol oxidase

The readings of peroxidase and polyphenol oxidase were increased in PGPF-treated plants than in the control (Fig. 4). The behaviour of the assessed enzyme was changed based on the inducer PGPF isolate. The *P. simplicissmum* GP17-2 treatment showed the highest level of defence-related enzyme activities followed by *T. asperellum* SKT-1, *Phoma* sp. GS8-1, *Phoma* sp. GS 8-3 and *F. equiseti* GF18-3. The results of disease severity were highly correlated with the results of enzyme activities. For example, GP17-2 was superior to all PGPF isolates in reducing the disease severity as well as in increasing the activities of peroxidase and polyphenol oxidase enzymes. Maurhofer et al. (1994) also reported varying levels of glucanase and chitinase in tobacco cultivars treated with different isolates of PGPR. Similarly, biochemical analysis of cucumber plants showed increased accumulations of POX and PPO in PGPF-treated plants in comparison with the control (Hassan et al. 2014). The exact role of defence-related enzymes in disease resistance was demonstrated previously (Hassan et al. 2014).

Expression levels of *PR1* and *PR2* genes

C_T values were evaluated for *PR1* and *PR2* genes in PGPF treatments under *S. cepivorum* infection to offer an outline of the relative abundance of PR genes (Fig. 5). The expression of *PR1* was significantly high in *P. simplicissmum* GP17-2 treatment with 6.9-folds of the control. *PR1* gene was upregulated with the expression levels 5.9, 5.7, 4.8 and 4.7-folds in treatments with *F. equiseti* GF18-3, *T. asperellum* SKT-1, *Phoma* sp. GS8-1 and *Phoma* sp. GS 8-3, respectively. Rapid induction of *PR2* gene (*β-1, 3-glucanases*) was noticed in PGPF treatments. Expression of *PR2* gene exhibited the highest values in *P. simplicissmum* GP17-2 treatment. In contrast, *PR2* gene was slightly induced in *Phoma* sp. GS 8-3 and the fungicide Folicur recording 3.4 and 2.4-folds, respectively.

The obtained results agree with that of Elsharkawy et al. (2012) who showed that the expression of the defence genes

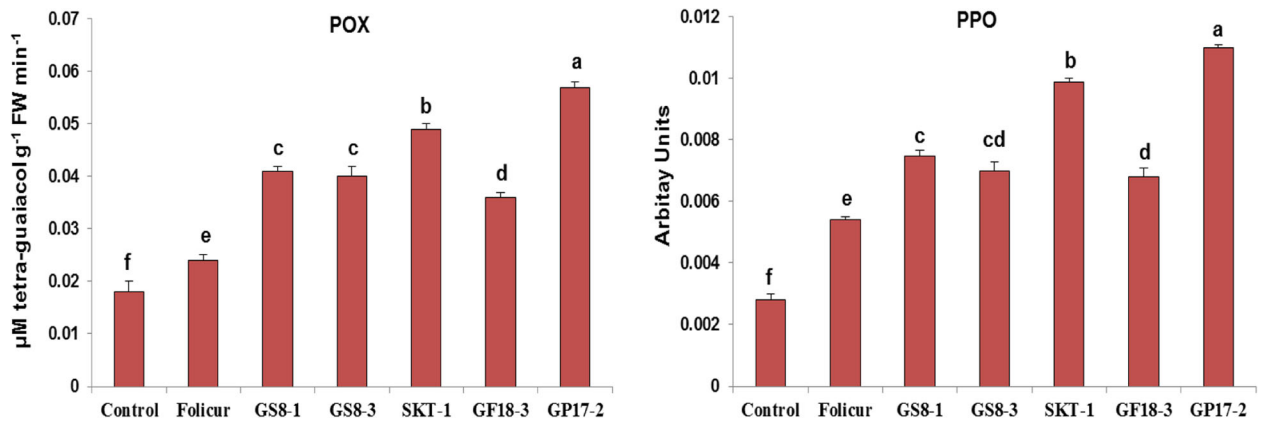

Fig. 4 Activities of peroxidase (POX) and polyphenol oxidase (PPO) enzymes in onion plants treated with PGPF isolates or the fungicide Folicur and infected with *Sclerotium cepivorum*

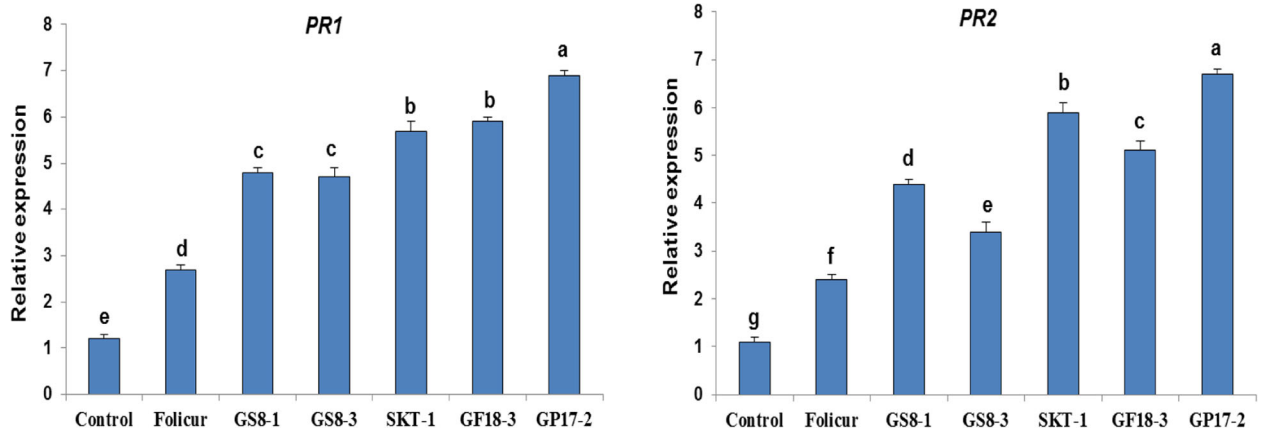

Fig. 5 Relative quantity expression of *PR1* and *PR2* genes in PGPF or Folicur treated plants compared with the control

were upregulated in *P. simplicissmum* GP17-2-treated plants. The main roles of PR1 proteins are still obscure (Urano et al. 2013), although Niderman et al. (1995) explained the antifungal behaviour of PR1 protein in tomato plants. Additionally, stimulation of *PR1* gene led to activated resistance of *Arabidopsis* plants against different viral and fungal pathogens (Hong and Hwang 2005; Elsharkawy et al. 2012).

Conclusion

The continuing quest for safe and potential biological control agents has directed the efforts toward the exploration of new biocontrol agents that can effectively substitute traditional and harmful pesticides. The obtained results indicated that the tested PGPF isolates can effectively control the white rot disease in onions. Moreover, these isolates significantly increased crop growth parameters. The assayed materials can represent new and potential biocontrol methods for the white rot disease in onions and maybe other pathogens.

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The authors declare that they have no competing interests.

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