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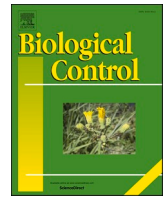
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Defense responses related to mycorrhizal-induced resistance in wheat against *Zymoseptoria tritici*

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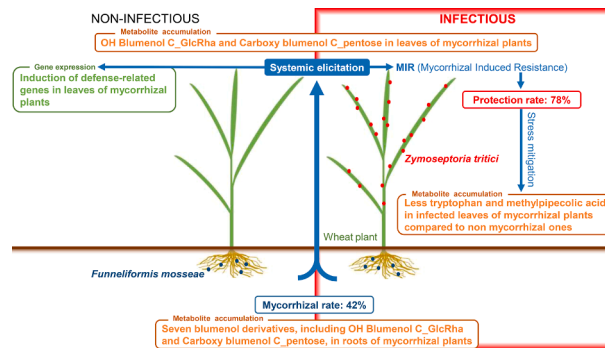
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HIGHLIGHTS

- Root inoculation with *F. mosseae* protects wheat leaves against STB.
- A systemic up-regulation of defense genes occurs in the leaves of mycorrhizal plants.
- A similar set of defense genes is induced by the pathogen itself in leaves.
- Blumenol derivatives are accumulated in mycorrhizal wheat plants.
- Metabolite balance is slightly affected in the leaves of mycorrhizal wheat plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Arbuscular mycorrhizal fungi (AMFs) are considered promising biocontrol agents that can provide protection to plants against bio-aggressors through the triggering of mycorrhiza-induced resistance (MIR). Here, the protective systemic effect of the AMF species *Funnelformis mosseae* was evaluated in a susceptible wheat cultivar against Septoria tritici blotch (STB), caused by the hemibiotrophic ascomycete *Zymoseptoria tritici*. Mycorrhizal inoculation led to a root colonization rate of 42 % in 50-day-old wheat plants, associated with an overexpression of genes encoding nutrient transporters in roots and the accumulation of blumenol derivatives in roots and leaves, attesting to a functional mycorrhizal symbiosis. Concomitantly, mycorrhizal wheat plants exhibited a foliar protection rate of 78 % against *Z. tritici*. An up-regulation of two defense-related genes (*PRI* and *POX*) in wheat leaves was also revealed consecutively to the AMF symbiosis establishment and prior to pathogen attack. These two genes and five other genes (*PR5*, *PAL*, *GST*, *CalS*, and *CAD*) were also up-regulated in leaves infected by

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Z. tritici alone during its biotrophic stage, whereas six genes (*PRI*, *PR4*, *PR5*, *PAL*, *POX*, and *CalS*) were up-regulated in mycorrhizal and *Z. tritici*-inoculated plants. Less accumulation of tryptophan and methylpipercolic acid was observed in infected leaves of mycorrhizal plants, indicating a stress alleviation in mycorrhizal wheat plants under *Z. tritici* attack. Our findings highlight the ability of *F. mosseae* to activate MIR and to protect wheat against *Z. tritici* by specifically remodeling gene expression in leaves.

1. Introduction

With a world production estimated at 781 million tons for 2022 (FAO, 2022), wheat, and in particular bread wheat (*Triticum aestivum* L.), is one of the most widely cultivated crops worldwide. Wheat crops deal with many abiotic and biotic stresses, which are getting more complex to manage along with the effects of global warming (Bento et al., 2021; Zhang et al., 2022), in the context of a constant growing food demand related to the rise of the global population (United Nations, 2022). One of the most economically damaging fungal wheat diseases is Septoria tritici blotch (STB), caused by the pathogenic fungus *Zymoseptoria tritici*, which can cause 50 % of yield loss in cases of severe epidemics (Ponomarenko et al., 2011; Fones and Gurr, 2015). *Z. tritici* is a hemibiotrophic fungus from the Dothideales order, able to propagate by both sexual ascospores and asexual pycnidiospores, which are spread by wind dispersion and rain splash (Kema et al., 1996). Its infection process takes place in two phases. During the first phase, fungal hyphae penetrate into the host tissues, generally through the stomatal apertures, and then grow inside the stomatal cavity to invade intercellular space in close contact with mesophyll cells (Kema et al., 1996). This latent asymptomatic phase, relying mostly on the fungus's own lipid storage (Rudd et al., 2015; Brennan et al., 2019), has been described as a biotrophic period (Keon et al., 2007). A sudden transition then leads to the second phase, described as the necrotrophic one, when the first signs of chlorotic lesions are starting to be visible. Host cells exhibit a reaction similar to an oxidative burst, with an increase in fungal cell-wall degrading enzyme production, leading to the maceration and death of mesophyll tissue (Kema et al., 1996; Siah et al., 2010; Brennan et al., 2019). Then, the host cell's collapse leads to the release of nutrients required for pycnidia formation in stomatal cavities and their emergence at the leaf surface (Kema et al., 1996; Siah et al., 2010; Rudd et al., 2015). The "switch" from biotrophy to necrotrophy usually occurs at least 10 days after infection, but this time lapse varies depending on the host cultivar and environmental conditions (Kema et al., 1996; Brennan et al., 2019).

The main methods to control STB include breeding for more resistant cultivars and fungicide application, mainly the sterol 14- α -demethylase inhibitors (DMIs) and the succinate dehydrogenase inhibitors (SDHIs) (Birr et al., 2021; Jørgensen et al., 2021). However, a greater number of *Z. tritici* genotypes resistant to DMI or SDHI have been reported (Selim et al., 2014; Birr et al., 2021), due to *Z. tritici* sexual reproduction and frequent genetic recombinations occurring in the field under the selection pressure of repeated fungicide treatments (Siah et al., 2010; Cheval et al., 2017; Kildea et al., 2021). Likewise, multidrug-resistant strains of *Z. tritici* are arising (Somai-Jemmali et al., 2014; Omrane et al., 2015; Kildea et al., 2019). In addition, other indirect damages need to be taken into account, including the non-intentional negative effects of fungicide use on the environment and human health (Gill and Garg, 2014) and the financial cost of massive fungicide use to control the disease (Torriani et al., 2015). Therefore, along with a more restrictive regulation on plant protection products to control and limit their use, new efficient and environmentally friendly control methods have to be developed. The use of biocontrol agents, such as living bacteria or fungi, is getting increasingly more attention in wheat disease management (Cordo et al., 2007; Kildea et al., 2008; Samain et al., 2017; Gehlot and Singh, 2018).

Among these potential biocontrol agents, arbuscular mycorrhizal fungi (AMFs) are of growing interest. These symbiotic fungi set up a mutualistic interaction with their host plant, thanks to the hyphal

colonization of the root cortex intercellular spaces and the formation of intracellular structures, the arbuscules, which are the main exchange sites between the two symbiosis partners. While AMFs improve the host plant's mineral and water nutrition, the host plant, in turn, provides photosynthetic carbohydrates and lipids to AMFs (Smith and Read, 2008; Wipf et al., 2019). These exchanges occur thanks to the activation of several membrane transporters located in the *peri*-arbuscular zone, such as Phosphate (PT, Phosphate Transporters), Nitrate (NRT, NitRate Transporters), Sucrose (SUT, SUcrose Transporters), or Sterol transporters (ABC, Atp Binding Cassette transporters) (Wipf et al., 2019). Overall, this symbiosis can result both in plant growth enhancement and in a better tolerance to abiotic and biotic stresses, including soil-borne and aerial diseases (Weng et al., 2022). Recently, it was shown that the AMF species *Funneliformis mosseae* was effective in controlling wheat powdery mildew, a foliar disease caused by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici* (Mustafa et al., 2016, 2017). Similarly, it has already been reported that plant protection was gained after mycorrhizal inoculation in several pathosystems involving hemibiotrophic agents causing aerial diseases, as for *Colletotrichum orbiculare* in cucumber (Lee et al., 2005) or for *Magnaporthe grisea* in rice (Campos-Soriano et al., 2012), both cucumber and rice being previously inoculated with the AMF *Rhizophagus irregularis*.

The mechanism underlying the protection obtained following mycorrhizal inoculation, called Mycorrhiza-Induced Resistance, or MIR, provides systemic protection against a variety of intruders, such as microbial pathogens, nematodes, parasitic plants, and insects (Jung et al., 2012; Weng et al., 2022). MIR shares similar features with systemic acquired resistance after pathogen infection (Cameron et al., 2013). MIR leads to a major reprogramming of primary and secondary metabolisms and to a modulation of defense-related phytohormone levels in mycorrhizal plants (Hause et al., 2007; Schliemann et al., 2008). It has been reported so far that MIR resulted from an active suppression of salicylic acid (SA)-dependent defense pathway components, causing systemic priming of jasmonic acid (JA)-dependent defenses (Benjamin et al., 2022; Fiorilli et al., 2024). In wheat, the induction of defense-related genes leads to the MIR in leaves of mycorrhizal plants without any pathogenic fungal challenge, as reported by Mustafa et al. (2017). An up-regulation of four target genes involved in wheat defense, encoding a peroxidase *POX*, a phenylalanine ammonia lyase *PAL*, a chitinase *CH11*, and a nonexpressor of pathogenesis-related proteins 1 *NPR1*, was observed.

The use of AMFs represents a promising biocontrol tool against cryptogamic diseases. However, the impact of mycorrhizal symbiosis on the protection of plants challenged by foliar hemibiotrophic pathogen fungi was poorly studied. Thus, the present study aims at evaluating the efficacy of the AMF species *F. mosseae* to protect wheat plants against *Z. tritici* and to provide insights on plant defense responses taking place with the MIR.

To this purpose, the evaluation of growth parameters and disease severity was performed on wheat plants grown in the presence or absence of the AMF species *F. mosseae* under controlled conditions. After assessment of root mycorrhizal rates, gene expression analyses were carried out to check the mycorrhizal symbiosis functionality by studying the expression of a set of specific nutrient transporter genes involved in nitrate, phosphate, sulfate, lipid, and glucid transports. To investigate MIR-associated defenses in mycorrhizal wheat, the expression of a panel of 24 defense-related genes was analyzed in leaves based on the molecular tool qPFD®, a quantitative RT-PCR microplate/low density DNA

chip (Brisset and Duge de Bernonville, 2011). Finally, a metabolomic investigation was performed on both roots and leaves of mycorrhizal and non-mycorrhizal wheat plants in order to monitor the effect of mycorrhizal symbiosis on metabolite accumulation two days post-pathogen-inoculation, during the biotrophic phase of the fungal pathogen.

2. Materials & methods

2.1. Plant culture and mycorrhizal inoculation

The winter bread wheat (*Triticum aestivum* L.) cv. Alixan used in this study was graciously supplied by RAGT (Rodez, France). This cultivar is considered susceptible to STB caused by *Z. tritici*, exhibiting an evaluated field resistance level of 4 on a scale from 1 (the most susceptible) to 9 (the most resistant). Pots were loaded with 600 g of a substrate made up of sand, perlite, and vermiculite (4:1:2, v/v/v) sterilized twice (120 °C and 1 bar for 30 min). The commercial mycorrhizal inoculum composed of the AMF *F. mosseae* strain FR140 was chosen in this study for its ability to colonize wheat roots (Mustafa et al., 2016, 2017). This inoculum provided by MycAgro Ltd. (Bretenière, France) consists of a granular form composed of mineral inert solid particles (clay, zeolite) and a mix of fragments of mycorrhizal roots and propagules at a concentration of at least 10 active propagules/g. For mycorrhizal plants, 60 g of the commercial mycorrhizal inoculum *F. mosseae* was mixed per pot with the substrate (10 % of the whole substrate quantity) (Mustafa et al., 2016). Five wheat seeds, which were previously soaked overnight in water and pre-germinated for two days in the same previously described sterilized substrate, were deposited per pot. In order to provide an equivalent quantity of organic matter, the same amount of the sterilized mycorrhizal inoculum (120 °C at 1 bar during 20 min) was added to the substrate dedicated to control non-mycorrhizal plants. Then, seedlings were put into growth chambers (Panasonic, MLR-352H) programmed to regulate temperature from 16 °C at night to 20 °C at day (15,000 Lux) with a photoperiod (Pd) night/day of 8 h/16 h and a relative humidity (RH) of 70 %. Plants were watered at field capacity three times a week. At the fourth week of plant growth, plants were supplied once a week with 100 mL per pot of a modified Hoagland's solution (Hoagland and Arnon, 1950) deprived of half of its phosphorus intake to promote mycorrhizal symbiosis establishment. Plants were kept growing until the 5th week to let enough time for the AMF to establish the symbiosis with their host.

2.2. *Z. tritici* spore production and plant pathogen-inoculation

The *Z. tritici* strain T02596, kindly provided by A. Siah (ISA, Junia, BioEcoAgro), was isolated in 2009 in France from bread wheat and was chosen for its high level of pathogenicity on cv. Alixan (Mejri et al., 2018). It was kept at -80 °C in cryopreservation tubes containing 50 % glycerol mixed with YES medium (Yeast Extract Sucrose). The fungus was grown on PDA medium (Potato Dextrose Agar) in Petri dishes incubated in growth chambers programmed as for plant growth, i.e., Pd: 8 h/16 h with 16 °C at night and 20 °C at day and RH: 70 %. After five days of incubation, fungal spores were collected by washing the cultures with 10 mL of sterile distilled water. The concentration of spore suspension was adjusted to 1×10^6 spores.mL⁻¹ and supplemented with 0.05 % of Tween 20 (Sigma Aldrich, USA). Then, 5-week-old plants were inoculated by spraying this spore suspension (1.33 mL/plant) on aerial parts, and control plants were sprayed with distilled water containing 0.05 % of Tween 20, all plants being at the same development stage with an emerging fifth leaf. All treated plants were immediately put into transparent and hermetic boxes to ensure 100 % RH. The boxes were then placed into growth chambers for three days to maintain a water-saturated atmosphere suitable for optimal *Z. tritici* spore germination.

2.3. Experimental design

Thirty pots of five wheat plantlets each, 15 of them inoculated and 15 non-inoculated with the commercial mycorrhizal inoculum *F. mosseae*, were grown in controlled conditions (Fig. 1). Among them, 24 pots (12 with and 12 without *F. mosseae*) were used after 5 weeks of culture for leaf inoculation with the pathogenic fungus *Z. tritici*. Leaves and roots were sampled 2 days post-infection (dpi) by *Z. tritici* for gene expression and metabolomic analyses. For each analysis, three different pots containing five plants each were harvested according to the experimental conditions, i.e., non-mycorrhizal and non-*Z. tritici*-inoculated plants (NM-NI), non-mycorrhizal and *Z. tritici*-inoculated plants (NM-I), mycorrhizal and non-*Z. tritici*-inoculated plants (M-NI), and finally, mycorrhizal and *Z. tritici*-inoculated plants (M-I). The 18 lasting pots (nine pots containing wheat plantlets inoculated only with *Z. tritici* and nine pots of wheat plantlets inoculated with both *F. mosseae* and *Z. tritici*) were kept in the growth chamber for the evaluation of symptoms at 18 days post-infection, corresponding to the end of the experiment. All roots of the five plants per pot were also harvested at 2 and 18 dpi to determine root mycorrhizal rates.

2.4. Estimation of disease severity

The symptoms of the disease were evaluated 18 dpi by estimating the necrotic and chlorotic leaf areas, and among them, the necrotic areas bearing pycnidia. The measurement of chlorotic and necrotic areas was realized by image analysis using Image J software (Abramoff et al., 2004; Schneider et al., 2012) on the fourth leaf of 45 wheat plants per condition. For all conditions, leaves were scanned on their adaxial faces. Firstly, the RGB (Red Green Blue) color values determining the color of non-infected scanned leaves were measured and recorded. Secondly, the RGB color values of non-infected plants were subtracted from the RGB color measured for *Z. tritici* infected plants, resulting in the elimination of healthy parts of the leaf and allowing only visible leaf necrotic and chlorotic areas. Percentages of necrotic and chlorotic areas per leaf were calculated on the basis of total leaf areas and used to calculate the protection rate as follows: [(Average of percentages of necrotic and chlorotic areas per leaf of NM-I wheat plants - Average of percentages of necrotic and chlorotic areas per leaf of M-I wheat plants) / Average of percentages of necrotic and chlorotic areas per leaf of NM-I wheat plants] x 100. The presence of pycnidia was evaluated macroscopically with a magnifying glass in the necrotic parts of symptomatic leaves and expressed as a percentage of necrotic areas bearing pycnidia.

2.5. Determination of root mycorrhizal rate

Wheat roots were sampled 2 and 18 dpi, corresponding to 5- and 8-week-old plants, respectively. The structures related to AMFs root colonization (arbuscules, vesicles, and hyphae) were observed on Trypan blue-stained roots with an optical microscope, using a protocol adapted from Phillips and Hayman (1970). Briefly, after sampling, roots were washed with distilled water to remove adhering substrate, followed by a step of clearing in KOH 10 % solution (v/v) at 70 °C for 1 h, and finally stained in a 0.05 % Trypan Blue solution (0.5 g of Trypan Blue powder dissolved in 1 L of a water, glycerol, and lactic acid mixture - 1:1:1, v/v/v) at 70 °C for 30 min. The stained root fragments were stored in a water/glycerol (1:1, v/v) solution until observation. As described by Mustafa et al. (2017), a total of 135 root fragments of 1 cm coming from 15 plants in three different pots were examined by optical microscope (x40 magnification, Nikon microscope, Champigny-sur-Marne, France) for each experimental condition. Root mycorrhizal rate was evaluated using the magnified intersections method (Mcgonigles et al., 1990), and three intersections per root fragment were examined.

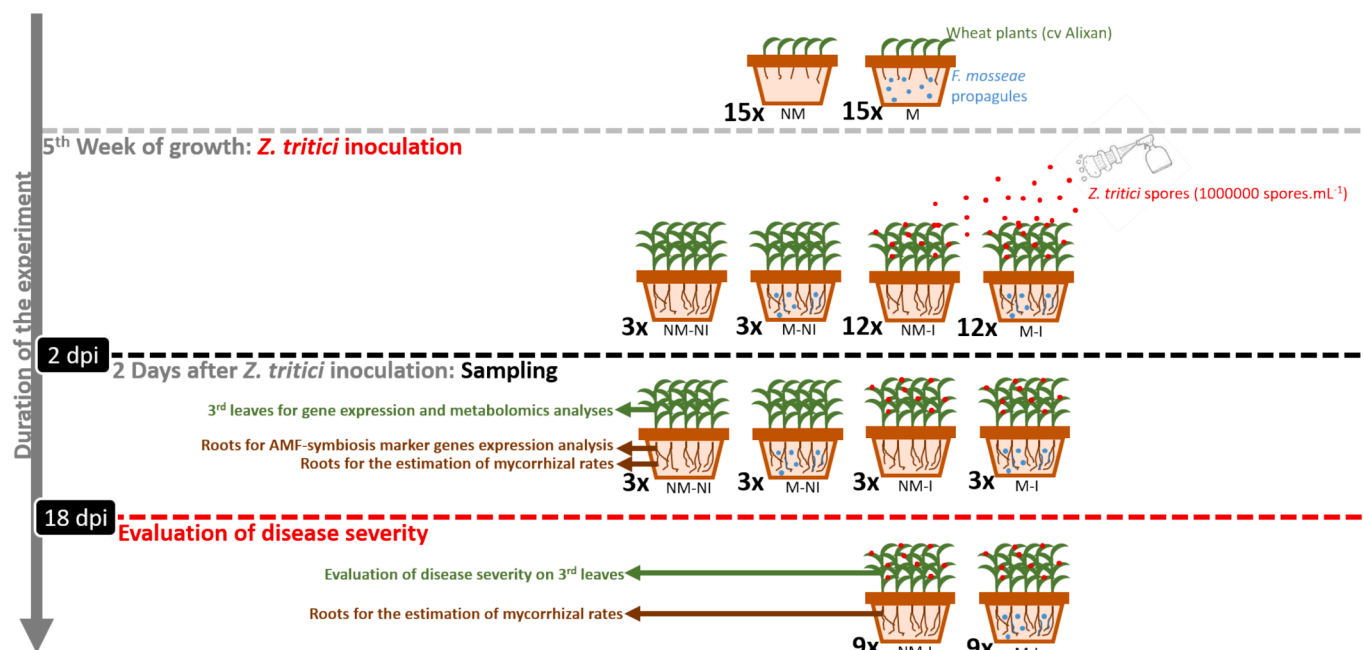


Fig. 1. Experiment design and samplings used to study the impact of *F. mosseae* inoculation on wheat plant defense against *Z. tritici* infection. Roots of wheat plantlets were first inoculated with *F. mosseae*, and then, leaves were inoculated with the pathogenic fungus *Z. tritici* at the 5th week of plant growth, resulting in 4 treatment conditions named: 1/ NM-NI (Non-Mycorrhizal and Non-*Z. tritici*-Inoculated plants); 2/ M-NI (Mycorrhizal and Non-*Z. tritici*-Inoculated plants); 3/ NM-I (Non-Mycorrhizal and *Z. tritici*-Inoculated plants); 4/ M-I (Mycorrhizal and *Z. tritici*-Inoculated plants).

2.6. Evaluation of plant growth parameters

Plant growth parameters were evaluated on 59-day-old mycorrhizal and non-mycorrhizal wheat plants. Plant length, stem number, and dry weight were noted for each plant for both 45 non-mycorrhizal and mycorrhizal plants. Aerial parts were sampled and dried in an oven at 60 °C for 72 h until they reached a constant weight to determine the dry biomass per plant.

2.7. Expression analysis of wheat defense genes and marker genes of arbuscular mycorrhizal symbiosis

The analysis of plant defense-related gene expression was carried out on the third leaf, whereas marker gene expressions of arbuscular mycorrhization were monitored in root tissues, all tissues being sampled from wheat plants at two dpi.

2.7.1. RNA extraction

Total RNA was isolated from powdered wheat leaves using a protocol adapted from Chang et al. (1993) and from powdered wheat roots using the QIAGEN RNeasy mini kit (Qiagen, Hilden, Germany).

For leaf tissues, 1 g of frozen powder was vigorously shaken with an aliquot of 1 mL of extraction buffer, containing 2 % CTAB (hexadecyltrimethylammonium bromide), 2 % PVP (polyvinylpyrrolidone K29-32), 100 mM Tris HCl (pH 8.0), 25 mM EDTA (ethylene-diamine-tetraacetic acid), 2 M NaCl, and 3 % β -mercaptoethanol, warmed at 65 °C for 15 min. Two successive extractions of nucleic acids were then performed, adding one equal volume of chloroform/isoamyl alcohol (24:1) solution and separating phases by centrifugation at 16,100 RCF at 4 °C for 20 min. To precipitate the RNA, 250 μ L of lithium chloride (10 M) were added to the recovered supernatant and placed at 4 °C overnight. After centrifugation at 16,100 RCF at 4 °C for 20 min, RNA pellets were washed twice with 500 μ L of frozen 70 % ethanol and resuspended into 25 μ L of RNase-free water (Qiagen, Hilden, Germany). DNase treatments were next performed by adding 5 μ L of a buffer containing 10X DNase buffer, RQ1 RNase-Free DNase, and RNasine® plus RNase Inhibitor (3:1:1, v/v/v; Promega, USA) for 10 min at room temperature. After

incubation, 400 μ L of a mixture of 10 mM Tris HCl (pH 8.0), 1 mM EDTA (pH 8.0), 1 M NaCl, and 0.5 % SDS were added to the sample. After vigorous mixing, RNA purification was done by supplementing the solution with one equal volume of chloroform/isoamyl alcohol (24:1) and separating the phases by centrifugation at 16,100 RCF at 4 °C for 10 min. The aqueous phase was recovered, added to two volumes of 100 % ethanol, and then put at -20 °C for 2 h to precipitate RNA. After a centrifugation of 45 min at 16,100 RCF and 4 °C, the pellet containing the RNA precipitates was washed four times with 500 μ L of 70 % frozen ethanol, dried for alcohol evaporation, and re-suspended in 30 μ L of RNase-free water (Qiagen, Hilden, Germany).

For root tissues, total RNA was isolated from 100 mg of grounded tissues using the QIAGEN RNeasy mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with the RNase-Free DNase treatment (Qiagen, Hilden, Germany) to remove genomic DNA contaminants and five supplemental steps of washing with RPE buffer (Qiagen, Hilden, Germany). At the end, total RNA was eluted with 50 μ L of RNase-free water (Qiagen, Hilden, Germany).

RNA (leaves and roots) quality was checked by running all RNA extracts on TAE Agarose gel 1 %, and the RNA concentration of each sample was quantified by measuring the absorbance at 260 nm following the manufacturer's instructions for the biophotometer (Eppendorf, Hambourg, Germany).

2.7.2. Real-time RT-PCR analysis of gene expression

In total, 1 μ g of total RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific – ancient ref. Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The expression level of wheat genes was monitored by quantitative PCR using primers targeting marker genes of arbuscular mycorrhizal symbiosis (only for root samples) or using the biomolecular tool targeting defense genes (only for leaf samples) described by Brisset and Duge de Bernonville, (2011). According to the manufacturer's protocol, amplification reactions were performed in a Biorad CFX Connect (Biorad, Hercules, CA, USA) using the SYBR SsoAdvanced Universal Green Supermix (Biorad, Hercules, CA, USA) with a thermal profile programmed as follows: 15 s at 95 °C

(denaturation) and 1 min at 60 °C (annealing/extension) for 40 cycles. Melting curve analysis was achieved from 65 to 95 °C at 0.5 °C/s, and amplification specificity was checked by melting peak visualization. Gene expression analysis focused on 9 wheat genes involved in arbuscular mycorrhizal symbiosis listed in Table S1 and on 24 wheat genes related to defense mechanisms listed in Table S2. Primers used for quantitative real-time RT-PCR (Tables S1 and S2) were from Fiorilli et al. (2018), Brisset and Duge de Bernonville (2011), or specially defined for this study by using the software Oligo Explorer (<https://www.genelink.com/tools/gl-oe.asp>). Internal reference genes *Tubβ*, *GAPDH*, and *Actin* for leaves (qPFD® tool, Brisset and Duge de Bernonville, 2011) and *ATPAAA*, *GAPDH*, and *Actin* for roots (Table S1) were used for normalization. Relative gene expression in leaf or root tissues was obtained by comparing gene expression of non-mycorrhizal and *Z. tritici*-inoculated, mycorrhizal and non-*Z. tritici*-inoculated, or mycorrhizal and *Z. tritici*-inoculated plants to that corresponding to non-mycorrhizal and non-*Z. tritici*-inoculated plants according to the $2^{(-\Delta\Delta Ct)}$ method described by Schmittgen and Livak (2008). Values of gene expression are the average of gene expressions in third leaves or root fragments coming from three distinct pots (biological replicates) containing five plants each with three technical repetitions per gene studied (technical replicates).

2.8. Metabolomic analysis

The metabolomic analysis was carried out on samples harvested at 2 dpi. Third leaves and root tissues were frozen in liquid nitrogen to be stored at -80 °C and freeze-dried for one day. Metabolite extraction was performed on ground freeze-dried plant material using 25 µL of methanol per mg dry weight. Metabolite extraction and metabolic profiling using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry were performed as described by Patel et al. (2022) with some modifications. The liquid chromatography system was coupled to an Exploris 120 Q-Orbitrap MS system (Thermo Scientific), operated with a heated electrospray ionization source in positive ion mode. The key parameters were as follows: spray voltage, 3.0 kV; nitrogen sheath gas flow rate, 40 arbitrary units (arb. unit); auxiliary gas flow rate, 10 arb. units; sweep; capillary temperature, 320 °C and auxiliary gas heater temperature, 320 °C. The scan modes were full MS with a resolution of 60 000 FWHM (at m/z 200) and the scan range was m/z 85–1200. The MS 2 spectra were obtained from data dependent acquisition (DDA) mode by the higher-energy collisional dissociation (HCD) technique with trigger ions from the inclusion ions list (targeted ions). This inclusion list was built by predicted compounds consistent with ions issued from the spectrum obtained by in-source fragmentation. The parameters of DDA were resolution, 30,000; isolation window, 0.5 m/z ; normalized collision energy, 15 %. Metabolite identifications were based on expertized analysis of corresponding mass spectra (MS1 and MS2) and comparison with published reports in the literature. Derivatives of blumenols, i.e., blumenin, blumenol C rhamnosyl-glucoside (blumenolC GlcRha), 11-hydroxyblumenol C Glucuronyl-Glucoside (11OHblumenolC GlcGlcA), 11-hydroxyblumenol C Rhamnosyl-Glucoside (11OHblumenolC GlcRha), blumenol A Glucoside (blumenolA Glc), and 11-carboxy-blumenol C pentoside (11carboxy-blumenolC pentose), were detected and identified on the basis of Wang et al. (2018), Schliemann et al. (2006), Liu et al. (1999) and Hill et al. (2018) (Table S3, and see additional note in Supp Data). Additional information was retrieved from the KEGG and PubChem databases. The identity of some metabolites was confirmed using the corresponding standards provided by Sigma-Aldrich (France), and a relative quantification of the selected metabolites was performed using the software Xcalibur (Thermo Fisher Scientific, Waltham, MA, USA).

2.9. Statistical analysis

Data used to evaluate disease severity were expressed as the mean

value \pm SE of nine different pots with five plants per condition ($n = 45$). Student's *t*-test and Mann-Whitney rank sum test were used to detect differences on leaf symptoms and plant growth parameters (plant length, stem number, and dry weight); Kruskal-Wallis one-way ANOVA and Tukey test were used to determine root mycorrhizal rate subsequently to arcsine transformation of data; Mann-Whitney rank sum test and Kruskal-Wallis one-way ANOVA, and Tukey test were used to estimate AMF symbiosis-related genes expression in the roots and defense-related gene expression in the leaves, respectively; whereas *t*-test, one-way ANOVA, and Tukey test were used to assess metabolite content in roots (pool of roots from three pots of five plants each, $n = 3$) and leaves (pool of leaves from three pots of five plants each, $n = 3$) using SIGMAPLOT from SPSS (San Jose; <https://www.sigmaplot.com>) at the usual probability level lower than 0.05.

For metabolomic analysis heatmaps, the average of significant metabolite contents was evaluated by pairwise comparison between treatments according to Tukey's honest significant difference method, followed by a Benjamini-Hochberg false discovery rate (FDR) correction.

3. Results

3.1. Wheat root colonization by the AMF *F. mosseae*

The establishment of the mycorrhizal symbiosis was evaluated by estimating the root mycorrhizal rate of wheat plants inoculated or not with *Z. tritici* after five weeks (34-day-old wheat plants; 2 dpi) and eight weeks of growth (50-day-old wheat plants; 18 dpi; wheat plants dedicated to STB symptom evaluation). No arbuscular mycorrhizal structures were observed in the roots of all non-mycorrhizal wheat plants (NM-NI). This result confirms the efficacy of mycorrhizal inoculum sterilization. Intraradical hyphae, arbuscules, and vesicles were extensively observed in the roots of mycorrhizal wheat plants. After five weeks of growth, roots of wheat inoculated with *F. mosseae* and inoculated or not with *Z. tritici* (2 dpi) showed a total mycorrhizal rate of 21.0 % (± 2.8 %), including 20.5 % (± 2.6 %) of arbuscules and 0.5 % (± 0.3 %) of vesicles (Table 1). Moreover, the total mycorrhizal rates were higher in the roots of 8-week-old wheat plants (18 dpi) compared to 5-week-old wheat plants (2 dpi), with plants showing a total mycorrhizal rate of 42 % (± 2.4 %), including 37.9 % (± 1.9 %) of arbuscules and 4.1 % (± 0.8 %) of vesicles, at the time of STB symptoms evaluation (Table 1).

3.2. Effects of mycorrhizal inoculation on wheat growth and protection against *Z. tritici*

Plant height, stem number, and dry weight were assessed for 59-day-old wheat plants inoculated or not with the AMF *F. mosseae* to estimate the impact of mycorrhizal inoculation on the early stages of plantlet growth. Both plant height and stem number were unaffected by the mycorrhizal inoculation. However, shoot biomass decreased in mycorrhizal wheat plants by 24 % compared to non-mycorrhizal ones, with dry weight being evaluated at 0.69 g (± 0.03 g) and 0.90 g (± 0.04 g) for mycorrhizal and non-mycorrhizal wheat plants, respectively (Table 2).

To evaluate the efficacy of the AMFs inoculation in reducing the severity of STB symptoms, mycorrhizal and non-mycorrhizal wheat plants were inoculated with *Z. tritici* after five weeks of plant growth, corresponding physiologically to the emergence of the fifth leaf for all plants, i.e., for both mycorrhizal and non-mycorrhizal wheat plants. The chlorotic and necrotic leaf areas, as well as the presence of pycnidia, were assessed on the fourth leaf of each plant at 18 dpi (Figs. 1 and 2). Chlorotic and necrotic leaf areas caused by *Z. tritici* were evaluated at 9 % (± 1 %) and 41 % (± 5 %) of the total leaf area in mycorrhizal and non-mycorrhizal wheat plants, respectively. The protection rate calculated on the basis of these observations reached 78 %. Disease reduction, observed in wheat leaves in response to root mycorrhizal inoculation,

Table 1

Wheat root mycorrhizal rates at 34 and 50 days (corresponding to 2 and 18 dpi, respectively) after inoculation with *F. mosseae*, and protection at 50 days against STB. The data are the means \pm SE of percentages of total root colonization observed on 135 root fragments. Different letters indicate significant differences between 34 and 50-day-old wheat plants ($n = 9$) according to a *t*-test, $P \leq 0.05$.

		34 DAYS (2 dpi)	50 DAYS (18 dpi)
		Time corresponding to molecular and metabolomic analyses	Time corresponding to the disease severity evaluation
ROOT MYCORRHIZAL RATES (%)	TOTAL COLONIZATION RATES	21.0 \pm 2.8 a	42.0 \pm 2.4b
	ARBUSCULAR COLONIZATION RATES	20.5 \pm 2.6 a	37.9 \pm 1.9b
	VESICULAR COLONIZATION RATES	0.5 \pm 0.3 a	4.1 \pm 0.8 a
PROTECTION RATE OBTAINED AGAINST <i>Z. tritici</i>			↓ 78 %

Table 2

Impact of mycorrhizal colonization with *F. mosseae* on plant growth parameters in 59-day-old wheat plants (cv. Alixan). The data are the means \pm SE of plant height, stem number, and dry weight measured on 45 mycorrhizal and non-mycorrhizal wheat plants ($n = 45$). Different letters indicate significant differences between treatments, i.e., mycorrhizal and non-mycorrhizal wheat plants, according to the *t*-test and Mann-Whitney rank sum test, $P \leq 0.05$.

	Non-mycorrhizal wheat plants	Mycorrhizal wheat plants
PLANT HEIGHT (cm)	38.7 \pm 0.6 a	39.8 \pm 0.6 a
STEM NUMBER	4,5 \pm 0.2 a	4,4 \pm 0.2 a
DRY WEIGHT (g/plant)	0.90 \pm 0.04b	0.69 \pm 0.03 a

was also confirmed by measuring the percentage of chlorotic and necrotic area-bearing pycnidia, which were 17 (± 4 %) and 29 % (± 4 %) in mycorrhizal plants and non-mycorrhizal ones, respectively, corresponding to a reduction of 41 % of pycnidial area.

3.3. Induction of AMF symbiosis-related genes in the roots of mycorrhizal wheat plants

To check the mycorrhizal symbiosis functioning, the relative expression of eight marker genes involved in nutrient exchanges was monitored in the roots of non-mycorrhizal and mycorrhizal wheat plants five weeks after inoculation with the AMF.

Four genes encoding nutrient transporters and channels were up-regulated in the roots of mycorrhizal plants in comparison with the roots of non-mycorrhizal plants (Fig. 3). In detail, *AMT3.1*, encoding an ammonium transporter, was strongly overexpressed by 125-fold in mycorrhizal plants. A significant accumulation of transcripts of *Kchan* and *PT11* encoding a potassium channel and a phosphate transporter, was also clearly detected in the roots of mycorrhizal plants, with an amount corresponding to 32-fold and 18-fold (Fig. 3) of the control ones, respectively. Finally, a slight up-regulation (2-fold) of *Sult*, encoding a sulfate transporter, was detected in the roots of mycorrhizal plants compared to the control. Among targeted genes encoding for lipid and carbohydrate transporters, only *ABC*, encoding an ABC-type transporter, was specifically up-regulated in the mycorrhizal roots, with *ABC* being overexpressed by 18-fold in comparison to the control (Fig. 3).

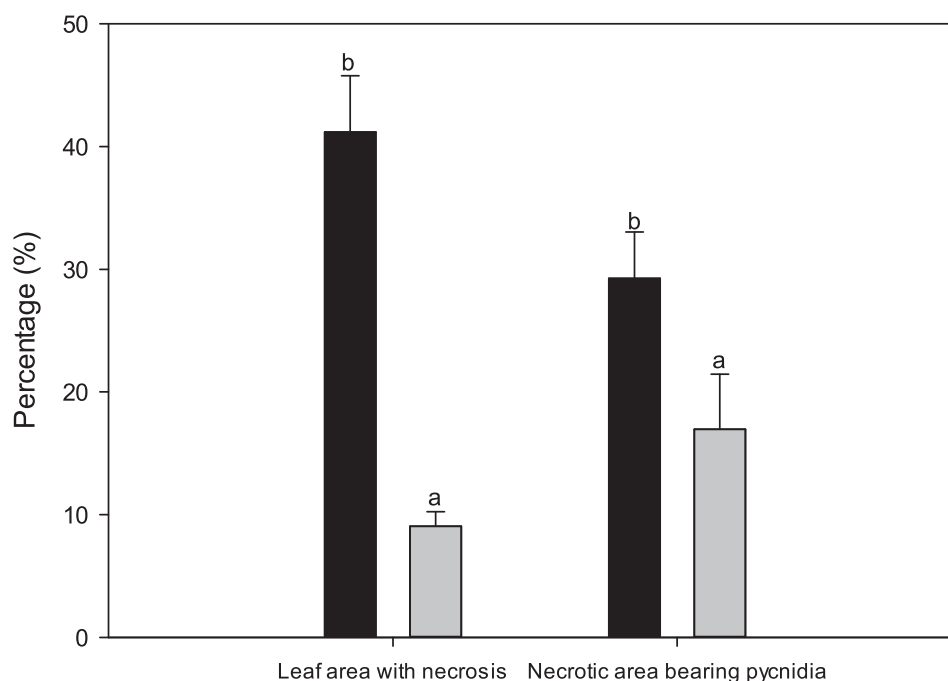


Fig. 2. Disease severity level on the fourth leaf of non-mycorrhizal (in black) or mycorrhizal (in grey) wheat plants inoculated with *Z. tritici* (18 dpi). Vertical bars indicate the mean value \pm SE obtained for 45 plants ($n = 45$), and different letters indicate a statistical difference between non-mycorrhizal and mycorrhizal *Z. tritici*-inoculated wheat plants according to the Mann-Whitney rank sum test, $P \leq 0.05$.

TARGETED PROTEIN	GENE	EXPRESSION		Scale bar
		GENE	LEVEL	
AMMONIUM TRANSPORTER 3 MEMBER 1	AMT3.1		125,41*	> 33
PHOSPHATE TRANSPORTER	PT11		18*	9-17
HIGH-AFFINITY SULFATE TRANSPORTER 2	Sult		2,20*	5-9
POTASSIUM CHANNEL	Kchan		31,74*	3-5
ABC-2 TYPE TRANSPORTER	ABC		17,91*	2-3
SUCROSE TRANSPORTER-LIKE PROTEINS	SUT2.1		0,76	0,5-2
SWEET TRANSPORTERS	SWEETB		1,99	0,4-0,5
CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE LIKE	CCamK1		1,27	0,3-0,4
				< 0,3

Fig. 3. Expression profile of marker genes related to arbuscular mycorrhizal symbiosis functioning in roots of mycorrhizal wheat plants, measured by quantitative reverse transcription-PCR analysis on three pools of five plants ($n = 3$), five weeks after inoculation with the AMF *F. mosseae*. Results are normalized with three internal reference genes (*ATPAAA*, *GAPDH*, and *Actin*), and the eight marker genes studied are listed in Table S1. Gene expression fold changes are given by shades of red or blue according to the scale bar. Significant data were marked with an asterisk ($n = 3$, Mann-Whitney rank sum test with $P \leq 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Effect of *F. mosseae* root inoculation on defense-related gene expression patterns in leaf tissues

For a better understanding of mechanisms involved in wheat protection observed against *Z. tritici* infection in mycorrhizal plants, a set of 24 plant defense-related genes was monitored in the leaves of 5-week-old non-mycorrhizal and mycorrhizal wheat plants inoculated or not with *Z. tritici*, at 2 dpi. The relative expression of defense-related genes was evaluated in non-mycorrhizal and *Z. tritici*-inoculated (NM-I), mycorrhizal and non-*Z. tritici*-inoculated (M-NI), or mycorrhizal and *Z. tritici*-inoculated (M-I) wheat plants compared to non-mycorrhizal and non-*Z. tritici*-inoculated (NM-NI) wheat plants and reported on a heatmap profile (Fig. 4). In non-infected conditions, a modulation in transcript accumulation was observed for the Pathogenesis-Related protein 1 (*PR1*) and the antioxidant peroxidase (*POX*) encoding genes in the leaves of mycorrhizal wheat plants by comparison to the control. Thus, the gene expression analysis highlighted the up-regulation of *PR1* (by 10-fold) and *POX* (by 51-fold).

The *Z. tritici* infection of non-mycorrhizal wheat plants also showed the up-regulation of the *PR1* (42-fold) and *POX* (122-fold). Nevertheless, *FAR* was strongly down-regulated by 0.16-fold, and five other defense-related genes were solely up-regulated by the infection with *Z. tritici*. These genes are coding for pathogenesis-related proteins (*PR5*, 11-fold) or are involved in the phenylpropanoid pathway (*PAL*, 9-fold) in antioxidant systems (*GST*, 2-fold), parietal compound biosynthesis (*CAD*, 4-fold and *CalS*, 5-fold). However, among the four defense-related genes studied in the phenylpropanoid pathway, three of them, i.e., *CHS* (chalcone synthase), *FNS* (flavone synthase), and *PPO* (polyphenol oxidase), were strongly down-regulated in response to *Z. tritici* infection in non-mycorrhizal plants, with values ranging from 0.06 to 0.18-fold.

Interestingly, this down-regulation of *FAR* and genes belonging to the phenylpropanoid pathway, observed in non-mycorrhizal wheat plants under *Z. tritici* attack, appeared to be less important in mycorrhizal ones, with only *FNS* firmly down-regulated by 0.15-fold. As noticed in infected wheat plants, the same set of defense-related genes, comprising *PR1* (39-fold), *PR5* (17-fold), *PAL* (6-fold), *POX* (110-fold), and *CalS* (4-fold), was up-regulated in mycorrhizal wheat plants infected with *Z. tritici*. In contrast to these genes, *PR4* (hevein-like protein) was significantly induced (59-fold) only in the leaves of mycorrhizal-infected wheat plants.

Briefly, each defense gene can be clustered according to the defense gene expression pattern obtained in leaves and reported in the heatmap in Fig. 4. Indeed, *PR1* and *POX* were up-regulated in leaves, whether it was in mycorrhizal and/or infected wheat plants. In contrast, *PR5*, *PAL*, *GST*, *CalS*, and *CAD* were up-regulated only in infected wheat leaves.

TARGETED DEFENSE PATHWAYS	GENE	EXPRESSION			Scale bar
		M-NI	M-I	NM-I	
PATHOGENESIS RELATED PROTEINS	PR1	9,68 ^b	39,29 ^a	41,78 ^a	> 33
	PR2	1,16	1,13	1,64	17-33
	PR4	24,51 ^{ab}	58,92 ^a	15,98 ^{ab}	9-17
	PR5	8,36 ^{ab}	16,95 ^a	10,62 ^a	5-9
	PR8	1,71	2,24	1,60	3-5
	PR15	1,72	2,68	5,39	2-3
PHENYLPROPANOID PATHWAY	PAL	3,64 ^{ab}	6,63 ^b	8,67 ^b	0,5-2
	CHS	1,16 ^a	0,54 ^a	0,15 ^b	0,4-0,5
	FNS	0,70 ^{ab}	0,15 ^{bc}	0,06 ^c	0,3-0,4
	PPO	1,55 ^a	0,86 ^{ab}	0,18 ^b	< 0,3
ISOPRENOID PATHWAY	HMGR	2,84	2,54	5,85	
	FPPS	1,65	1,01	0,75	
	FAR	0,98 ^a	0,49 ^{ab}	0,16 ^b	
ANTIOXIDANT SYSTEMS	APOX	1,04	1,40	1,83	
	GST	1,24 ^{ab}	0,90 ^a	2,06 ^b	
	POX	50,65 ^b	110,36 ^b	122,05 ^b	
	WRKY	5,32	3,35	6,17	
PARIETAL COMPOUNDS	CalS	2,88 ^{ab}	3,58 ^b	4,62 ^b	
	CesA	1,20	1,23	1,51	
	CAD	1,73 ^{ab}	1,60 ^a	4,20 ^b	
SALICYLIC ACID SIGNALING	EDS1	1,51	1,90	3,02	
JASMONIC ACID SIGNALING	JAR	2,18	1,39	1,69	
ETHYLEN SIGNALING	ACC3	1,33	0,70	0,68	
	EIN3	1,43	1,23	1,52	

Fig. 4. Defense gene expression profile monitored at 2 dpi in leaves of non-mycorrhizal (NM) or mycorrhizal (M) wheat plants non-inoculated (NI) or inoculated (I) with *Z. tritici* ($n = 3$) measured by quantitative reverse transcription-PCR analysis (qPFD® tool, Brisset and Dugé de Bernonville, 2011). The results were normalized with three internal reference genes (*Tubβ*, *GAPDH*, and *Actin*), and the 24 defense-related genes studied are listed in Table S2. Defense gene expression fold changes are given by shades of red or blue according to the scale bar. The data represent the mean value of three pools of five biological replicates per condition. No significant data were colored in white, and different letters indicate a statistical difference between treatments ($n = 3$, Kruskal-Wallis one-way ANOVA and Tukey test with $P \leq 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Lastly, *CHS*, *FNS*, and *PPO* of the phenylpropanoid pathway were specifically and strongly down-regulated in infected wheat leaves, as well as *FAR* of the isoprenoid pathway, whereas *PR4* was the only gene to be found up-regulated uniquely in mycorrhizal and infected wheat plants.

3.5. Effect of *F. mosseae* inoculation on metabolite accumulation in the leaf and root tissues of wheat

Concomitantly with gene expression analysis, metabolomic analyses were performed on root and leaf tissues. On the basis of peak area measurements for selected metabolites, significant differences in metabolite contents were evaluated by pairwise comparison between two treatments (i.e., non-mycorrhizal and mycorrhizal wheat plants) in roots and between four treatments (i.e., non-mycorrhizal and non-*Z. tritici*-inoculated, non-mycorrhizal and *Z. tritici*-inoculated, mycorrhizal and non-*Z. tritici*-inoculated, and mycorrhizal and *Z. tritici*-inoculated wheat plants) in leaves. Then, 63 metabolites in roots and 61 metabolites in leaves were reported on a heatmap for both roots (Fig. S1) and leaves (Fig. S2), with a scale ranging from red for a high content to blue for a low content.

3.5.1. In roots

To investigate the impact of mycorrhizal symbiosis on root metabolites, metabolomic analysis was achieved on the roots of 5-week-old mycorrhizal and non-mycorrhizal wheat plants. Differential analysis between mycorrhizal and non-mycorrhizal wheat roots shows that only six blumenol derivatives (blumenin, blumenol GlcRha, 11OHblumenol GlcGlcA, 11OHblumenol GlcRha, blumenolA Glc, and 11carboxy-blumenolC pentose) were detected in a larger amount in mycorrhizal wheat plants (i.e., 3-, 33-, 25-, 32-, 5-, 110-, and 60-fold, respectively) than in non-mycorrhizal wheat plants (Fig. 5). It is worth noting that primary metabolite contents were unaltered by mycorrhizal symbiosis (Figs. S1 and S2).

3.5.2. In leaves

Metabolomic analysis was carried out on leaves 2 dpi in order to monitor the early response of mycorrhizal wheat plants, in comparison to non-mycorrhizal ones, under infectious conditions (*Z. tritici* attack). Only three categories of metabolites, including amino acids (AA), blumenol derivatives (B), and hormones (H), were differentially accumulated in leaves (Fig. 6a). Among these three categories, four metabolites

were clearly differentially accumulated in wheat leaves according to the treatments applied: the amino acid tryptophan, the methylpiperolic acid, and two blumenol derivatives (11OHblumenolC GlcRha and 11carboxy-blumenolC pentose). The two blumenol derivatives were specifically more accumulated in the leaves of mycorrhizal wheat plants than in the leaves of non-mycorrhizal ones (10-fold and 5-fold more, respectively) (Fig. 6a and 6b). Concerning the amino acid tryptophan, its content was higher in the leaves of infected plants than in non-infected plants by a factor of two, with a slightly higher level in non-mycorrhizal and infected plants (Fig. 6b). Lastly, methylpiperolic acid was clearly higher in infected wheat leaves compared to non-infected ones. Moreover, methylpiperolic acid content was 3-fold higher in non-mycorrhizal-infected plants than in mycorrhizal-infected ones.

4. Discussion

The current research aims to contribute to the development of eco-friendly strategies in plant protection for more sustainable agriculture, in particular to protect the wheat crop against STB. In this frame, previous work showed that root inoculation with the AMF species *F. mosseae* was able to protect wheat in controlled conditions against the biotrophic pathogen fungus *B. graminis* f.sp. *tritici* by inducing wheat defense genes in leaves (Mustafa et al., 2016, 2017). Here, we have investigated the efficacy of *F. mosseae* inoculation to mediate MIR against the hemibiotrophic *Z. tritici* through the induction of plant defense responses in aerial parts.

Our results showed that *F. mosseae* is able to colonize the roots of the bread wheat cv. Alixan, as observed by Mustafa et al. (2016, 2017) with two other cultivars, Orvantis and Lord. Arbuscules are transient fungal structures corresponding to the main exchange sites for nutrient trade-off in root cortical cells at the interface plant/AMF (Wipf et al., 2019). The strong arbuscular rates observed in our experimental conditions indicate an extensive exchange taking place between the AMF *F. mosseae* and the wheat plant cv. Alixan. It is generally assumed that mycorrhizal inoculation improves plant growth (Al-Karaki et al., 2004; Mohammadi et al., 2011; Mitra et al., 2019). However, in our experimental

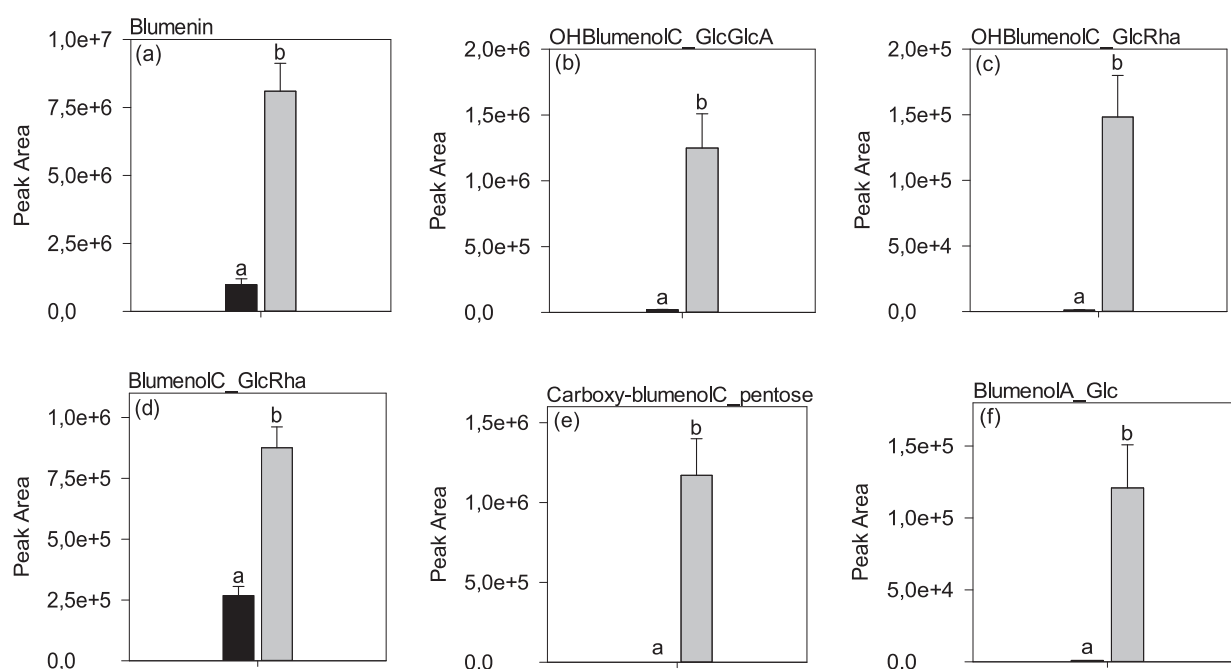


Fig. 5. Effect of *F. mosseae* inoculation on blumenol derivative contents in wheat roots identified in the heatmap, i.e., (a) Blumenin, (b) OH Blumenol C.GlcGlcA, (c) OH Blumenol C.Glc, (d) BlumenolC.GlcRha, (e) Carboxy-blumenol C.pentose, and (f) BlumenolA.Glc. The data represent the mean value \pm SE of three pools of five biological replicates per condition ($n = 3$, t test with $P \leq 0.05$). Bars in black or gray color represent, respectively, the mean values obtained in the roots of wheat plants, non-inoculated or inoculated with the AMFs.

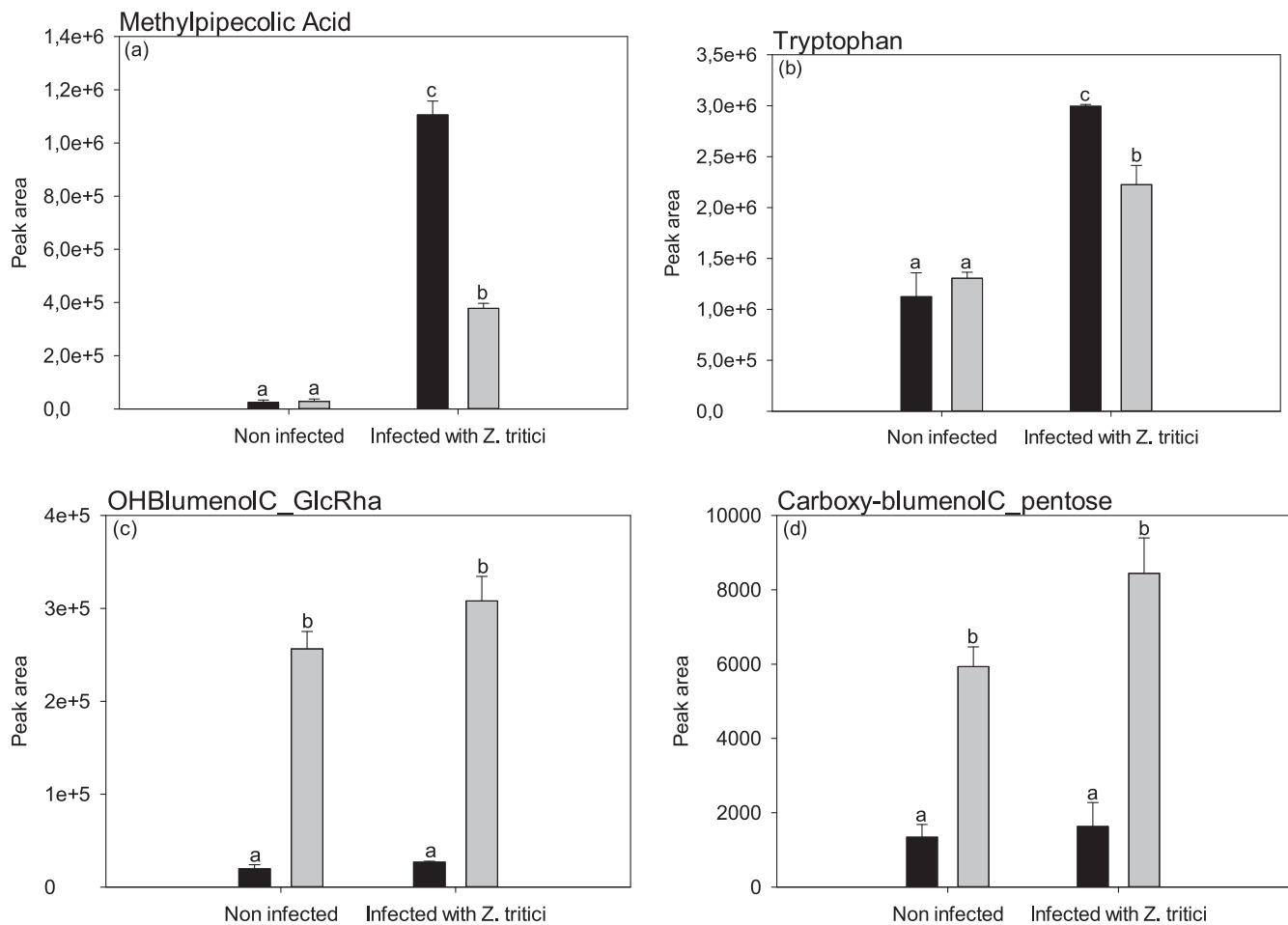


Fig. 6. Effects of *Z. tritici* infection in third leaves of wheat plants inoculated with *F. mosseae* (bars in gray color) or not (bars in black color) on the content of metabolites identified in the heatmap, i.e., (a) methylpipercolic acid, (b) tryptophan, and two blumenol derivatives ((c) OHblumenol C_GlcRha and (d) carboxy-blumenol C pentose). The data represent the mean value \pm SE of three pools of five biological replicates per condition ($n = 3$, ANOVA and Tukey test with $P \leq 0.05$).

conditions, no increase in the primary metabolite content of roots or leaves was detected in 34-day-old mycorrhizal wheat plants. At the same time point, the roots of mycorrhizal wheat plants exhibited a strong up-regulation of the gene encoding an ABC transporter, assuming an important export of lipid C from the host plant to the AMFs (Wang et al., 2017). Later, a decrease in shoot biomass after 59 days of culture was measured, whereas no alteration was detected concerning plant length or stem numbers. Previous studies showed that different wheat cultivars can exhibit variable growth responses to mycorrhizal inoculation (García de Leon et al., 2020). For instance, the shoot biomass was improved in *T. aestivum* cv. Chinese Spring inoculated with *F. mosseae* (Fiorilli et al., 2018), whereas no gain was observed in the wheat cultivars Skyfall, Avalon, and Cadenza when inoculated with the AMF species *R. irregularis* (Elliott et al., 2020). A high mycorrhizal rate could also lead to an imbalance in the mutualistic relationship (Smith and Read, 1997), for instance, impacting the plant growth-defense tradeoffs by redirecting carbohydrates for the plant defense to the detriment of growth (Huot et al., 2014). Nevertheless, mycorrhizae improve nutrient trafficking. In mycorrhizal wheat roots, the up-regulation of ABC goes along with a strong up-regulation of *PT11* encoding a phosphate transporter. Indeed, it has been hypothesized that interchanges in C and P between the AMF and its host plant were driven by P (Maeda et al., 2006; Fitter, 2006; Javot et al., 2007), suggesting that the supply of plant lipid C to the AMF depends on the AMF's ability in delivering P to the host plant (Wipf et al., 2019). Similarly, genes encoding an ammonium transporter (*AMT3.1*) and a K transporter (*Kchan*) were strongly up-regulated in mycorrhizal wheat roots. The inducible ammonium

transporter (*AMT3.1*) has been described as the main plant transporter involved in N transfer from the AMF to host plants in sorghum (Koegel et al., 2017). Furthermore, the inducible K transporter (*Kchan*) has been linked to an increase in K uptake by tomato plants during mycorrhizal symbiosis with the AMF *R. irregularis* (Liu et al., 2019). The concomitant increases in *PT11*, *AMT3.1*, and *Kchan* expression during the mycorrhizal symbiosis in wheat corroborated that N and K were closely related to P in nutrient exchanges during the AMF symbiosis.

After five weeks of culture, the root colonization by *F. mosseae* also imbalanced six blumenol derivatives (blumenin, blumenol C_GlcRha, 11OHblumenol C_GlcGlcA, 11OHblumenol C_GlcRha, blumenol A_Glc, and 11-carboxy-blumenol C pentose), which were specifically detected in higher amounts in mycorrhizal wheat plants than in non-mycorrhizal ones. Blumenol derivatives are apocarotenoids (from the carotenoid pathway), also named C13 cyclohexenone derivatives. Apocarotenoids accumulation in roots is specifically linked to the establishment and maintenance of the mutualistic symbiosis occurring between the AMF and its host plant (Peipp et al., 1997; Schliemann et al., 2008; Fiorilli et al., 2019; Kaur and Suseela, 2020). However, among the three major types of blumenols, i.e., blumenol A, B, and C, only blumenol C derivatives respond clearly to AMF symbiosis in plants (Wang et al., 2018; Fiorilli et al., 2019). Thus, it has been observed a continuous accumulation of two cyclohexenone derivatives in barley roots (Peipp et al., 1997), seven C 13 cyclohexenone blumenol structures in *Senecio jacobaea* (Hill et al., 2018), and blumenol C 9-*O*-(2'-D ~-glucuronosyl)-~-glucoside (Blumenin) in four cereals, including *Hordeum vulgare* (barley), *T. aestivum* (wheat), *Secale cereale* (rye), and *Avena sativa* (oat)

(Maier et al., 1995), when they were inoculated with *R. irregularis* (formerly *G. intraradices*). Interestingly, in our experimental conditions, among the most differential blumenol derivatives found in mycorrhizal roots, the 11carboxy-blumenolC pentose and the 11OHblumenolC GlcRha have also been detected in leaves, suggesting a root-to-shoot transport of these apocarotenoids through the xylem sap flow. These two blumenol C derivatives have already been described as being carried via the crude sap to the aerial parts of a broad range of mono- and dicotyledonous plants, such as *Nicotiana attenuate*, *Solanum lycopersicum*, *S. tuberosum*, *H. vulgare*, *T. aestivum*, *Medicago truncatula*, and *Brachypodium distachyon*, inoculated with *R. irregularis* (Wang et al., 2018). These AMF-dependent blumenol C derivatives found in leaves were positively correlated to root fungal colonization (Fester et al., 1999; Hill et al., 2018; Kaur and Suseela, 2020) and were proposed as markers of mycorrhizal symbiosis (Wang et al., 2018; Mindt et al., 2019). Our results demonstrated that a functioning mutualistic symbiosis takes place between *F. mosseae* and the bread wheat cv. Alixan in our experimental conditions.

High levels of Blumenol C produced on behalf of AMF symbiosis are suspected to have antifungal activities and to participate in plant defense (Kadam et al., 2020). Indeed, it was reported that blumenol C was found in high quantity in the essential oil with antibacterial activity from *Premna microphylla* Turczaninow (Zhang et al., 2017) or found to be involved in detoxification processes in *Palicourea luxurians* (Kornpointner et al., 2022). A very limited set of metabolites have been reported to be consistently altered in shoots of mycorrhizal plants in the absence of stress (Hill et al., 2018), and, to our knowledge, no specific compounds coming from mycorrhizal roots to induce defense-related genes in shoots have still been clearly identified. However, it has been hypothesized that increases in blumenol content, under control of AMF, act as systemic signals mediating a reprogramming of several processes in leaves, such as those involved in plant defenses (Wang et al., 2018; Kaur and Suseela, 2020) and, as depicted here, in wheat inoculated with *F. mosseae*. Indeed, mycorrhizal wheat plants showed a leaf protection rate of 78 % at 18 dpi compared to non-mycorrhizal ones when facing STB disease. We also observed an up-regulation of two defense-related genes (*PR1* and *POX*) in leaves consecutively to the AMF symbiosis establishment without any pathogen attack, pointing out its systemic effect on plant defense-related genes in the aerial parts, as described during MIR (Pozo and Azcón-Aguilar, 2007; Cameron et al., 2013). These observations seem to result rather in a systemic elicitation than in a priming of plant defense mechanisms. This is consistent with findings reported by Mustafa et al. (2017), stating that a systemic induction of *PAL*, *POX*, *CH11* (encoding chitinase 1, equivalent to PR8), and *NPR1* (encoding the non-expressor of pathogenesis-related proteins 1, involved in hormone signaling) was observed in leaves of mycorrhizal wheat cv. Orvantis in non-infectious conditions. This is consistent with our results of defense-related gene expression monitoring in leaves of mycorrhizal wheat, reporting an up-regulation of the *PR1* SA-dependent marker (Gruner et al., 2013), encoding a protein with antimicrobial properties (Breen et al., 2017). Concomitantly, *POX*, encoding a peroxidase involved in cell detoxification, was also up-regulated in the leaves of wheat inoculated with *F. mosseae*. Among defense-related genes induced by *F. mosseae* in healthy wheat plants, *PR1* and *POX* were also induced by *Z. tritici* in leaves of non-mycorrhizal plants, pointing out a common gene induction in leaves triggered by *Z. tritici* infection or by *F. mosseae* root inoculation. In addition, *GST*, encoding the Glutathione S-transferase involved in detoxication processes; *PR5*, encoding the thaumatin-like and classified as a partly-dependent SA gene (Gruner et al., 2013), and *CalS*, encoding a callose synthase and contributing to strengthen the cell wall, were also up-regulated in infected leaves of wheat plants, suggesting an induction of the defense responses against any penetration attempt of the pathogenic fungus. Interestingly, some defense-related genes involved in the phenylpropanoid pathway were strongly down-regulated two days after *Z. tritici*-inoculation, such as *CHS*, encoding a chalcone synthase; *FNS*, encoding a flavone synthase;

and *PPO*, encoding a polyphenol oxidase, mainly known to produce pigments taking part in plant development and defense. They belong to the flavonoid pathway, sharing the first steps with the general phenylpropanoid pathway under the control of *PAL* (Liu et al., 2021). Another pathway, the monolignol pathway, with *CAD* acting at its final steps in producing lignins, shares the same first steps of the general phenylpropanoid pathway (Sundin et al., 2014; Liu et al., 2021). In infected plants, while there is a down-regulation of all three targeted genes of the flavonoid pathway, i.e., *CHS*, *FNS*, and *PPO*, an overexpression of *PAL* and *CAD* was noticed, suggesting a redirection of the general phenylpropanoid pathway in detriment of the flavonoid pathway to promote the monolignol pathway in producing lignins, thus reinforcing the cell wall and restricting *Z. tritici* spread. In agreement, Schenke et al. (2011) have observed a strong suppression of flavonoids production, in particular a strong down-regulation of *CHS* (Schenke et al., 2019), when *Arabidopsis thaliana* was treated by the bacterial elicitor flg22, mimicking a pathogen attack. This was explained by a shift in flavonoid production in favor of pathogen defense compound production. In addition, we cannot exclude that the strong down-regulation of *CHS*, *FNS*, and *PPO* occurring in the susceptible wheat cv. Alixan infected by *Z. tritici* could be the result of the pathogen fungus itself, possibly by releasing some effectors, to suppress defense mechanisms promoted by the flavonoid pathway (Mirzadi Gohari et al., 2015; Seybold et al., 2020). In a same way, *FAR*, which is a plant terpene synthase gene encoding the (E,E)-alpha-farnesene synthase and playing an essential role in defense against nematodes and insects (Lin et al., 2017), was also strongly down-regulated in the presence of the pathogenic fungus. However, this down-regulation of genes involved in the phenylpropanoid and isoprenoid pathways was clearly alleviated in mycorrhizal wheat. AMF inoculation probably allows maintaining plant fitness during pathogenic infection.

The expression of defense genes promoted by *F. mosseae* and also induced by *Z. tritici*, as well as the mitigation of the impact of *Z. tritici* on the phenylpropanoid pathway in mycorrhizal wheat plants, provide insights into the capacity of mycorrhizal symbiosis to help plants in fighting a pathogenic attack. However, on the panel of defense genes studied, no additional genes were upregulated than those already induced in mycorrhizal or infected wheat plants when mycorrhizal wheat plants endure *Z. tritici* infection, except for *PR4*. Taking all this together, our data suggest that AMF protective effects seem to rely mainly on the AMF capacity to induce plant defense-related genes prior to a future pathogen attack. Therefore, when infected with *Z. tritici*, the wheat inoculated with *F. mosseae* showed fewer symptoms on leaves compared to non-mycorrhizal wheat plants at 18 dpi. Indeed, pycnidia and leaf necrosis were drastically reduced in the leaves of mycorrhizal wheat plants. In addition, tryptophan was in higher content in infected leaves, with a greater amount in the leaves of the non-mycorrhizal wheat plants than the mycorrhizal ones. Tryptophan is an aromatic amino acid involved in plant tolerance to biotic and abiotic stresses (Yang et al., 2020; Sadak and Ramadan, 2021). Its accumulation is relative to the plant stress level due to its role as the precursor of melatonin, a signal molecule that regulates plant responses to environmental stresses (Sadak and Ramadan, 2021) and triggers the metabolic pathways of oxidative stress (Queiroz et al., 2023; Hussain et al., 2024). As observed for tryptophan, foliar levels of methylpipercolic acid were found to be related to the level of STB symptoms on leaves. Methylpipercolic acid is a derivated form of pipercolic acid known to be the putative precursor of the osmolyte homostachydrine, also called pipercolic acid betaine (Servillo et al., 2016). Homostachydrine is a betaine-derived osmolyte that helps to maintain protein integrity by scavenging reactive oxygen species and contributes to controlling osmotic homeostasis in plants under environmental stresses (Iqbal and Nazar, 2015). Pipercolic acid is also reported to play a crucial role in cellular redox homeostasis (Wang et al., 2021). The pipercolic acid pathway was probably used to produce the osmolyte homostachydrine (Servillo et al., 2016) to help plants overcome stress.

Depending on the plant and AMF species studied, some molecules were detected as signals coming from mycorrhizal roots and leading to plant defense coordination in shoots when infected by a pathogen. Thus, Sanmartín et al., 2020 identified oxylipins and the lignan yatein as signal molecules produced in the roots of tomato plants mycorrhized with *R. irregularis* and translocated to the shoot to induce plant defense against *Botrytis cinerea*. Still in tomato plants, Rivero et al., 2021 revealed that root inoculation with *F. mosseae* allowed the priming of the accumulation of defensive compounds in shoots, principally related to alkaloids, fatty acid derivatives, and phenylpropanoid-polyamine conjugates, leading to the repulsion of the leaf-chewer *Spodoptera exigua* in the damaged area of the leaves. In citrus plants mycorrhized with *R. irregularis* and facing the spider mite *Tetranychus urticae*, the reduction of damage noticed in infested leaves was correlated to a local accumulation of defensive compounds, such as coumaric acid and diconiferyl alcohol, linked to the priming of JA-dependent responses occurring in mycorrhizal plants (Manresa-Grao et al., 2022). Unlike these observations made on tomato or citrus plants, we did not observe any specific metabolite signal coming from mycorrhizal roots to shoots of the cv Alixan wheat plants in response to the pathogen attack. Despite this, the mycorrhizal wheat plants showed better resistance to STB disease, with leaves exhibiting fewer symptoms and fewer tryptophan and methylpicoelic acid contents.

The inoculation of the common wheat cv. Alixan by *F. mosseae* improved wheat resistance to STB by promoting the induction of a specific defense-related gene profile in non-infectious conditions. Hence, this mutualistic symbiosis arms the wheat plant against possible foliar diseases by a systemic elicitation of defenses. In contrast to a foliar application of biocontrol products, root inoculation with AMFs allows maintaining a long-term effect by eliciting continuously plant defense-related genes (Genre et al., 2005, 2008). Thus, continuously penetrating root cortical cells to generate new arbuscules, AMFs seem to elicit *de novo* plant defense-related genes as long as the symbiosis remains active between AMFs and the host plant. As AMFs symbiosis authorizes a long-term plant defense elicitation, it should be advantageous to use plant inoculation with AMFs coupled with foliar biocontrol treatments to amplify the plant defense responses and prevent a pathogen attack when needed. For instance, Abdel-Kader et al. (2023) observed a higher reduction in disease severity using *F. mosseae* in soil treatments coupled to foliar spray with *T. harzianum* or *P. fluorescens*, compared to treatment with only one of these microorganisms, when managing wheat foliar diseases (powdery mildew, STB, and stem rust) under field conditions. By this way, the use of *F. mosseae* to inoculate wheat plants represents a promising biocontrol tool against *Z. tritici*. Moreover, the protective effect could be improved by combining the mycorrhizal inoculation with foliar spraying of resistance inducers such as surfactin (Le Mire et al., 2018; Platel et al., 2023) to promote eco-friendly control of STB disease. In addition, field tests have to be carried out to corroborate the results obtained in our controlled conditions, considering the impact of the natural environment on the mutualistic association in roots and protection against aerial pathogenic fungi.

CRediT authorship contribution statement

Thierry Allario: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Yuko Krzyzaniak:** Methodology. **Maryline Magnin-Robert:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Benoît Tisserant:** Methodology. **Joël Fontaine:** Writing – review & editing. **Pierre-Emmanuel Courty:** Methodology. **Alessandra Maia-Grondard:** Methodology. **Ali Siah:** Methodology. **Raymonde Baltenweck:** Methodology. **Philippe Huguency:** Methodology. **Béatrice Randoux:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization, Project administration. **Anissa Lounès-Hadj Sahraoui:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2025.105729>.

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