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Coating seeds with *Trichoderma* strains promotes plant growth and enhance the systemic resistance against *Fusarium* crown rot in durum wheat

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Abstract

Background: Fusarium crown rot is one of the major diseases that cause significant yield losses of wheat, and *Trichoderma* strains were known as an effective biocontrol agent.

Main body: The aim of this study was to evaluate the potential of coating durum wheat seeds of the cultivar "Karim" with 3 different Tunisian strains of *Trichoderma* sp. (S.INAT, SIO1, SIO2) and the *Trichoderma*-based commercial product Trianum-T22 on seed germination, seedling growth, and plant defense response against the pathogen *Fusarium culmorum*. The strains were identified using molecular tools based on sequencing ITS region of ribosomal DNA. The results confirmed at 99% of homology that the strains were *T. harzianum*. Under controlled conditions, the coating seeds were released with 400 μl of spore suspension at 10^7 spores/ml. The seed coating with Trianum-P, and S.INAT showed the highest seed germination rates ranging from 85 to 90% while S.IO1 and S.IO2 presented the lowest germination rates with 66 and 68%, respectively. At 20 days post-infection (dpi) with *F. culmorum*, the treated plants with S.INAT and Trianum-T22 reduced the disease incidence by 53.59 and 51.79%, respectively than the control. Besides, S.INAT induced two-folds the phenolic compounds level compared to infected control. Further, the peroxidase activity was enhanced by 50% in average since 10 dpi in plants treated with S.INAT and SIO2 than the control.

Conclusion: The results suggest that seed coating with *T. harzianum* S.INAT was a promising tool for crop production and protection under field conditions due to both direct antagonist activity and the indirect growth promotion. This strain seems to induce the systemic resistance of plants against foot crown rot disease.

Keywords: Seed coating, *Trichoderma* sp., *Fusarium culmorum*, Wheat, Growth promotion, Antioxidative, Systemic resistance

Background

One of the major problems in agricultural production in the world is soil borne diseases that cause significant economic losses in yield and quality of many important crops (El-Sobky et al. 2019). In Tunisia, durum wheat, which constitutes the largest part of the staple food in Tunisia and represents around 60% of cereal area, is very susceptible to *Fusarium* crown rot (FCR). The FCR has a wide distribution across the cereal growing areas in Tunisia, and is caused by a complex of fungal species including *Fusarium culmorum* (W. G. Sm.), *F. pseudograminearum*, *F. verticillioides*, *F. avenaceum*, *Microdochium majus*, and *M. nivale* leading to important yield losses up to 44% (Chekali et al. 2011).

Currently, disease control measures are carried out on a large-scale using agrochemicals such as chemical

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fungicides which are expensive, increases potentially the consequence of environment pollution and health risk for humans and living organisms, with a little effect on biological control and result in the emergence of resistant pathogens (El-Sobky et al. 2019). Use of the most chemical fungicides as seed coatings could not effectively control FCR. The efficiency of the fungicide does not maintain much beyond the seedling stage due to the natural degradation of chemicals (Li et al. 2016). Also, *Fusarium* mycotoxins pose a significant health risk for humans and animals through food and feed prepared from contaminated cereal crops (Błaszczyk et al. 2017). An alternative approach is to rely on the application of natural antagonists, able to counteract the pathogenic and mycotoxigenic potential of natural populations of *Fusarium*, rather than acting on their saprophytic phase, or capable of stimulating natural resistance responses by the host plant (Scherm et al. 2013). *Trichoderma* spp. have been drawing the interest of researchers and intensively investigated since they biocontrol pathogens by different mechanisms such as antibiotic production, mycoparasitism, production of cell wall degrading enzymes and competition for nutrients or space (Tian et al. 2018 and El-Sobky et al. 2019). Besides, they are known to induce the plant defense responses, to trigger plant growth by producing growth hormones, antibiosis compounds and cell wall degrading enzymes, and to induce broad spectrum systemic resistance responses (ISR) in leaves (Li et al. 2016). The great economic and industrial interest in *Trichoderma* spp. has resulted in formulations of regulated and commercialized products for agricultural use (Filizola et al. 2019). Seed coating with *Trichoderma* spp. is a promising approach, as an integral component of agricultural practice in seed–plant–soil system that can replace chemical seeds treatments, in order to establish seed bio-priming through making beneficial microorganisms (Nagaraju et al. 2012) accessible to the roots of crops (Tavares et al. 2013), and capable of colonizing the rhizosphere at the critical "early germination" stage and therefore facilitating early, healthy and rapid development with improving nutrient uptake and tolerance to stresses (Nagaraju et al. 2012 and Lutts et al. 2016). Furthermore, this approach stands out as the best alternative sustainable disease management strategy since it either can be used to improve soil microbiome and structure, bioremediation of infected soils, eliminate or reduce the need to treat the broad area in which a crop is installed, and can represent a saving in the time and expense to making such wide-ranging applications (Chatterjee et al. 2018). Therefore, the search of novel indigenous *Trichoderma* strains with high biocontrol potential for plant disease management is required for the ubiquitous occurrence of *Trichoderma* (Li et al. 2016). It is highly necessary to screen the candidate *Trichoderma* strains for potential use as a coating seed treatment for the control of *Fusarium* in durum wheat.

In this context, the objectives of the present investigation were (i) to isolate endogenous (Tunisian) strains of *Trichoderma,* followed by molecular characterizations of *Trichoderma* strains using the internal transcribed spacer rDNA (ITS) method; (ii) to evaluate and select *Trichoderma* strains with high antagonistic activity against basing on the in vitro dual culture plate technique; (iii) to explore the effect of the seed coating approach with these strains on seed germination, seedling growth and the biocontrol capacity against FCR of durum wheat in plants greenhouse assays; and (iiii) to evaluate the potential of the strains on the induction of plant biochemical response (phenolic content and peroxidases activity) in the absence and presence of the disease.

Materials and methods

Isolation of the pathogen

The fungal pathogen *F. culmorum* (FC) was isolated from brown lesions in wheat roots sampled from severely infected fields from north Tunisia, kindly provided by Dr. Samia Gargouri (National Institute of Agronomic Research of Tunisia). The pathogen inoculum used for plant inoculation were prepared as previously described by Mnasri et al. (2017). To produce macroconidia, a mixture of barely grains (3:1 by volume) was soaked in water overnight in 250 ml glass bottles. Water was decanted and seeds were autoclaved. Afterwards, seeds were soaked in *Fusarium* liquid culture, and were kept for 2 weeks at 25 °C in the dark. Conidia were washed from the kernels and the concentration of the conidial suspension was set to 1×10^5 ml^{-1}.

Isolation and identification of the beneficial antagonist *Trichoderma* strains

Trichoderma strains were isolated from the rhizosphere of tomato roots sampled from infected fields with *Fusarium oxysporum* f. sp. *radicis-lycopersici* of different regions of Tunisia (Table 1) and maintained after purification on Potato dextrose agar (PDA) medium to be used later as an inoculum. The liquid culture of each pure solid culture of *Trichoderma* strain was produced by scraping the spores in the sterile distilled water from Petri dishes and transferring them to a 250-ml Erlenmeyer flask containing 100 ml of PDB (Potato-Dextrose-Broth) medium. The liquid culture was then incubated

Table 1 Strains of *Trichoderma* and origin

Strains	S.INAT	S.IO1	S.IO2
Origin	Tekelsa	Sidi bouzid	Sfax

on a rotary shaker at 110 rpm and 25 °C for 7–10 days and the mycelium was collected by filtration.

The *Trichoderma* strains were taken from 1-week-old fungal liquid cultures and the DNA was extracted using the innuPREP DNA Mini Kit (Analytik Jena, Jena, Germany). The purity and quality of the DNA was checked using 1.5% agarose gel electrophoresis and spectroscopically by reading the absorbance at 260 and 280 nm. The universal primers ITS1 (5′-TCGGTAGGTG AACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTG ATATGC-3′) were synthesized by Carthagenomics (RAN BioLinks SARL, Tunisia) and used to amplify the ITS (Internal Transcribed Spacer) region of ribosomal DNA (White et al. 1990). PCR was performed in a total reaction volume of 25 μl, containing 10 ng of the template DNA, 1.25 U Taq DNA polymerase and 1× Taq polymerase buffer ((Promega, Madison, WI, USA), 0.5 mM of each primer, 200 μM of each of the four dNTP. The MiniOpticon Real-Time PCR System built on the MJ Mini™ cycler (Bio-Rad Laboratories, CA, USA) was used for PCR with the following program; 5 min at 94 °C, followed by 30 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C (Table 2) and extension for 1 min at 72 °C. PCR products were then purified and sequenced by Carthagenomics (RAN BioLinks SARL, Tunisia). Sequences were submitted to GenBank database through Submission Portal (a World Wide Web sequence submission server available at NCBI home page: http://www.ncbi.nlm.nih.gov). The sequenced data were compared to the GenBank database, using the BLAST tool available on the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/BLAST/).

In vitro antagonistic activity of T. harzianum strains against F. culmorum

The in vitro antagonist activity against FC was evaluated using the dual culture technique according to Li et al. (2016). Mycelial discs of 5 mm diameter of 1-week old *Trichoderma* and 1-week-old FC were placed on the opposite sides of Petri dishes containing potato-dextrose agar at equal distance. The control plates contained only Mycelial discs of FC. The experiment was conducted with 3 repetitions for each antagonist and for the control. The plates were incubated at 25 ± 2 °C for 5 days in the dark and at the end of the experiment, the diameter of mycelia growth was measured to determine the inhibition percentage as following:

$$\text{Inhibition}\ (\%) = \frac{(C - T)}{C} \times 100;$$

Where *C* is the radial growth of FC (mm) alone (control); and *T* is the radial growth of FC (mm) in the presence of *Trichoderma* strains.

Table 2 The different seed coating treatments

N°	Seed coating treatments
1	Coated control
2	Coated Seeds with S INAT
3	Coated Seeds with S. IO1
4	Coated Seeds with S. IO2
5	Coated Seeds with Trianum-P

Plant experiments

Seed coating treatment

Seeds of durum wheat (*Triticum durum* L.) cv. Karim were chosen for all experiments according to its susceptibility to the soil-borne fungal pathogens (Chekali et al. 2011). Prior to use, seeds were surface-sterilized, soaked for 2 min in an aqueous solution of 0.6% sodium hypochlorite, then for 2 min in 70% ethanol, and rinsed three times with sterile distilled water. Afterwards, the seeds were coated as described by Ben-Jabeur et al. (2019), The coating technique for each treatment consisted of mixing 40 μl of the coating product Agicote Rouge T17 (AEGILOPS Applications, France) with 400 μl of the suspension of each *Trichoderma* culture (Table 2) at a concentration of 10^7 spores.ml^{-1} (water was used as a control), at a concentration of 10^6 spores.ml^{-1}. Then, the coating mixture was applied progressively to 10 g of wheat seeds in continuous rotation, until complete adhesion and absorption, to assure homogeneous distribution of the coating mixture among seeds. The product Trianum-P was used as a reference for this experiment; it is a biofungicide marketed by koppert based on *T. harzianum*-T22 (3.65%) and other components (96.35%), contained at least 1.0 × 10^7 colony forming units per gram dry weight, and known to promote plant growth and to control plant diseases (Lascaux and Piron, 2009 and Gveroska, 2017).

Effect of seed coating treatments on seed germination and growth promotion

After seed coating treatment, the coated seeds were sown in Petri dishes with Hoagland medium (1% agar). Plates were placed in a growth chamber at 22 ± 2 °C with a 12-h photoperiod. The percentage of seed germination was measured at 7 days post-coating (dpc). Subsequently, coated seeds were grown in pots of 16 cm diameter containing a mixture of standard substrate: perlite (1:1, v/v), with a density of four seeds per pot. Pots were maintained under controlled conditions in the growth chamber (22 ± 3 °C, 40–50% relative humidity, and a photoperiod of 16:8 h light/dark). Plants were irrigated with the nutritive solution (Hoagland) during the course of experiments. Experiments were performed

using a completely randomized design (CRD) with 6 treatments, and 3 replicates and the pots were rotated 3 times a week to assure uniform growth conditions in the growth chamber. For each treatment, plant samples were taken at 10 dpc to measure the shoot height, the root length, and the plant biomass. The seedling vigor index (VI) was calculated according to the following formula: seedling vigor index = [seedling length (cm) × germination percentage (%)] (Buriro et al. 2011).

Effect of seed coating treatments on *F. culmorum* disease control

At stage of 3 to 4 leaves, plants were inoculated by irrigation with the suspension of *FC* at a concentration of 10^5 macroconidia.ml^{-1}. Control seedlings were irrigated with water only. For each treatment, the incidence was recorded at 10 dpi as the percentage of plants showing browning symptoms on the base of the stem. The evaluation of the disease severity of FCR was scored in the seedling stage at 20 dpi and rated on a 0–5 scale based on the typical symptoms of browning as previously described by Koycu (2019) as follows: 0, healthy plant; 1, necrotic area is lower than 25%; 2, necrotic area is between 25 and 50%; 3, necrotic area is between 51 and 75%; 4, necrotic area is greater than 75%; 5, plant is dead. Disease incidence and severity were averaged among the replicates. Afterwards, the reduction rates (RR) of the disease index (incidence or severity) of FCR by the treatments were calculated as following:

$$\mathrm{RR}\ (\%) = \frac{\text{Disease index in control infected plants - Disease index in treated infected plants}}{\text{Disease index in control infected plants}} \times 100$$

Effect of seed coating treatments on the antioxidative metabolites in wheat seedlings in the absence and presence of *F. culmorum*

At 10 dpi representing the time of the emergence of FCR symptoms, samples were taken from both inoculated and non-inoculated plants derived from coated seeds for the quantification of total phenolic content and peroxidases activity which are metabolites involved in the plants' defense response, antioxidative pathway, and lignification. Total phenolic content was estimated by Folin-Ciocalteu method (Singleton et al. 1999). Samples of 500 mg of fresh leaves were ground with 2 ml of methanol (80%) at 4 °C and then centrifuged at 1000 rpm for 10 min. A 100 μl of the supernatant was added to the reaction mixture containing 50 μl of sodium carbonate (20%), 1750 μl of sterile distilled water and 250 μl of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany). The reaction mixture was well mixed and incubated for 30 min at 40 °C and then cooled to room temperature. The optical density was measured at 760 nm and the amount of phenol was determined using Catechol as the standard and expressed as mg.g^{-1} fresh weight (FW). Peroxidase activity was measured according to (Egley et al. 1983); samples of 200 mg of fresh roots and third equal-size leaves were homogenized in 5 ml of 50 mM K-phosphate buffer (pH 5.5). After centrifugation at 12, 000×*g* for 20 min at 4 °C, the supernatant was collected as the crude enzyme solution. A reaction mixture was prepared by adding 2.9 ml of 50 mM K-phosphate buffer (pH 5.5), 1 ml of H2O2 (0.6 M) and 1 ml of 50 Mm guaiacol to 0.1 ml of crude enzyme solution. Protein content of the crude enzyme solution was determined at 595 nm with bovine serum albumin as the standard using the Bradford assay (Bradford, 1976) by mixing 790 μl of extraction buffer, 10μl of crude enzyme solution and 200μl of Bradford reagent (Biomatik, Tunisia). Peroxidases activity was determined with guaiacol at 470 nm and expressed in unit mg^{-1} protein.

Statistical analyses

In order to assess the significance among the effect of *Trichoderma* species, data were subjected to analysis of variance (ANOVA) using SPSS software (20.0). The antagonism effect of the *Trichoderma* strains on the pathogen (Fc) growth and the effect of seed coating treatments on growth traits of wheat were determined through a one-factor ANOVA (strains) and (treatments), respectively. The effects of the treatments and infection and their interaction on FCR incidence and severity, and on phenolic content and peroxidases activity were determined through a two-factor (treatments × infection) ANOVA. The comparison of means was performed using the Duncan's multiple range test (DMRT). All graphics creation was generated in the RStudio environment, using RStudio software 1.1.463 (2009–2018 RStudio, USA).

Results and discussion

Identification of *Trichoderma* strains

Macroscopic morphology was investigated in the 3 of *Trichoderma* strains. They showed to be compatible with the description of the genus according to the findings of the literature, and revealed colonies of 1–2 concentric rings with green conidial production in mature colonies (Fig. 1). The mycelium, initially of a white color, acquired green, yellow shades, or remained white, due to the abundant production of conidia (Siddiquee, 2017 and Filizola et al. 2019), which in consistent with the characteristics previously described for this fungus (El-Sobky et al. 2019). The preliminary identification of these strains, based on colony morphology observations, was insufficient to reliably determine species. Thus, the identification was further ascertained by molecular analysis based on the sequencing of the ITS region of

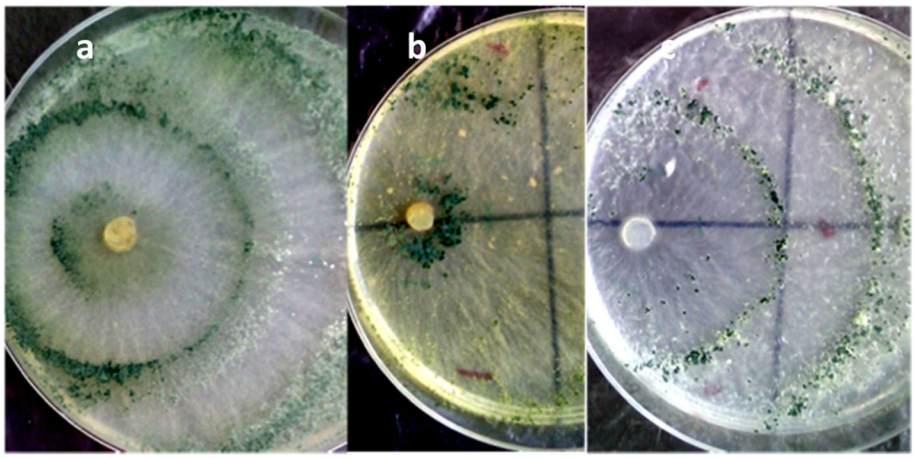

Fig. 1 Macroscopic aspects of *T. harzianum* strains inoculated in Petri dishes containing PDA as culture medium. **a** S.INAT. **b** SIO1. **c** SIO2

ribosomal DNA. PCR based on ITS primers was used to amplify ITS region and the sequencing data was entered on NCBI site to search BLAST and compare these data with published ITS data. The identity of the isolates was identified as belonging to species *T. harzianum* with homology percentage of 99% with the published sequence of *Trichoderma* spp. and sequences were deposited in GenBank (Table 3), which was reported as one of the common species with a widest distribution (El-Sobky et al. 2019).

Evaluation of the antagonistic activity of *Trichoderma* strains on *F. culmorum* mycelium

In the dual-culture experiment (Fig. 2), the three *Trichoderma* strains significantly hampered the mycelial growth of *F. culmorum*; however, they exhibited different inhibition rates against it. The maximum inhibition was recorded by the strain S.INAT (61%), with an inhibition zone of 15 mm, followed by S.IO1 (50.3%), and then S.IO2 (37.66%).

Testing of the strain of *Trichoderma* with the highest antagonistic activity is important for developing biocontrol agents. The dual culture experiment indicated that the three *T. harzianum* species were able to repress the mycelium growth of *F. culmorum* with a great variability in the level of the antagonist potential among them. As previously reported, the antagonistic function of the *T. harzianum* species is owing to their competition for nutrients, antibiosis, and mycoparasitism, including their active metabolism in the production of large amounts of secondary metabolites such as chitinase, b-1, 3-glucanase, protease, peptaibols, polyketides, pyrones, terpenes, and polypeptides, that act as potent weapons against other non-beneficial fungi (Li et al. 2016 and El-Sobky et al. 2019). This research also proved possible parasitism of *T. harzianum* and competition for space and nutrients. The strain S.INAT displayed the most promising antagonistic potential to prevent the growth of *F. culmorum*, which deserve attention and further analysis of their ability to control disease development in controlled conditions as well as field experiments. The observed variations regarding the degree of antagonism among the different isolates against the same *Fusarium* strain may be indicative of the existence of specificity between the antagonist and the potential phytopathogen, suggesting the involvement of various genes and genetic factors interacting with the environment, and highlight the fact that more than one mechanism of action might be simultaneously involved in antagonistic actions (Filizola et al. 2019).

Table 3 Molecular identification of *T. harzianum* strains

Strains	Identification	Accession number
SIO1	*T. harzianum* SIO1	MT605289
SIO2	*T. harzianum* SIO2	MT605288
S.INAT	*T. harzianum* S. INAT	KU710282

Effect of seed coating treatments on seed germination and seedling growth

The analysis of variance showed that seed coating treatments significantly affected seed germination, and root and shoot length, plant dry matter, and vigor index (P = 0.001) in wheat seedlings (Figs. 3 and 4). At 7 dpc, the seed coating with Trianum-P, and S.INAT resulted in the highest seed germination rates (85–90%), while the control as well as S.IO1 and, S.IO2 resulted in the lowest germination rates (66–68%) (Fig. 3).

At 10 dpc, the seed coating with the reference product Trianum-P had the more remarkable effect on all growth traits compared to the other seed coating treatments (Fig. 4). Compared to the coated control, the strains

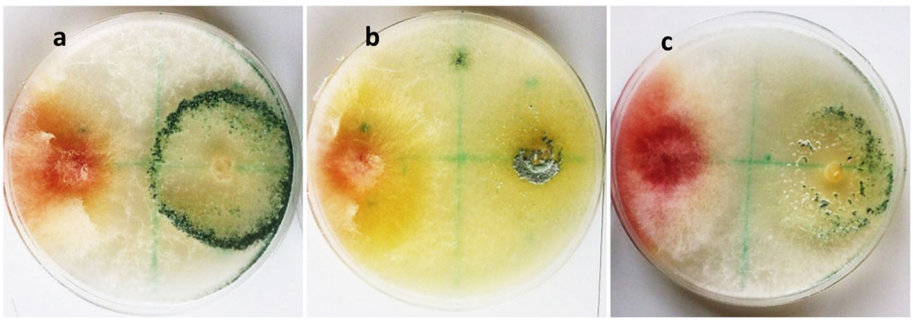

Fig. 2 In vitro antagonist activity of *T. harzianum* strains and their inhibition rates against *FC* in the dual-culture. **a** S. INAT. **b** SIO1. **c** SIO2

S.INAT and S.IO2 increased all the growth traits. Specifically, the strain S.INAT resulted in the highest root and shoot length, and the vigor index, while the strain S.IO2 resulted in the highest plant dry matter. The strain S.IO1 was the less effective in the wheat growth promotion (Fig. 4).

Taken into consideration that *Trichoderma* has a pivotal function as antagonist and growth promoters (Li et al. 2016) and that some strains can produce antifungal metabolites while others act as promoters of plant development (Filizola et al. 2019), all the strains were subjected to experiments to evaluate their impact on plant's growth as seed coating treatments. In general, the coating method facilitates the contact between the treatments and the seed, helps beneficial microorganisms to colonize the roots at early stages of growth which give the plants the ability to better assimilate the nutrients (Tavares et al. 2013 and Ben-Jabeur et al. 2019). As expected, the referential product Trianum-P strongly stimulated the seed germination and the root and shoot development of seedling, with higher phenolic accumulation and peroxidases activity. This not only add evidence to the potential of the commercialized strain T22 of *Trichoderma* to promote plant growth and to control plant diseases (Lascaux and Piron, 2009 and Gveroska, 2017), but also emphasizes the effective future use of this product as a seed coating treatment. Among the tested endogenous *Trichoderma* strains, the seed coating with S.INAT and S.IO2 seemed to be the most promising seed coating treatments resulting in the promotion of all the growth traits associated with higher phenolic accumulation and peroxidases activity in wheat. Specifically, the strain S.INAT resulted in the highest root and shoot length, vigor index, and phenolic accumulation, while the strain S.IO2 resulted in the highest plant dry matter and peroxidases activity. Indeed, in the absence of stress, peroxidases, and phenolic compounds are involved in the wall-building processes such as lignification and reinforcement of plant structural components (Scherm et al. 2013 and Akbari-Vafaii et al. 2014). Therefore, the stimulation of peroxidases and phenolic accumulation constitutes one of the mechanisms of *T. harzianum* in promoting growth. To summarize, the observed growth promotion could be attributed to (i) seed bio-priming through making S.INAT and S.IO2 capable of colonizing

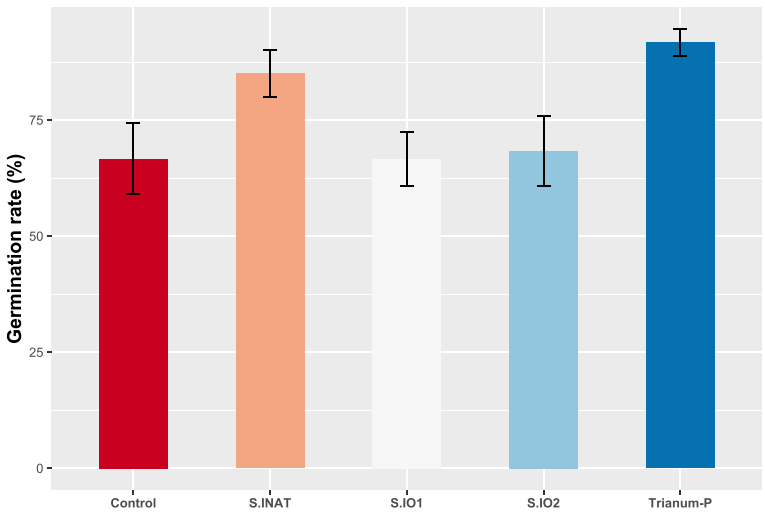

Fig. 3 Effect of seed coating treatments on seed germination at 7 dpc

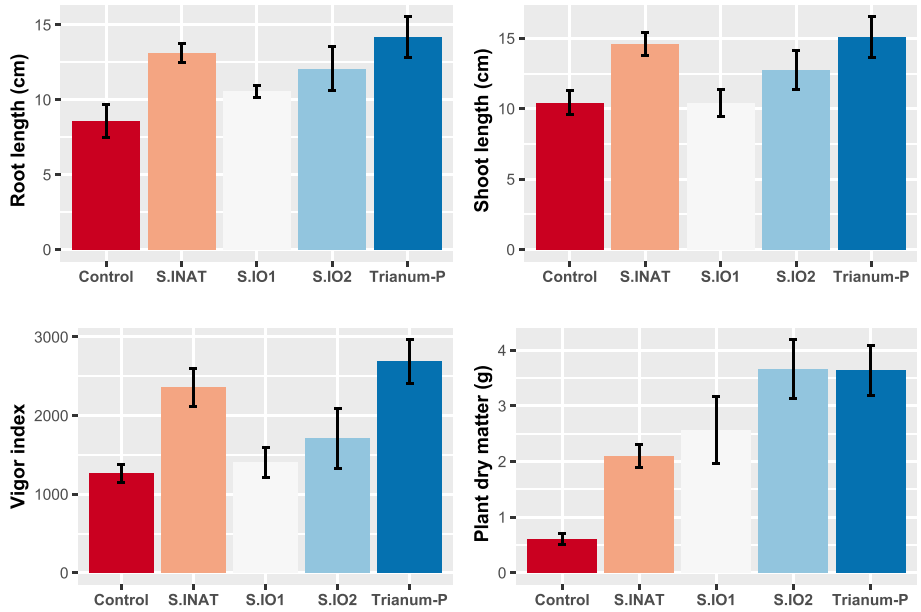

Fig. 4 Effect of treatments on root and shoot length, vigor index, and plant dry matter at 10 dpc

the roots at the "early germination" stage and hence facilitating early, healthy and rapid development with improving nutrient uptake (Singh, 2010; Saba et al. 2012; Lutts et al. 2016 and Ben-Jabeur et al. 2019), and (ii) the antagonist effect of *T. harzianum* on decreasing the activity of deleterious root micro flora and inactivation of toxic compounds in the root zone leading to a better root development (Roberti et al. 2008).

Effect of seed coating with *T. harzianum* strains on foot crown rot disease control and the antioxidative defense response in wheat

Effect of seed coating with T. harzianum on foot crown rot disease control

The analysis of variance revealed a highly significant effect of the treatments for FCR disease incidence (p = 0.001) and severity (p = 0.01) (Table 4). In inoculated control plants, the incidence reached 93.3% at 10 dpi, and the severity reached 3.3 at 20 dpi. In general, all seed coating treatments reduced the pathogenic effect of *F. culmorum* in wheat depicted by a lower incidence and severity of the disease than the control (Fig. 5). Specifically, plants derived from seed coating with S.INAT and Trianum-P were the more remarkable treatments as S.INAT resulting in the highest reduction rate of the disease incidence (RR = ± 53.59%), and Trianum-P resulting in the highest reduction rate of the disease severity by (RR = ± 59.69%) (Table 4).

Moreover, the effect of treatments on growth promotion under the biotic stress was further ascertained based on the pictures showing an enhanced plant development, described by a higher leaf number and/or a higher length of the emerging third leaf, than the control. Captivatingly, the strain S.INAT resulted in a higher leaf number and area at 10 dpi, and the highest leaf number at 20 dpi.

Effect of seed coating with T. harzianum on the antioxidative system in wheat seedlings

Analysis of variance (Fig. 6) revealed that seed coating treatment and infection affected significantly, the peroxidase activity and the phenolic content (p = 0.001), while their interaction (seed coating treatment × infection) affected significantly only the peroxidase activity (p = 0.001). In the absence of the infection, the plants derived from seed coating with all treatments (S.INAT, S.IO1, S.IO2, and Trianum-P) had a higher phenolic content and peroxidases activity, compared to coated control; with a more remarkable increase of phenolic content than peroxidases activity. The S.IO2 was recorded as the most strain inducing peroxidases, while S.INAT was the most strain inducing phenolic accumulation. When challenged with the pathogen Fc, a slight increase of peroxidases activity was recorded in infected control plants than the control plants, while phenolic content remained unchanged. In infected treated plants, the peroxidases activity was magnified and the increase of phenolic content was activated, compared to the infected control plants. The increase rate of both metabolites changed relatively to the different treatments; the strain S.INAT induced the highest phenolic content and peroxidases activity under infection.

At 20 dpi, control plants showed the symptoms of basal browning along with a lower foliar development as

Table 4 Results of one-factor ANOVA for disease incidence and disease severity on plants inoculated with *F. culmorum*. The F values are shown (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$)

	Disease incidence (%) at 10 dpi	Reduction rate %	Disease severity at 20 dpi	Reduction rate %
Control	$93.3^{a} \pm 2.8$	–	$3,3^{a} \pm 0.57$	–
SINAT	$43.3^{c} \pm 2.8$	53.59	$1.66^{b} \pm 0.57$	49.69
SIO1	$62.3^{b} \pm 2.5$	33.22	$3.0^{a} \pm 0.0$	9.09
SIO2	$59.6^{b} \pm 2.5$	36.12	$1.66^{b} \pm 0.57$	49.69
Trianum	$45.0^{c} \pm 5$	51.76	$1.33^{b} \pm 0.57$	59.69
ANOVA				
Treatments	111.6***		9.125**	

previously reported (Roberti et al. 2008 and Scherm et al. 2013). These symptoms were preceded by an increase in peroxidases activity at 10 dpi, with no recorded change in phenolic content. This biochemical response is considered as the triggered defense mechanism occurring at this stage of *F. culmorum*-wheat interaction in response to pathogenic oxidative and cell death processes. In fact, it is well-established that upon infection plant cells respond with a hypersensitive reaction by the generation of reactive oxygen species (ROS), which induce antioxidative metabolites as catalase, peroxidases, and phenolic compounds (Harrach et al. 2013; Rajeswari, 2014 and Akbari-Vafaii et al. 2014). In this study, phenolic compounds were not induced in control plants which underline the sensitivity of the variety "karim" towards the foot crown rot disease. This is corroborating with the findings showing that phenolic and polyphenolic compounds are present in larger amounts only in Fusarium-resistant plants (Jogaiah et al. 2013 and Akbari-Vafaii et al. 2014).

The seed coating treatments with *Trichoderma* strains simultaneously enabled plants to more efficiently scavenge ROS or prevent their production by inducing both peroxidases and phenolics, consequently decreased the disease incidence and severity, and promoted the growth of plants under the biotic stress. This could be attributed to: (i) the beneficial antagonist potential of *T. harzianum* that contains spores and produces mycelium growing at the same rate as the roots and protecting them from diseases (Dendouga et al. 2016), and (ii) the induction of the systemic acquired resistance (SAR) depicted by a magnified antioxidant and/or radical scavenging activities especially phenolics which have been shown to exhibit antifungal activity against *Fusarium* spp. and antioxidant activities (Jogaiah et al. 2018). This result is consistent to previous studies showing that seed treatment of maize with *T. harzianum* T22 and Th-8 strains reduces *Fusarium verticillioides* colonization and induces systemic resistance in maize against this pathogen (Ferriego et al. 2014a, b). Again, in this study, the strain S.INAT outperformed the other seed treatments in terms of disease control and growth promotion as well as peroxidases activity and phenolic content. This suggests that phenolics most likely played a key role in

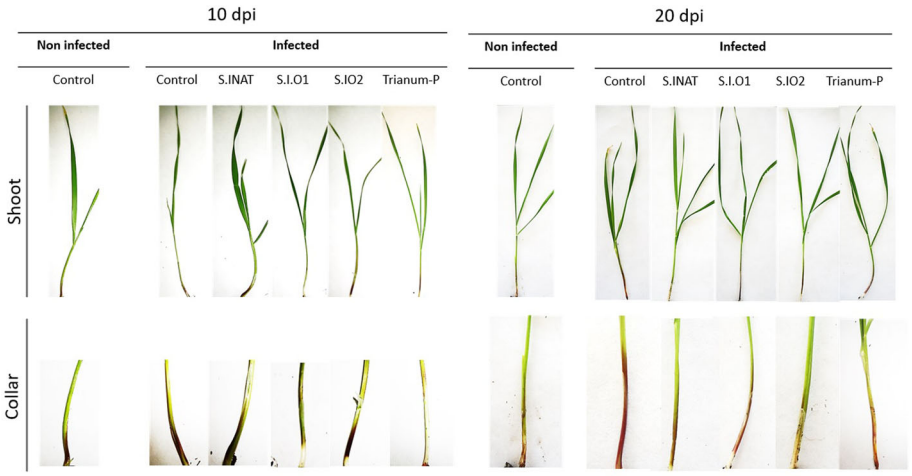

Fig. 5 Incidence percentage of FCR in wheat plants and disease severity of FCR in wheat crown following treatments

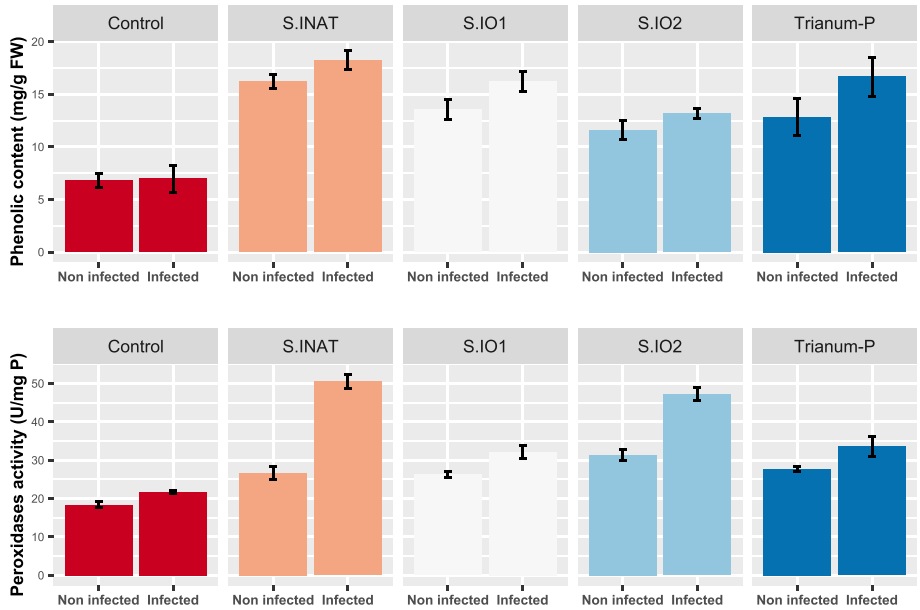

Fig. 6 Effect of both treatments and infection with *F. culmorum* on total phenolic content and peroxidases activity in wheat leaves at 10 dpi

the observed resistance which has been reported to be involved in the cell wall reinforcement and may supply structural resistance to invading pathogens (Schöneberg et al. 2018). This is in line with the findings of Lorenc-Kukuła et al. (2007) who demonstrated that the increase in resistance of transgenic flax (Linum usitatissimum) seedlings to *F. culmorum* was mediated by three key enzymes of flavonoid biosynthesis.

Conclusion

In conclusion, the results showed that the *T. harzianum* strain studied had successful biocontrol activity and ability to compete against *F. culmorum in vitro* as well *in vivo*. Further, it confirmed the seed-priming activity of these strains in improving the seedling vigor and enhancing the disease protection against FCR. Also, it is suggested that S.INAT was the most effective against foot crown rot disease. Further experimentations are necessary to find out whether the induced resistance was due to either an induced state of immunity or a priming effect.

This study lays the foundation for using *T. harzianum* S.INAT as a candidate fungus for biocontrol of foot crown rot in field conditions paving the way for the exploitation of this strain in the seed coating approach at the aim to improve crop production.

Abbreviations

dpi: Days post-infection; FCR: *Fusarium* crown rot; ISR: Systemic resistance responses; FC: *F. culmorum*; CRD: Completely randomized design; VI: Vigor index; RR: The reduction rates; ANOVA: Analysis of variance; DMRT: Duncan's multiple range test; PDB: Potato-dextrose-broth; dpc: Days post-coating; ROS: Reactive oxygen species; SAR: Systemic acquired resistance

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Authors' contributions

Z.K: carried out the experiment, collected the data, performed the analysis, and wrote the paper. MBJ: helped in the writing paper. MM: helped in collecting data. SG: reviewed the manuscript. KH: helped in the identification of the Trichoderma strains. WH: reviewed the manuscript. All authors have read and approved the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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