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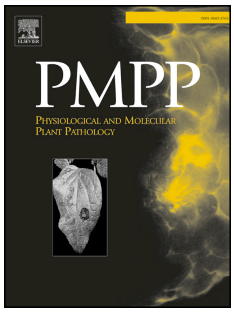

Function of the endophytic fungus *Acrophialophora jodhpurensis*, methionine, and nitric oxide in wheat resistance induction against *Fusarium graminearum* via interplay of reactive oxygen species and iron

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Function of the endophytic fungus *Acrophialophora jodhpurensis*, methionine, and nitric oxide in wheat resistance induction against *Fusarium graminearum* via interplay of reactive oxygen species and iron

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Abstract

In this study, the effect of *Acrophialophora jodhpurensis*, methionine, and sodium nitroprusside (as a NO donor) in induction of resistance against *Fusarium graminearum*, as a hemibiotrophic phytopathogenic fungus causing wheat root and crown rot, was investigated *in vivo*. Also, the effect of *A. jodhpurensis* was investigated on the mycelial growth, spore germination and hyphal structures of *F. graminearum in vitro.* The obtained results showed that *A. jodhpurensis* reduced mycelial growth and spore germination of the pathogen. Also, the spores and hyphae of the pathogen were swollen and deformed in the presence of *A. jodhpurensis*. Wheat root colonization by *A. jodhpurensis* and foliar application of methionine and sodium nitroprusside reduced the disease severity of *F. graminearum* and increased the plant growth parameters, such as shoot and root weight compared to the controls. Application of *A. jodhpurensis*, methionine and sodium nitroprusside had a significant effect in induction of resistance against *F. graminearum* via increasing superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), iron ions, phenolic contents, induction of antioxidant enzymes such as catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities, membrane stability index (MSI) and relative water content (RWC) compared to the plants only inoculated with *F. graminearum* and uninoculated controls. Simultaneous application of *A. jodhpurensis* and sodium nitroprusside was more effective than simultaneous application of *A. jodhpurensis* and methionine. Also, simultaneous application of *A. jodhpurensis*, methionine and sodium nitroprusside treatments showed more effect on the plant growth parameters and resistance induction against the pathogen compared to the other treatments. Therefore, the combined *A. jodhpurensis*,

methionine and sodium nitroprusside treatment can be used for increasing the plant growth parameters and protecting wheat plants against *F. graminearum.*

Keywords: Root and crown rot, *Triticum aestivum*, Growth parameters, Disease index, Defense responses

1. Introduction

The genus *Fusarium* is one of the most important pathogens on numerous agricultural crops including small grain cereals, worldwide (Shahbazi et al. 2021; Zakaria, 2023). The fungus *Fusarium graminearum* is the causal agent of serious diseases on wheat in many cereal-producing regions of the world. This pathogen causes dry rot and necrosis of the crown, root tissue, and basal stem (Chakraborty et al. 2010; Kazan and Gardiner, 2018).

Biocontrol is one of the effective and environmentally safe methods to control destructive phytopathogens, such as *F. graminearum*. In this disease management strategy, beneficial microorganisms protect plants from phytopathogens via direct and indirect mechanisms. Direct interaction is between the biocontrol agent and the pathogen, which consists of several mechanisms such as antibiosis, via secretion of lytic enzymes and production of toxins. But indirect interaction occurs via different mechanisms, including production of phytohormones, phosphate solubilization, stimulating plant secondary metabolites, and induction of resistance, which leads to plant growth promotion and decreases the disease progress in the plant tissues (Legein et al. 2020). The ability of plants to prevent or delay colonization and development of the pathogen can be defined as disease resistance. Several defense mechanisms are involved in resistance of plants under biotic and abiotic stresses,

such as strengthening the cell wall, production of reactive oxygen species (ROS), defense-related enzymes, accumulation of phenolics and lignin, production of antimicrobial metabolites, and activation of hypersensitive response (Llorens et al. 2017; Fontana et al. 2021).

Fungal endophytes live inside the tissues of plants for at least a part of their life cycle without causing disease symptoms. These fungi induce the plant immune system against pathogen attacks (Constantin et al. 2019; Daroodi et al. 2021a, b), induced resistance using endophytic fungi has been investigated against *Fusarium* spp. causing wheat root and crown rot by many researchers. For example pre-inoculation of wheat plants with *Piriformospora indica* induced resistance against *F. graminearum* and *F. culmorum* (Rabiey et al. 2015), and also against *F. pseudograminearum* (Dehghanpour-Farashah et al. 2019b). Also, seed inoculation with the fungal entomopathogen *Metarhizium brunneum* (Jaber, 2018), *Trichoderma* sp. (Kthiri et al. 2020) and *Meyerozyma guilliermondii* (Kthiri et al. 2021) induced wheat resistance against *F. culmorum.*

Methionine is a sulfur-containing amino acid, which has nutritional importance and exists at low levels in plants. Also, it has an important role in the initiation of mRNA translation. In addition, methionine is a fundamental metabolite in plant cells, which indirectly regulates cellular processes. It is the precursor of S-adenosylmethionine (SAM), which SAM participates in primary and secondary metabolism. Therefore, it controls the level of important metabolites including polyamines, ethylene, and biotin (Amir, 2010). The efficiency of methionine in induction of resistance has been investigated against several fungal phytopathogens, such as the downy mildew pathogen *Sclerospora graminicola* in pearl millet (Sarosh et al. 2005), *Plasmopara viticola* infection in grapevine (Boubakri et al.

2013), powdery mildew caused by *Sphaerotheca fuliginea* in cucumber (Kang, 2008), powdery mildew caused by *Oidium neolycopersici* and *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Saito et al. 2017 a, b), and postharvest black spot rot caused by *Alternaria alternata* in jujube fruit (Liu et al. 2022). Also, it enhanced plant tolerance to abiotic stresses, such as wounding in rice (Nakazato et al. 2000), salt in maize (Perveen and Hussain, 2021), and water-deficiency in sunflower (Mehak et al. 2021).

Nitric oxide (NO), also called nitrogen monoxide, is a colorless toxic gas with a small size and a very short half-life (Parankusam et al. 2017; Noorbakhsh and Taheri, 2016). It is formed via oxidation of nitrogen. Nitric oxide has critical chemical signaling functions, and it involves in growth, development, reproduction, biotic and abiotic stress responses, and plant immunity (Keshavarz-Tohid et al. 2016; Sánchez-Vicente et al. 2019). Also, the excessiveness of NO causes nitro-oxidative damage to lipids and proteins and inhibits photosynthetic electron transport and thylakoid ascorbate peroxidase, which leads to plant cell death (Misra et al. 2014). The role of NO was investigated in the induction of resistance against phytopathogens in various plants. For example, against *Botrytis cinerea* in *Nicotiana benthamiana* (Asai and Yoshioka, 2009), *Sclerotinia sclerotiorum* (Perchepied et al. 2010), *Golovinomyces* and *Erysiphe pisi* (Schlicht and Kombrink, 2013) in *Arabidopsis thaliana.* Also, against *Macrophomina phaseolina* in *Corchorus capsularis* (Sarkar et al. 2014), *Rhizoctonia solani* in bean (Keshavarz-Tohid et al. 2016) and tomato (Noorbakhsh and Taheri, 2016), and *Fusarium pseudograminearum* in wheat (Dehghanpour-Farashah et al. 2019b).

Many studies have been reported the synergistic effect of beneficial fungi and resistance inducers in reducing disease severity of phytopathogens and increasing plant growth

parameters. Several reports revealed that simultaneous application of beneficial fungi and resistance inducers had more effect on protecting plants against pathogens and increasing plant growth parameters compared to the single treatments. For example, simultaneous application of sodium nitroprusside **(**SNP), polyamines and *Piriformospora indica* against *Fusarium pseudograminearum* (Dehghanpour-Farashah et al., 2019b), zinc sulfate, thiamine and *P. indica* against *Rhizoctonia solani* (Kheyri and Taheri, 2021), thiamine and *P. indica* against *Rhizoctonia solani* (Kheyri et al. 2022) revealed higher level of protection and defense activation against each pathogen compared to the single treatments.

To our knowledge, induction of resistance using *A. jodhpurensis*, methionine, and sodium nitroprusside **(**SNP; as a NO donor) and the effect of simultaneous application of *A. jodhpurensis*, methionine, and SNP against *F. graminearum* has not been investigated in any pathosystem, till now. Thus, the aims of this study were to (i) investigate the effect of *A. jodhpurensis* on the mycelial growth, spore germination and hyphal structures of *F. graminearum* (ii) determine the possibility of increasing growth parameters and protecting wheat plants against *F. graminearum* using the endophytic fungus *A. jodhpurensis*, methionine, and SNP, (iii) examine the involvement of ROS, iron ions, enzymatic antioxidants, total phenolics, cell membrane stability and relative water content in the induced defense responses of wheat against *F. graminearum*.

2. Materials and methods

2.1. Fungal isolates

In this research, the isolates of *Fusarium graminearum, Acrophialophora jodhpurensis*, and *Serendipita indica* (formerly *Piriformospora indica*) were obtained from culture collection

of Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad in Iran. The isolate of *F. graminearum* was previously isolated from infected wheat plants (Dehghanpour-Farashah et al. 2019a), and the isolate of *A. jodhpurensis,* was obtained from healthy roots of tomato (Daroodi et al. 2022). The isolate of *S. indica* used in this study as a positive control was previously used in our several studies on different pathosystems to investigate its potential in induction of plant defense responses against various fungal pathogens (Dehghanpour-Farasha et al. 2019, Kheyri et al. 2021 and 2022, Nassimi and Taheri 2017).

2.2. Effect of methionine and SNP on the mycelial growth of *F. graminearum* and *A. jodhpurensis*

The inhibitory effect of methionine and SNP on the mycelial growth of *F. graminearum* and *A. jodhpurensis* was investigated. Different concentrations of SNP (100, 150, and 200 µM) and methionine (10 and 20 mg/L) were added to the flasks containing sterilized PDA medium. The PDA medium without these compounds was used as control. Then, the plates were inoculated in the center with 5 mm diameter mycelial plugs taken from 7 days old cultures of *F. graminearum* and *A. jodhpurensis.* The plates were incubated at 28°C, and the colony diameter of *F. graminearum* and *A. jodhpurensis* was recorded before the control plates were completely covered by the mycelia of each fungus. The experiment was repeated three times with three replications for each repetition.

Inhibitory percentage of the compounds on mycelial growth of the fungi was calculated using the following formula (Asran-Amal et al. 2010):

$$L = ((C - T)/C) \times 100$$

In this formula, L is the inhibition of radial mycelial growth, C and T are the colony diameters of the fungi in the control and in presence of the compounds, respectively.

2.3. Effect of *A. jodhpurensis* on the mycelial growth of *F. graminearum*

The inhibitory effect of *A. jodhpurensis* on the mycelial growth of *F. graminearum* was investigated using the dual culture method on PDA medium. Briefly, a 5 mm diameter mycelial plug obtained from 7 days old cultures of *A. jodhpurensis* was cultured on one side of the Petri dish containing PDA. The PDA medium without fungus was used as control. After two days, a 5 mm diameter mycelial plug obtained from 7 days old cultures of *F. graminearum* was cultured on the other side of each Petri dish in the opposite side of the *A. jodhpurensis*. The plates were incubated at 28°C, and the colony diameter of *F. graminearum* was recorded before the control plates were completely covered by the mycelia of *F. graminearum* (S´anchez-Fern´andez et al. 2016). The inhibitory percentage of *A. jodhpurensis* on mycelial growth of *F. graminearum* was calculated as before. The experiment was repeated three times with three replications for each repetition.

2.4. Effect of *A. jodhpurensis* on mycelial structure of *F. graminearum*

The mycelial structure of *F. graminearum* in dual culture with *A. jodhpurensis* was investigated using light microscopy. A thin plug of *F. graminearum* from the edge of colony in dual culture with *A. jodhpurensis* was investigated by an Olympus microscope (BH2, Tokyo, Japon) after 7 days, before the control plates were completely covered with the mycelia of *F. graminearum*. Then, the hyphal staining was carried out using aniline blue as described by Koneman et al. (1978).

2.5. Effect of *A. jodhpurensis* on spore germination of the pathogen

For growth-free supernatant of *A. jodhpurensis* preparation, the fungus *A. jodhpurensis* was cultured on PDA medium at 28°C for 7 days. Then, two mycelial plugs (10 mm × 10 mm) of this fungus were transferred into a flask containing 100 mL potato dextrose broth (PDB) medium. The flasks were incubated on a rotary shaker at 30°C and 150 rpm. After 10 days, the culture was filtered by Whatman filter paper (no. 1) for removing mycelia, then a 0.2 µm pore biological membrane filter was used for sterilizing (Xiao et al. 2013).

For preparing the inoculum of *F. graminearum***,** five grams of wheat straw powder were mixed with 125 mL of tap water in a 250 mL Erlenmeyer flask. The flasks were autoclaved at 121°C for 20 min during three days successively to sterilize completely and inhibit the presence of heat-resistant microorganisms. Then, the flasks were inoculated with three mycelial plugs of *F. graminearum* (one cm^2 diameter) obtained from 7 days old culture of the pathogen and incubated on a rotary shaker at 30 °C and 150 rpm for 7 days (Taheri et al. 2022).

Then, the growth-free supernatant was mixed with spore suspensions of *F. graminearum* (10^5 spores mL^{-1}) at a ratio of 1:1, 2:1 5:1 and 10:1 (v:v) in tubes (Zhang et al. 2022). Also, spore suspension of *F. graminearum* with only sterile water was used as control. The mixtures were kept at 24°C with 90% relative humidity. After 12h, mixtures of *F. graminearum* spore suspensions and the growth-free supernatant of *A. jodhpurensis* (100 µl) were placed on a glass slide. Then, the germination of the spores was evaluated by observation of 100 spores through the light microscope (BH2, Tokyo, Japon). The spore germination (spores mL^{-1}) was examined as a percentage using the following formula (Li et al., 2015):

$$\text{Total germination rate (\%)} = (\text{Germinated spores}/\text{Total spores}) \times 100$$

2.6. Preparation of inoculum

Inoculum of *F. graminearum* was prepared as mentioned above according to the method described by Taheri et al. (2022). Fresh spores were harvested and spore concentration was adjusted to 10^5 spores mL^{-1} using a haemocytometer.

The 1/10-strength potato agar (PA) medium was used in order to induce sporulation of *A. jodhpurensis* (Su et al. 2012). The ascospores were washed using sterile distilled water and spore concentration was adjusted to 10^5 spores mL^{-1} using a haemocytometer.

The spore suspension of *S. indica* was obtained by using the method of Nassimi and Taheri (2017). In this method, the surface of 14 days old PDA plate was flooded using sterile distilled water, and chlamydospores were separated by a small spatula. The chlamydospore concentration was adjusted to 10^5 spores mL^{-1} using a haemocytometer.

2.7. Plant growth conditions and inoculation with *A. jodhpurensis* and *S. indica*

Seeds of wheat cultivar "Falat" were surface sterilized using 1% sodium hypochlorite for 2 min and washed three times using sterile distilled water. For germinating, the sterilized seeds were placed on wet sterilized filter paper in a Petri dish at room temperature. Then, the germinated seeds were transferred to cultivation trays containing sterilized perlite under greenhouse conditions (30 ± 4 °C; 16/8h light/dark photoperiod).

After one week, wheat seedlings were inoculated by immersing the roots of wheat seedlings in *A. jodhpurensis* spore suspension (10^5 spores mL^{-1} containing 0.05% Tween 20). For

positive control, the roots of wheat seedlings were immersed in chlamydospore suspension of *S. indica* (10^5 spores mL^{-1} containing 0.05% Tween 20), also for negative control, the roots of wheat seedlings were immersed in 0.05% Tween 20. Then, the seedlings were planted in the 12 × 10 cm pots containing a mixture of sterilized perlite, farm soil, and sand (1:2:1) and the pots were incubated under greenhouse conditions.

2.8. Detecting *A. jodhpurensis* and *S. indica* colonization in the wheat roots

Wheat roots were investigated using a light microscope for detecting hyphae of *A. jodhpurensis* and *S. indica* at 14 and 21 days after inoculating the beneficial fungi. For this purpose, the plants were removed from the soil and washed under running tap water. Staining of the roots was done using cotton blue. The stained roots were investigated using an Olympus microscope (BH2, Tokyo, Japan) (Vierheilig et al. 1998).

To evaluate wheat root colonization by the beneficial fungi, the hyphae of *A. jodhpurensis* and *S. indica* reisolated from wheat roots. Briefly, the wheat roots were harvested at 14 and 21 days post-inoculation and were washed under running tap water. Then, the roots were treated using 2% sodium hypochlorite (for 2 min) and then 70% ethanol (for 2 min). Then, the roots were washed three times using sterile distilled water and dried in laminar flow, and finally the roots were cut into 1 cm × 1 cm fragments. The root fragments were dried using sterile filter paper and four pieces were placed in each Petri dish (10 cm diameter) containing PDA medium supplemented with 0.05% streptomycin. The Petri dishes were incubated at 28°C for 10 days. Root colonization was determined by counting fungal colonies (Dingle and Mcgee 2003).

2.9. Effect of *A. jodhpurensis,* methionine and a NO donor (SNP) on wheat root and crown rot caused by *F. graminearum*

For investigating effect of *A. jodhpurensis,* methionine, and SNP on progress of the disease caused by *F. graminearum in vivo,* inoculation of wheat plants by *A. jodhpurensis* was done using spore suspension as described above. After 21 days (when the plant roots were colonized by the endophytic fungus very well), the 10 mg L^{-1} concentration of methionine and 100 µM concentration of SNP (which had no inhibitory effect on *A. jodhpurensis* and *F. graminearum* growth *in vitro*) were used for spraying on wheat seedlings. At this time, the stem base and leaf primordial of each seedling were treated with 250 µL of *F. graminearum* spore suspension (10^5 spores mL^{-1} containing 0.05% (v/v) tween 20) (Dehghanpour-Farashah et al. 2019). The inoculated plants were kept in the greenhouse at 90% relative humidity and 22°C temperature.

Disease evaluation was done 21 days after inoculating the pathogen. The plants were carefully pulled out from the pots and washed. Disease severity was determined using the scales described by Fernandez and Chen (2007), including 0= no necrotic lesion; 1 = necrosis 1 to 25%; 2 = necrosis 26 to 50%; 3 = necrosis 51 to 75%; 4 = necrosis longer than 75% and 5 = seedling damping-off. Disease index (DI) was calculated using the following formula (Taheri and Tarighi, 2010):

DI (%) = [sum (frequency × score)]/ [(total number of plants) × (maximal score)] × 100

Each experiment was repeated three times with three replications for each repetition.

2.10. Effect of *A. jodhpurensis,* methionine, and SNP on the plant growth

To evaluate the effect of *A. jodhpurensis*, methionine, and NO donor (SNP) on wheat growth parameters, the wheat seeds were planted and seedlings were inoculated as described before. After 21 days, fresh and dry weights of the seedlings were measured. To investigate the dry weight, the samples were placed in an oven at 70℃ temperature for 48 h. Three replications and three repetitions were maintained for each treatment.

2.11. Histological detection of oxidative burst and iron accumulation

For detecting hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), and iron ions accumulation in wheat seedlings, the leaf samples with different treatments at various time points (0, 6, 12, 24, 48, and 72 hpi) after *F. graminearum* inoculation were stained using 3, 3′-diaminobenzidine (DAB), nitroblue tetrazolium (NBT), and potassium ferrocyanide staining, respectively. Decolorizing the leaf segments was done using alcohol and glycerin (8:2) and accumulation of H_2O_2, $O_2^{\cdot-}$, Fe^{3+}, and Fe^{2+} was evaluated using an Olympus microscope (BH2, Tokyo, Japon). The image J software (http://rsb.info.nih.gov/ij/index.html) was used for quantifying staining intensities. The experiments were performed using three leaf samples for each treatment at each time point and each assay was repeated three times.

Detection of H_2O_2 was done according to the method described by Thordal-Christensen et al. (2002). The leaf samples at various time points after inoculating the pathogen were floated for 75 min in a solution of 1 mg/mL DAB-HCl (DAB: 3,3-diaminobenzidine; pH 3.8). A reddish–- brown polymer was produced by DAB polymerization at the site of H_2O_2 accumulation, which was macroscopically visible and microscopically checked by an Olympus microscope (BH2, Tokyo, Japan).

For detection of $O_2^{\cdot-}$, the leaf samples were submerged for 1 h in a solution of 0.1 mg mL^{-1} nitroblue tetrazolium (NBT) (Sigma, St Louis, MO, USA), in 25 mM Hepes buffer, pH 7.6 at 22°C in the dark. The leaf samples were transferred to distilled water and washed several times to stop the reaction. After decolorizing the leaf segments, a dark blue insoluble formazan compound was produced by the reaction of NBT with $O_2^{\cdot-}$, which was macroscopically visible and microscopically checked by an Olympus microscope (BH2, Tokyo, Japan).

For iron ions staining, the leaf segments were submerged for 24 h in 7 % (w/v) potassium ferrocyanide (for Fe^{3+} detection) or for 48 h in 7 % (w/v) potassium ferrocyanide (for Fe^{2+} detection) in aqueous 3 % (v/v) hydrochloric acid at room temperature (Smith et al. 1997). When potassium ferrocyanide reacted with Fe^{3+} a dark blue precipitate was produced, and in the reaction of potassium ferrocyanide with Fe^{2+} a white precipitate was produced that was converted to blue due to the oxidation by oxygen in the air. The blue precipitates were microscopically checked by an Olympus microscope (BH2, Tokyo, Japan).

2.12. Measurement of cell death

Wheat seedlings were inoculated using *A. jodhpurensis*, methionine, SNP, and the *F. graminearum* as described before. Cell death in the pieces of the plant crown (0.5 g) at 0, 6, 12, 24, 48, and 72 h post *F. graminearum* inoculation (hpi), was evaluated using the Evans blue staining method (Baker and Mock, 1994). Briefly, the pieces of the plant crown (0.5 g) with different treatments were immersed in 0.25 % (w/v) Evans blue solution (Sigma) for 20 min. Pieces of the plant crown were washed well with deionized water to remove unbound

dye. Then, the pieces of the plant crown were homogenized in methanol 50 % (v/v) and SDS 1 % (w/v) solution and were centrifuged at 14,000 g for 15 min. The optical density was determined using a spectrophotometer at 600 nm and cell death was expressed as a percentage, with 100 % corresponding to absorbance of dead crown (boiled for 30 min). At least three replications and three repetitions were used for each treatment.

2.13. Extraction and measurement of total phenolics

Extraction of phenolic contents for each treatment was done at various time points (0, 6, 12, 24, 48, and 72 h) post the pathogen inoculation (hpi) using 80% methanol according to the method described by Seevers and Daly (1970). The leaves of wheat plants (0.2 g) were sampled and homogenized in 3.2 mL of 80% methanol. The mixture was centrifuged at 10000 g at room temperature for 5 min. The supernatant was collected as the phenolics source and total phenol evaluation was done using Foline Ciocalteu reagent. Briefly, the extract (62.5 µL) was added to 62.5 µL of Folin Ciocalteau reagent 50% and 1 mL of distilled water and the solution was kept at 25°C. After 3 min, 125 µL saturated solution of 5% Na_2CO_3 was added and the reaction mixture was incubated at 25°C for 1 h. The optical density of the blue solution was investigated using a spectrophotometer at 725 nm. Gallic acid was used as a standard solution. Then, the results of the analysis were expressed as mg gallic acid equivalent (GAE) g^{-1} fresh weight. At least three replications and three repetitions were used for each treatment (Seevers and Daly, 1970).

2.14. Protein extraction and activity assay of antioxidant enzymes

Wheat plants were treated by *A. jodhpurensis*, methionine, SNP, and the *F. graminearum* as described before. For each treatment, three replications and three repetitions were maintained.

For extraction of total protein, the leaf segments (300 mg) were sampled at 0, 6, 12, 24, 48, and 72 hpi. The leaf samples were ground in liquid nitrogen using mortar and pestle, 3 mL of 100 mM potassium phosphate buffer (pH 6.8) was added, and homogenized thoroughly. Then, the mixture was centrifuged at 14,000 g for 20 min at 4°C, and supernatant was used as enzyme source. For measuring soluble protein concentration, bovine serum albumin (BSA, Sigma) was used as a standard (Bradford, 1976).

Catalase (CAT) activity was assayed by measuring H_2O_2 consumption at 240 nm for 3 min. The reaction mixture included potassium phosphate buffer (100 mM, pH 6.8), H_2O_2 (70 mM), and enzyme extract (10 μL) in a total volume of 1.51 mL (Aebi, 1984).

Activity of guaiacol peroxidase (GPX) was calculated using guaiacol as a hydrogen donor by determining absorbance of the mixture at 470 nm. The reaction mixture included potassium phosphate buffer (100 mM, pH 6.8), guaiacol (10 mM), H_2O_2 (70 mM), and enzyme extract (10 μL) in a total volume of 1.18 mL (Chance and Maehly, 1955).

Activity of ascorbate peroxidase (APX) was determined by measuring ascorbate consumption via determining absorbance of the mixture at 290 nm for 3 min. The reaction mixture contained potassium phosphate buffer (50 mM, pH 7), ascorbate (0.5 mM), H_2O_2 (0.1 mM), and EDTA (0.1 mM) in a total volume of 1 mL (Nakano and Asada, 1981).

The CAT, GPX, and APX activities were expressed as μmol min^{-1} mg^{-1} protein.

Activity of superoxide dismutase (SOD) was assayed by measuring the reduction of nitro blue tetrazolium chloride (NBT) at 560 nm. The reaction mixture contained potassium

phosphate buffer (50 mM, pH 7.8), methionine (13 mM), riboflavin (2 μM), EDTA (0.1 mM), nitro blue tetrazolium (NBT: 75 μM), and enzyme extract (100 μL) in a total volume of 3 mL. The control was prepared without the enzyme extract, but a blank was prepared without NBT and the enzyme extract. The SOD activity was expressed as U SOD mg−1 protein (Sadasivam and Manickam, 1996).

2.15. Estimation of membrane stability index (MSI)

For evaluating the cell membrane stability, pieces of the plant crown (One cm^2) with different treatments were sampled at 21 hpi and washed using distilled water, then placed in test tubes containing 25 mL distilled water at room temperature for 24 h. Electrical conductivity of the solution (EC1) was measured using a conductivity meter (JENWAY, USA). The test tubes were transferred to autoclave at 121˚C for 20 min and after cooling to room temperature, the final electrical conductivity (EC2) was measured by a conductivity meter. The percentage of cell membrane stability index (MSI) was calculated by the following formula Singh et al. (2008):

$$MSI = (\frac{1 - EC1}{EC2}) \times 100$$

2.16. Evaluation of relative water content

For investigating the relative water content, the weight of first leaf segments of wheat plants with different treatments at 21 hpi was measured (fresh weight: FW). Then, the leaf samples were immersed in distilled water and incubated at room temperature for 24 h. Then the

samples were weighted (turgid weight: TW). Finally, the leaf segments were dried in oven at 70°C for 48 h and weighed (DW) (Wheatherley, 1950).

$$RWC = (\frac{FW - DW}{TW - DW}) \times 100$$

2.17. Statistical analysis

The experiments were done in a complete randomized design three times independently with three replications in each repetition. The data were normalized for each experiment using Minitab 17 software. Then, the mean of replications was calculated for each repetition. The means of all data obtained for different treatments at various time points were analyzed by Minitab 17 software using one-way analysis of variance (ANOVA). Also, a two-way factorial design (time and treatment) was used for analyzing defense activities at various time points. And also, the means were separated by Fisher test at the level of $P \leq 0.05$. The presented data for each assay were means (± standard error) of three experiments. All diagrams were drawn using Excel 2013. The image J software (http://rsb.info.nih.gov/ij/index.html) was used for quantifying staining intensities of H_2O_2, $O_2^{\cdot -}$, Fe^{2+}, and Fe^{3+} in the plant cells.

3. Results

3.1. Effect of methionine (Me) and sodium nitroprusside (SNP) on mycelial growth of *F. graminearum* and *A. jodhpurensis*

The results showed that 20 mg L^{-1} concentration of methionine inhibited the mycelial growth of *F. graminearum,* while had no inhibitory effect on *A. jodhpurensis.* Also, 150 and 200 µM

concentrations of SNP inhibited the mycelial growth of *A. jodhpurensis*, whereas only 200 μM concentration of SNP inhibited the mycelial growth of *F. graminearum.* Methionine at concentration of 10 mg L^{-1} and SNP at 100 μM concentration had no inhibitory effect on *A. jodhpurensis* and *F. graminearum* growth. Therefore, 10 mg L^{-1} concentration of methionine and 100 μM concentration of SNP were selected for induction of resistance experiments (Table 1).

3.2. Antagonistic activity of *A. jodhpurensis* against *F. graminearum* in dual culture

The isolate of *A. jodhpurensis* inhibited *in vitro* growth of *F. graminearum* in dual culture on PDA. The inhibitory percentage (IP) of *F. graminearum* growth was 52%. Also, pigment production decreased in hyphae of *F. graminearum* in dual culture by *A. jodhpurensis* on PDA (Fig. 1A and 1B).

3.3. Microscopic observation of *F. graminearum* hyphae affected by *A. jodhpurensis*

Light microscopic analyses of the pathogen hyphae were carried out in dual culture with the antagonistic fungus after 7 days. The results showed that the mycelium formation of *F. graminearum* changed in presence of *A. jodhpurensis*. Deformed, vacuolated, empty, fractured, lysed and swollen hyphae with balloon-shaped cells were observed in the mycelia of *F. graminearum* treated with the antagonistic fungus (Figs 1C and 1D) compared to the controls (Fig 1E).

3.4. Effect of metabolites produced by *A. jodhpurensis* on *F. graminearum* spore germination

The growth-free supernatant of *A. jodhpurensis* reduced the spore germination of *F. graminearum*. The spores of *F. graminearum* in the presence of growth-free supernatant of *A. jodhpurensis* were swollen and malformed (Figs 1F and 1G) compared to the controls, which were normal (Fig 1H). Only 12% of *F. graminearum* spores treated with growth-free supernatant of antagonistic fungus (1:10) produced germ tubes at 12 h, while 85.3% of spores in the control produce germ tubes at 12h. The germination percent of spores in concentrations of 1:1, 1:2, and 1:5 were 66, 49.3 and 34%, respectively.

3.5. Detecting *A. jodhpurensis* and *S. indica* in wheat roots and colonization estimation

Microscopic detection of *A. jodhpurensis* and *S. indica* was done in the inoculated wheat roots using spore suspension of the endophytic fungi at 14 and 21 days post-inoculation (dpi). Intracellular hyphae of *A. jodhpurensis* (Fig. 2A and 2B) and intracellular pear-shaped chlamydospores of *S. indica* (Fig. 2C and 2D) were observed. Also, the colonization percentage of wheat roots by *A. jodhpurensis* and *S. indica* in the roots inoculated using spore suspension was 33.33 and 46.66%, respectively at 14 dpi and was 60 and 63.33%, respectively at 21 dpi (Fig. 2E).

3.6. Effect of *A. jodhpurensis* (Aj), methionine (Me), and sodium nitroprusside (SNP) on wheat root and crown rot caused by *F. graminearum* (Fg)

Investigating the effect of Aj, Me, and SNP treatments showed considerable protection against *F. graminearum* in wheat seedlings compared to the plants without treatments. The MeSNPAj treatment had a higher effect on the reduction of disease severity and the disease index of *F. graminearum* decreased on wheat plants by more than 45% compared to the

controls (without treatment). Also, the FgSi (*Serendipita indica* as positive control) and FgAj treatments had no significant difference in reduction of the disease severity (Fig. 2F).

3.7. Effect of *A. jodhpurensis* (Aj), methionine (Me) and sodium nitroprusside (SNP) on plant growth promotion

Treatment of wheat plants with Aj, Me, and SNP increased plant growth characteristics *in vivo.* The biomass enhancement was obvious in the seedling treated with Aj, Me, and SNP compared to the uninoculated controls. Shoot and root weight increased in the plants treated with Aj, Me, and SNP compared to the controls. The MeSNPAj treatment had a higher effect on plant growth parameters. Also, the shoot fresh weight in FgSi (*Serendipita indica* as positive control) treatment was higher than FgAj, while shoot dry weight and root fresh and dry weights had no significant difference (Figs. 2G, 2H, 2I, 2J).

3.8. Hydrogen peroxide detection

In the Fg and FgMe treatments, an increasing trend of H_2O_2 production was observed until 24 hpi, and then decreased until 48 hpi, followed by an increase of H_2O_2 production from 48 to 72 hpi. In the FgAj treatment, production of H_2O_2 increased until 48 hpi and then decreased until 72 hpi. In the FgSNP treatment, an increasing trend of H_2O_2 production was observed until 6 hpi, but it decreased from 6 hpi to 12 hpi, followed by an increasing status till 24 hpi, and finally decreased until 72 hpi. In the FgMeSNP, FgMeAj, FgSNPAj, FgMeSNPAj and FgSi treatments, H_2O_2 production increased until 24 hpi, and then decreased until 72 hpi. Also, production of H_2O_2 in the FgSi treatment was higher than that of the FgAj treatment at

24 hpi, while in the FgAj treatment was higher than the FgSi treatment at 48 hpi. In the negative control treatment, production of H_2O_2 showed a stationary estate (Figs. 3A and 3B).

3.9. Superoxide detection

In the Fg, FgSNP, and FgMe treatments, production of $O_2^{\cdot-}$ increased until 12 hpi, and then decreased until 24 hpi, followed by an increase from 24 to 72 hpi. In the FgAj, FgMeSNP, FgSNPAj, FgMeSNPAj, and FgSi treatments, an increasing trend of $O_2^{\cdot-}$ production was observed until 12 hpi, and it decreased from 12 hpi to 48 hpi, followed by an increasing status till 72 hpi. In the FgMeAj treatment, an increasing trend of $O_2^{\cdot-}$ production was observed until 12 hpi, then it decreased until 24 hpi, followed by an increase of $O_2^{\cdot-}$ production from 24 to 48 hpi, and finally decreased until 72 hpi. Also, production of $O_2^{\cdot-}$ in both FgSi and FgAj treatments was the same at 12 hpi, while the FgAi treatment was higher than the FgSi treatment from 24 hpi to 72 hpi. In the negative control treatment, production of $O_2^{\cdot-}$ had stationary estate (Figs 4A and 4B).

3.10. Detection of Fe^{3+} and Fe^{2+}

In the FgAj, FgMeAj, FgSNPAj, and FgMeSNP treatments, production of Fe^{3+} increased until 12 hpi, and then decreased until 24 hpi, followed by an increase of Fe^{3+} production from 24 to 72 hpi. In the Fg, FgSNP, FgMe, and FgMeSNP treatments, an increasing trend of Fe^{3+} production was observed until 12 hpi, but it decreased from 12 to 24 hpi, and then an increase of Fe^{3+} production was observed until 48 hpi, finally decreased until 72 hpi. In the FgSi treatment, Fe^{3+} production increased until 12 hpi and then decreased until 24 hpi, followed

by a stationary estate until 72 hpi. Also, the production of Fe^{3+} in both FgSi and FgAj treatments was the same at 12 hpi, while in the FgAi treatment was higher than that of the FgSi treatment from 24 hpi to 72 hpi. The highest Fe^{3+} production was observed in the Fg treatment at 48 hpi. In the negative control treatment, the production of Fe^{3+} had a stationary estate (Figs. 5A and 5B).

In the Fg treatment, production of Fe^{2+} increased until 24 hpi and then decreased until 72 hpi. In the FgAj treatment, an increasing trend of Fe^{2+} production was observed until 6 hpi, but it decreased till 48 hpi, and then an increase of Fe^{2+} production until 72 hpi was observed. In the FgSNP, FgMe, FgSNPAj, and FgMeSNPAj treatments, the production of Fe^{2+} increased until 6 hpi, and then it decreased until 24 hpi, and finally production of Fe^{2+} increased until 72 hpi. The FgMeSNP and FgMeAj treatments had an increasing trend of Fe^{2+} production until 6 hpi, but it decreased until 12 hpi, and then it increased from 12 to 72 hpi. In the FgSi treatment, Fe^{2+} production increased until 6 hpi, followed by a decrease of Fe^{2+} production until 12 hpi was observed, then it increased from 12 to 48 hpi, and finally Fe^{2+} production decreased until 72 hpi. Also, the production of Fe^{2+} in the FgSi and FgAj treatments was at the same level. In the negative control treatment, the production of Fe^{2+} had a stationary estate (Figs 6A and 6B).

3.11. Measurement of cell death

Cell death in the treatments of Fg, FgSNP, FgMe, FgMeSNP, FgMeAj, FgSNPAj, FgMeSNPAj, and FgSi increased until 6 hpi and then decreased until 12 hpi, followed by an increasing status until 48 hpi, and then it decreased until 72 hpi. In the FgAj treatment, cell

death increased until 24 hpi and then it decreased from 24 hpi to 72 hpi. In the negative control treatment, cell death had a stationary estate (Figs 7A).

3.12. Total phenolics quantification

Accumulation of phenolics increased after inoculating wheat plants with *F. graminearum* compared to the non-inoculated plants. Also, significantly higher phenolic contents were observed in the plants treated with methionine, SNP and *A. jodhpurensis*. In the wheat plants only treated with *F. graminearum* (Fg), phenolics increased from 0 to 6 hpi, then decreased until 12 hpi followed by an increasing status until 24 hpi and then decreased until 72 hpi. In the FgMe, FgSNP, FgMeSNP, FgSi, and MeAj treatments increase of phenolic contents was observed from 0 to 6 hpi, then decreased until 12 hpi, followed by an increasing status until 48 hpi and finally decreased until 72 hpi. But in the FgAj treatment, increase of phenolics was observed until 6 hpi and then decreased until 12 hpi, followed by an increasing trend from 12 hpi to 24 hpi and finally decreased until 72 hpi. The FgMeSNPAj and FgSNPAj treatments had an increasing trend of phenolics until 6 hpi, then decreased until 24 hpi followed by an increasing status until 48 hpi, and then both treatments exhibited a decreasing trend until 72 hpi. The highest level of total phenolics belonged to FgMeSNPAj and FgSNPAj treatments at 48 hpi. Also, the total phenolics in the FgSi and FgAj treatments were the same at 6 hpi, but that of the FgSi treatment was higher than the FgAi treatment at 12 hpi, and in the FgAj treatment was higher than the FgSi treatment at 24 hpi. In plants without treatment (the negative control) a stationary estate was observed at the lowest level in all times points examined (Fig. 7B).

3.13. Determination of antioxidant enzymes activity

The CAT activity in the wheat seedlings only inoculated with the pathogen (Fg) increased until 24 hpi, but it decreased until 48 hpi, followed by an increase of the enzyme activity from 48 to 72 hpi. The FgAj treatment caused an increasing trend of CAT activity until 6 hpi and then it decreased until 12 hpi, followed by an increasing status of enzyme activity from 12 to 48 hpi was observed, and then it decreased until 72 hpi. In the FgSNP and FgMeSNP treatments, the CAT activity increased until 48 hpi and then it decreased until 72 hpi. In the FgMe treatment activity of this antioxidant increased until 12 hpi, but it decreased until 72 hpi. In the FgMeAj, and FgMeSNPAj treatments, the enzyme activity increased until 12 hpi, then it decreased until 48 hpi, and finally it increased until 72 hpi. The CAT activity in the FgSNPAj treatment increased until 12 hpi and then it decreased until 24 hpi, followed by an increasing trend of CAT activity from 24 to 72 hpi. The FgSi treatment showed an increasing status until 12 hpi and then it decreased until 24 hpi, followed by an increasing trend of enzyme activity from 24 to 48 hpi was observed, finally it decreased until 72 hpi. Also, the CAT activity in the FgAj treatment was higher than the FgSi treatment at 6 hpi but the FgSi treatment showed higher CAT activity than the FgAi treatment at 12 hpi. In the negative control treatment, the CAT activity had a stationary trend in all time points tested (Fig. 7C). The GPX activity in the Fg and FgMeSNPAj treatments increased until 12 hpi, then it decreased until 24 hpi, followed by an increasing trend of the enzyme activity from 24 to 48 hpi, and finally decreased until 72 hpi. But the FgAj treatment had increasing status until 6 hpi, but it decreased until 48 hpi, followed by an increasing status of GPX activity from 48 to 72 hpi. Also, in the FgSNP and FgMeAj treatments, the GPX activity increased until 6 hpi, then it decreased until 12 hpi, followed by an increasing trend of GPX activity until 48 hpi,

finally it decreased until 72 hpi. In the FgMe treatment, the GPX activity increased until 48 hpi, then it decreased until 72 hpi. The FgMeSNP treatment had an increasing trend until 6 hpi and then it decreased until 24 hpi, followed by an increasing status of GPX activity until 48 hpi, finally it decreased until 72 hpi. In the FgSNPAj treatment, the GPX activity increased until 6 hpi, followed by a decreasing status of enzyme activity from 6 to 12 hpi was observed, then it increased until 72 hpi. In the FgSi treatment, the GPX activity increased until 12 hpi, and then it decreased until 24 hpi, followed by an increasing trend of GPX activity from 24 to 48 hpi, finally it decreased until 72 hpi. Also, the GPX activity in the FgAj treatment was higher than the FgSi treatment at 6 hpi and 72 hpi, while the FgSi treatment was higher than the FgAi treatment at 12 hpi and 48 hpi. In the negative control treatment, activity of this enzyme showed a stationary trend in all time points (Fig. 7D).

The APX activity in the Fg, FgAj, FgSNP, FgMe, FgMeSNP, FgSNPAj, and FgMeSNPAj treatments increased until 12 hpi, but it decreased from 12 hpi to 24 hpi, followed by an increasing trend of enzyme activity until 72 hpi. But the FgMeAj and FgSi treatments had an increasing status of APX activity from 0 hpi to 12 hpi and then decreased until 48 hpi, followed by an increasing trend until 72 hpi. Also, the APX activity in the FgAj treatment was higher than the FgSi treatment at 6, 12 and 72 hpi, while the FgSi treatment was higher than the FgAi treatment at 24 hpi. However, in the negative control plants, considerable changes were not observed in the APX activity (Fig. 7E).

The SOD activity in the Fg and FgMeAj treatments increased until 12 hpi and then decreased from 12 hpi to 24 hpi, followed by an increasing trend of enzyme activity until 48 hpi, and finally it decreased until 72 hpi. But the FgAj, FgSNP, FgMeSNP, and FgSi treatments had an increase of the SOD activity until 12 hpi, but it decreased until 24 hpi, followed by an

increase of SOD activity from 24 to 72 hpi. In the FgMe, and FgSNPAj treatments, an increase of SOD activity was observed until 6 hpi and then decreased until 24 hpi, followed by an increasing status of enzyme activity until 48 hpi, and then it decreased until 72 hpi. Also, in the FgMeSNPAj treatment, the SOD activity increased until 6 hpi, but it decreased until 24 hpi, followed by an increase of enzyme activity until 48 hpi, and finally it decreased until 72 hpi. Also, the SOD activity in the FgSi and FgAj treatments was nearly the same at 6 hpi and 72 hpi, but it was higher in the FgSi treatment compared to the FgAj treatment at 12, 24 and 48 hpi. In the negative control treatment, the SOD activity showed a stationary estate in all time points tested (Fig. 7F).

3.14. Relative water content (RWC) and cell membrane stability index (MSI)

Investigating the effect of *A. jodhpurensis* (Aj), methionine (Me), sodium nitroprusside (SNP), and *F. graminearum* (Fg) on membrane stability index (MSI) and relative water content (RWC) showed that RWC and MSI levels in the plants with Aj, Me and SNP treatments were higher than those of the plants inoculated with Fg alone and negative control plants only treated with water. In this study, *S. indica* (Si) was used as a positive control, which results revealed that the RWC levels in the plants with FgAj, FgMeAj, FgSNPAj, and FgSi treatments had no significant differences. Also, the MSI levels in the plants with FgAj, FgMeAj, and FgSi treatments had no significant differences, but the MSI level in the FgSNPAj treatment was higher than that of other treatments. The FgMeSNPAj and Fg treatments had the highest and the lowest levels of RWC and MSI compared to the other treatments, respectively (Figs. 7G and 7H).

4. Discussion

In this research, the effects of biological and chemical defense activators such as *A. jodhpurensis* (Aj), methionine (Me), and sodium nitroprusside (SNP) were investigated on development of the disease caused by *F. graminearum,* activation of induced resistance and plant growth parameters in wheat seedlings. Dual culture assay showed antagonistic effect of *A. jodhpurensis* against *F. graminearum*. In agreement with our findings, the fungus *A. jodhpurensis* was effective against *Rhizoctonia solani* (Daroodi et al. 2021a), *Alternaria alternata* (Daroodi et al. 2022) and *Fusarium equiseti* (Han et al. 2022) in dual culture. Also, the obtained data indicated that the fungus *A. jodhpurensis* was effective in decreasing spore germination and altering hyphal structures of the pathogen, which these results were similar to our previous studies against *R. solani* (Daroodi et al. 2021a), and *A. alternata* (Daroodi et al. 2022).

The results revealed that the disease development was reduced significantly in the seedlings inoculated with Aj, Me, and SNP treatments, which Aj treatment showed more effect than Me and SNP. Also, the simultaneous application of *A. jodhpurensis* with methionine or sodium nitroprusside treatments showed increasing the plant growth parameters and induced resistance against pathogen higher than single treatments due to the synergistic effect of the beneficial fungus and chemical inducers. The combined *A. jodhpurensis* and sodium nitroprusside treatment was more effective than the combined *A. jodhpurensis* and methionine treatment. And also the simultaneous application of *A. jodhpurensis*, methionine and sodium nitroprusside showed the highest effect on the plant growth parameters and induce resistance against pathogen compared to other treatments. Also, our previous studies

showed that the disease severity of *R. solani* (Daroodi et al. 2021a) and *A. alternata* (Daroodi et al. 2021b; Daroodi et al. 2022) reduced on tomato plants via the Aj application.

Many studies reported the effects of endophytic fungi, Me, and SNP on reducing disease development of various phytopathogens via induced resistance in various plants. For example, effect of *Penicillium citrinum* against Fusarium wilt in banana plantlets (Ting et al. 2012), *Phomopsis liguidambaris* against *Colletotrichum gloeosporioides* in peanut (Zhang et al. 2020), *Piriformospora indica* against *R. solani* in rice (Nassimi and Taheri, 2017), and bean (Kheyri and Taheri, 2021), against *Fusarium pseudograminearum* (Dehghanpour-Farashah et al., 2019b), and *Zymoseptoria tritici* (Ashrafi et al. 2021) in wheat were reported. The Me was effective against *Sclerospora graminicola* in pearl millet (Sarosh et al. 2005), *Plasmopara viticola* in grapevine (Boubakri et al. 2013), *Sphaerotheca fuliginea* in cucumber (Kang, 2008), *Oidium neolycopersici* and *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Saito et al. 2017 a, b), and *Alternaria alternata* in jujube (Liu et al. 2022). Also, the SNP was effective against *Ralstonia solanacearum* in tomato plants (Hong et al. 2013), *R. solani* in tomato (Noorbakhsh and Taheri, 2016), tobacco mosaic virus in tobacco plants (Zhang et al. 2019), and *F. pseudograminearum* in wheat (Dehghanpour-Farashah, et al. 2019b).

Also, the data obtained in this research showed that the Aj, Me, and SNP treatments increased growth parameters in wheat seedlings. Consistent with these findings, our previous studies revealed improvement of growth parameters using application of the endophytic fungus Aj in tomato-*R. solani* (Daroodi et al. 2021a) and tomato-*A. alternata* (Daroodi et al. 2021b; Daroodi et al. 2022) pathosystems. Also, enhancement of plant growth parameters were reported against *R. solani* in rice via application of *P. indica* (Nassimi and Taheri, 2017), in bean using thiamine and *P. indica* (Kheyri and Taheri, 2022), in wheat against *Fusarium*

pseudograminearum using the SNP and *P. indica* (Dehghanpour-Farashah et al., 2019b), and *Byssochlamys spectabilis* in the medicinal plant *Bletilla striata* (Chen et al. 2021). Also, the SNP application improved plant growth parameters in safflower under drought stress (Chavoushi et al. 2020), in soybean (Jabeen et al. 2021), and in lentil (Yasir et al. 2021) under salt stress. Many researchers reported enhancement of growth parameters via application of L-methionine in various plants such as Lettuce (Khan et al. 2019), white shrimp (Wang et al. 2019), cabbage (Haghighi et al. 2020), tomato (Almas et al. 2021), sunflower (Mehak et al. 2021), and wheat (Maqsood et al. 2022).

The current study showed that Aj, Me, and SNP were capable of inducing defense responses in wheat plants against *F. graminearum*. The accumulation of H_2O_2, $O_2^{\cdot-}$, Fe^{2+}, and Fe^{3+}, and production of total phenolics, antioxidative enzymes (including CAT, GPX, APX, and SOD activity), MSI, and RWC increased in response to the host-pathogen interaction. Furthermore, seedling treatment using Aj, Me, and SNP before the *F. graminearum* inoculation primed these defense responses in wheat.

One of the earliest plant defense responses to abiotic and biotic stresses is ROS accumulation (Torres et al., 2006), which causes oxidative stress. Then, the oxidative stress leads to lipid peroxidation, damage to DNA or RNA, protein, and finally cell death (Mishra et al., 2011). Also, ROS plays a key role as a second messenger in cellular processes (Yan et al., 2007). The destructive or signaling role of ROS depends on the balance between ROS production and their scavenging in living cells (Sharma et al., 2012). Enzymatic and non-enzymatic antioxidant systems are involved in induction of plant defense against phytopathogens via scavenging ROS and protecting plant cells from oxidative damage. The production of

defensive H_2O_2 increased in *Pinus sylvestris* root against *Heterobasidion annosum* species (Mucha et al. 2012), in cucumber plants against *A. alternata* (Wang et al. 2020), in tomato seedlings against *Botrytis cinerea* (Asselbergh, 2007), *Fusarium oxysporum* f.sp. *lycopersici* (Mandal et al. 2008) and *R. solani* (Nikraftar et al. 2013). Also, the accumulation of $O_2^{\cdot-}$ increased in potato plants against *Phytophthora infestans* (Chai and Doke, 1987), in rice against *Pyricularia oryzae* (Sekizawa et al. 1990), and in *Pinus sylvestris* root against *Heterobasidion annosum* species (Mucha et al. 2012).

In this study accumulation of H_2O_2 and $O_2^{\cdot-}$ increased in wheat plants treated with Aj, Me and SNP after the pathogen inoculation. In FgMeSNPAj treatment, higher levels of H_2O_2 and $O_2^{\cdot-}$ production were observed compared to the other treatments due to the synergistic effect of the beneficial fungus and chemical inducers. Also, the Aj treatment had more effect on the ROS accumulation than chemical inducers (Me and SNP). Similarly, accumulation of H_2O_2 increased in rice-*R. solani* pathosystem via application of the endophytic fungus *P. indica* (Nassimi and Taheri, 2017), and also in wheat-*Fusarium pseudograminearum* pathosystem using the SNP and *P. indica* (Dehghanpour-Farashah et al., 2019b). The production of $O_2^{\cdot-}$ and H_2O_2 increased in bean-*R. solani* pathosystem via the application of thiamine and *P. indica* (Kheyri et al. 2022), and the production of $O_2^{\cdot-}$ and H_2O_2 increased against *R. solani* (Daroodi et al. 2021a) and *A. alternata* (Daroodi et al. 2021b) in tomato plants inoculated with Aj. Also, the application of methionine improved H_2O_2 contents in sunflower under water deficit conditions (Mehak et al. 2021).

Also, the accumulation of H_2O_2 inhibits the growth of biotrophic pathogens (Glazebrook, 2005). Therefore, the accumulation of ROS can be helpful to reduce progress of the *F.*

graminearum as a hemibiotrophic pathogen at the early time points after attack. While accumulation of ROS could be facilitate colonization and plant infection by this hemibiotrophic pathogen in its necrotrophic phase. Our results showed that H_2O_2 and $O_2^{\cdot-}$ accumulation in plants inoculated with Aj, Me, SNP and Fg had an increasing trend in the wheat tissues at the early time points after pathogen inoculation, when *F. graminearum* is in the biotrophic phase of its life cycle, and this increasing trends in Aj, Me and SNP treatments and pathogen were higher than only Fg treatment. But H_2O_2 and $O_2^{\cdot-}$ accumulation in the plants with Aj, Me and SNP treatments showed a decreasing trend at the later time points, while H_2O_2 and $O_2^{\cdot-}$ accumulation in the plants only inoculated with the pathogen showed an increasing trend at the later time points, which could help the pathogen to develops its necrotrophic phase.

Iron (Fe) is an essential micronutrient for plants and pathogenic fungi. It plays a vital role in growth and development, and it is required for cellular processes like photosynthesis and respiration (Connorton et al. 2017; anchez‐Sanuy et al. 2022). Iron exists in two forms, ferrous (Fe^{2+}) and ferric (Fe^{3+}), in nature. Liu et al. (2007) reported that iron is a central mediator linking three phenomena of localized cell wall oppositions, the oxidative burst, and production of pathogenesis-related proteins. Iron can catalyze dangerous free radicals production via redox cycling (Santos et al. 2019), and the activities of antioxidant enzymes like APX, GPX, CAT, and SOD are related to iron (Das and Roychoudhury, 2014, Taheri, 2022). The Fe^{2+} can react with H_2O_2 during the Fenton reaction and produce harmful hydroxyl radicals (Novo and Parola, 2008). Also, in response to the pathogen attack, Fe^{3+} is deposited in the cell wall, which mediates oxidative stress. Indeed, the secretion of Fe^{3+}

provokes and leads to intracellular iron depletion after a pathogen attack. Finally, intracellular iron depletion promotes the transcription of pathogenesis-related genes in concert with H_2O_2 (Liu et al. 2007). Also, Greenshields et al. (2007) reported that the accumulation of free and reactive Fe^{3+} in the apoplast mediates defensive H_2O_2 production. Also, in this study the levels of ROS production were related to the levels of iron production at the early time points after the pathogen attack. Production of H_2O_2, $O_2^{\cdot-}$, and iron have been investigated in *Pinus sylvestris* root cortical cells infected with *Heterobasidion annosum*, which the obtained results showed that H_2O_2 accumulation was positively correlated with Fe^{2+}, whereas was negatively associated with $O_2^{\cdot-}$ production (Mucha et al. 2012).

Iron equilibrium must be controlled in the living cells (Verbon et al. 2017). The iron ions can delay or increase host defense responses, depending on the virulence strategy of the pathogen. Generally, high levels of iron can facilitate ROS production and initiate hypersensitive response (HR) cell death via ferroptosis or other mechanisms in plants infected with biotrophic pathogens, but high levels of iron can increase susceptibility to necrotrophic pathogens in plants and enhance ROS production and cell death, that would be useful in resistance (Herlihy et al. 2020). Also, Ye et al. (2014) reported that high levels of iron at infection sites can be considered as a critical immune response for many plants, particularly in Poaceae, whereas maize plants under iron deficiency are unable to produce ROS at infection sites of *Colletotrichum*, which correlates with enhanced susceptibility to this hemibiotrophic fungal pathogen. In this research, we observed that resistance to *F. graminearum* in wheat seedlings treated with Aj, Me and SNP is correlated with

accumulation of H_2O_2, iron and $O_2^{\cdot-}$ at the early time points after the pathogen inoculation, when *F. graminearum* is in the biotrophic phase of its life cycle. The levels of iron in the plants only inoculated with the pathogen showed an increasing trend at the later time points, which could be involved in facilitating wheat infection by this hemibiotrophic pathogen at its necrotrophic phase. Consistent with these findings, our previous studies revealed that accumulation of iron increases defensive H_2O_2 production (Daroodi et al. 2021a, b). Also, Kheyri et al (2022) investigated the role of iron in bean-*R. solani* pathosystem using thiamine and *P. indica* pretreatments before the pathogen inoculation. Their results showed that H_2O_2 accumulation was positively correlated with the levels of iron. Many researchers reported that high levels of H_2O_2 in plant cells could be correlated to cell death and cellular defense (Mandal et al. 2008; Taheri et al. 2014). Also, our data showed that the cell death in wheat plants only inoculated with the pathogen was higher than that of the inoculated plants, which were pretreated with Aj, Me and SNP. While, more levels of H_2O_2 accumulation were observed in Aj, Me and SNP treatments compared to the plants only inoculated with the pathogen. These results might be related to higher levels of antioxidant enzymes production in Aj, Me and SNP treatments compared to the plants only inoculated with the pathogen. Similarly, Murgia et al. (2004) reported that overexpression of the H_2O_2-detoxifying enzymes such as ascorbate peroxidase can suppress the cell death induced by H_2O_2. In addition, H_2O_2 might be used in lignification via the involvement of peroxidase, which is previously reported as a main defense mechanism activated by riboflavin treatment in rice-*R. solani* pathosystem (Taheri and Tarighi 2010).

Phenolics are a group of secondary metabolites associated with plant defense against phytopathogens (Taheri and Tarighi, 2011; Nikraftar et al. 2013). These defense compounds act as radical scavengers (Grace, 2005) and diminish the toxic effects of oxidative stress, leading to plant protection under stress conditions (Noctor and Foyer, 1998). Also, phenolics and peroxidases were reported as defense components against *R. solani* (Taheri and Tarighi, 2012; Nikraftar et al. 2013). In this study, Aj, Me, and SNP treatments increased the accumulation of phenolics in wheat plants. More levels of phenolics production were observed in FgMeSNPAj treatment compared to the other treatments used in this study. Also, these results were related to the ROS production. In agreement with this finding, the endophytic fungus Aj increased phenolics accumulation against *R. solani* (Daroodi et al. 2021a), and *A. alternata* (Daroodi et al. 2021b) in tomato plants. Also, other beneficial fungi and chemical defense inducers decreased the disease index of phytopathogens in various plants by phenolics accumulation. Examples of this case include *C. globosum* against *Bipolaris sorokiniana* in wheat (Biswas et al., 2003), and *Cephalosporium maydis* in maize (Elshahawy and Khattab, 2022), *T. atroviride* against *R. solani* in cucumber (Nawrocka et al., 2018), *Epicoccum nigrum* (as an endophyte) against *Pectobacterium carotovora* subsp. *atrosepticum* in potato (Bagy et al., 2019), *P. indica*, SNP and polyamines against *F. pseudograminearum* in wheat (Dehghanpour-Farashah et al., 2019b), and *P. indica*, thiamine, and zinc sulfate against *R. solani* in bean (Kheiry and Taheri, 2021). Also, the accumulation of phenolics was increased via exogenous application of L-methionine in bitter gourd under drought stress (Akram et al. 2020).

Results of the present study showed that the Aj, Me, and SNP treatments increased the activity of antioxidant enzymes such as CAT, GPX, APX, and SOD in wheat seedlings. Also, production of antioxidant enzymes was positively correlated with accumulation of ROS and iron at the early time points after the pathogen attack. Antioxidant enzymes have a key role in basal defense and induced resistance against phytopathogens via scavenging ROS, therefore these enzymes protect plant cells from oxidative damage. Catalase and peroxidase rapidly catalyze decomposition of H_2O_2 into water (Gaetani et al. 1996; Zhang and Kirkham, 1994), but The SOD catalyze decomposition of $O_2^{\cdot -}$ into O_2 and H_2O_2 (Boguszewska et al. 2010). Also, phenolics can be oxidized by peroxidases to form quinines, which are toxic directly for fungal pathogens (Gogoi et al., 2001). Therefore, induction and accumulation of antioxidant enzymes are often related to induction of resistance (Van Loon et al. 1998; Dehghanpour-Farashah et al. 2019b).

Several authors reported that the activity of antioxidant enzymes increased via plant treatment using beneficial fungi and chemical inducers. Increasing CAT and APX activities have been reported using *Trichoderma aureoviride* and *T. hamatum* against *Fusarium solani* in Cassava (da Silva et al., 2016). Increasing GPX activity is reported using *P. indica* against *R. solani* in rice (Nassimi and Taheri, 2017). Also, increasing CAT and GPX activities are demonstrated via *P. indica*, SNP and polyamines treatment against *Fusarium pseudograminearum* in wheat (Dehghanpour-Farashah et al., 2019b)**.** Inducing CAT, GPX, APX, and SOD activities via *P. indica*, thiamine, and zinc sulfate ($ZnSO_4.7H_2O$) treatments against *R. solani* in bean (Kheyri and Taheri, 2021), increasing CAT, GPX, APX, and SOD

activities via application of *A. jodhpurensis* against *R. solani* (Daroodi et al. 2021a) and *A. alternata* (Daroodi et al. 2021b) in tomato plants are also reported.

Accumulation of CAT, SOD and peroxidase (POD) in Lentil under salt stress (Yasir et al. 2021) increased using SNP treatment. Methionine induced activities of SOD, POD and CAT enzymes in sunflower (Mehak et al. 2021) and wheat, both under water deficit conditions (Maqsood et al. 2022). Also, application of L-methionine induced new proteins activities (peroxidase, chitinase and superoxide dismutase isozyme) and increased resistance against *Fusarium oxysporum* f. sp. *lycopersici* in tomato plants (El-Fawy et al. 2021).

The RWC and MSI are physiological characteristics related to plant defense against biotic and abiotic stresses. The RWC is an important indicator for describing water status in plants, which indicates the balance between water supply to the leaf tissue and transpiration rate and is related to the cell volume (Lugojan and Ciulca, 2011). Also, the RWC provides a measurement of water deficiency in the leaves, which may show a degree of stress (Torres et al. 2019). The MSI shows the stress tolerance ability of plant cells (Ahmad et al. 2022) and is considered as one of the main selection indices of stress tolerance in cereals (Tripathy et al. 2000). In this research, the levels of RWC and MSI were increased in plants treated by Aj, Me and SNP compared to the uninoculated plants or the plants only inoculated by the pathogen. Also, higher levels of RWC and MSI were observed in FgMeSNPAj treatment compared to the other treatments, which these results were related to other defense responses in wheat seedlings. Furthermore, the fungus Aj had more effect on the levels of RWC and MSI compared to the chemical inducers (Me and SNP). Similarly, many studies reported enhancement of RWC and MSI using beneficial fungi and/or chemical inducers in various

plants. For instance, the arbuscular mycorrhizal fungus *Glomus clarum* increased RWC in cowpea plants (Abdel-Fattah and Shabana, 2002), mycorrhizal biofertilizers improved RWC and MSI in maize plants (Naghashzadeh, 2014), *P. indica,* SNP and polyamines increased RWC and MSI in wheat (Dehghanpour-Farashah et al. 2019b) and *P. indica*, zinc and thiamine increased these defense-related responses against *R. solani* in bean plants (Kheiry and Taheri, 2021).

In overall, this research demonstrated that the endophytic fungus *A. jodhpurensis* and exogenous application of methionine and SNP are capable of inducing resistance and protecting wheat seedlings against *F. graminearum*. So, application of this beneficial endophytic fungus together with chemical resistance inducers such as methionine and SNP could be used as a novel and effective method for decreasing destructive effects of phytopathogens.

CRediT authorship contribution statement

Zoha Daroodi: Methodology, Resources, Writing - original draft, Formal analysis, Software, Visualization. **Parissa Taheri:** Conceptualization, Data curation, Project administration, Formal analysis, Funding acquisition, Validation, Writing - review & editing.

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Table 1. Effect of methionine and sodium nitroprusside on mycelial growth of *Acrophialophora jodhpurensis* and *Fusarium graminearum*, growth of fungi were investigated when the control Petri dishes were completely covered with mycelium of fungi. Statistical analysis was performed using Minitab 17 software according to Fisher analysis. The presented data for each assay are the means (± standard error) of three experiments.

Treatment	Concentration	Growth inhibition percentage of *A. jodhpurensis*	Growth inhibition percentage of *F. graminearum* (%)
Methionine	10 mg/L	0 c	0 c
Methionine	20 mg/L	0 c	9.69± 0.6 a
Sodium nitroprusside	100 µM	0 c	0 c
Sodium nitroprusside	150 µM	19.39± 1.21 b	0 c
Sodium nitroprusside	200 µM	31.51± 2.64 a	3.03± 3.033 b

Figure caption

Fig. 1. Effect of *Acrophialophora. jodhpurensis* on the mycelial growth, spore germination and hyphal structures of *Fusarium graminearum*. Biocontrol effect of *A. jodhpurensis* in dual culture test on potato dextrose agar medium (A), the colony of *F. graminearum* in control (B), cytoplasm lysis of *F. graminearum* mycelia (C) and deformed and swollen mycelia in dual culture with *A. jodhpurensis* (D), the hyphae of *F. graminearum* in control (E), swollen (F) and malformed (G) spores of *F. graminearum* in the presence of growth-free supernatant of *A. jodhpurensis*, the spore of *F. graminearum* in control (H). Scale bar = 30 μm. Error bars correspond to standard error (SE) of three experiments.

Fig. 2. Detection of *Acrophialophora jodhpurensis* (A and B) and *Serendipita indica* (formerly *Piriformospora indica:* C and D) in colonized roots of wheat at 14 (A and C) and 21 dpi (B and D), the colonization percentage of wheat roots by *A. jodhpurensis* and *S. indica* (E), effect of different treatments, including *Acrophialophora jodhpurensis* (Aj), methionine (Me), and sodium nitroprusside (SNP) on disease index of *Fusarium graminearum* (Fg: F), shoot fresh weight (G), root fresh weight (H), shoot dry weight (I), and root dry weight (J) on wheat seedlings at 21 post-inoculation (dpi) with *F. graminearum*. H: *A. jodhpurensis* hyphae, Ch: chlamydospores of *S. indica*, Fg: *F. graminearum*, FgMe: *F. graminearum* and methionine, FgSNP: *F. graminearum* and sodium nitroprusside, FgAj: *F. graminearum* and *A. jodhpurensis*, FgMeAj: *F. graminearum*, methionine and *A. jodhpurensis*, FgSNPAj: *F. graminearum*, sodium nitroprusside and *A. jodhpurensis*, FgMeSNPAj: *F. graminearum*, methionine, sodium nitroprusside and *A. jodhpurensis*, FgMeSNP: *F. graminearum*, methionine and sodium nitroprusside, FgSi: *F. graminearum* and *Serendipita indica*

(formerly *Piriformospora indica*: as positive control) and negative control: water treatment. Scale bar = 50 μm. Error bars correspond to standard error (SE) of three experiments.

Fig. 3. Detection of H_2O_2 in wheat leaves with different treatments at various hours post inoculation (hpi) by *Fusarium graminearum*. Detecting H_2O_2 by 3, 30-diaminobenzidine (DAB) staining (scale bar = 50 μm) (A) and the staining intensities of H_2O_2 using Image J software (B). Fg: *Fusarium graminearum*, FgMe: *F. graminearum* and methionine, FgSNP: *F. graminearum* and sodium nitroprusside, FgAj: *F. graminearum* and *Acrophialophora jodhpurensis*, FgMeAj: *F. graminearum*, methionine and *A. jodhpurensis*, FgSNPAj: *F. graminearum*, sodium nitroprusside and *A. jodhpurensis*, FgMeSNPAj: *F. graminearum*, methionine, sodium nitroprusside and *A. jodhpurensis*, FgMeSNP: *F. graminearum*, methionine and sodium nitroprusside, FgSi: *F. graminearum* and *Serendipita indica* (formerly *Piriformospora indica*: as positive control) and negative control: water treatment.

Fig. 4. Detection of $O_2^{\cdot-}$ in wheat leaves with different treatments at various hours post inoculation (hpi) by *Fusarium graminearum*. Detecting $O_2^{\cdot-}$ by nitroblue tetrazolium (NBT) staining (scale bar= 50 μm) (A) and the staining intensities of $O_2^{\cdot-}$ using Image J software (B). Fg: *Fusarium graminearum*, FgMe: *F. graminearum* and methionine, FgSNP: *F. graminearum* and sodium nitroprusside, FgAj: *F. graminearum* and *Acrophialophora jodhpurensis*, FgMeAj: *F. graminearum*, methionine and *A. jodhpurensis*, FgSNPAj: *F. graminearum*, sodium nitroprusside and *A. jodhpurensis*, FgMeSNPAj: *F. graminearum*, methionine, sodium nitroprusside and *A. jodhpurensis*, FgMeSNP: *F. graminearum*, methionine and sodium nitroprusside, FgSi: *F. graminearum* and *Serendipita indica* (formerly *Piriformospora indica*: as positive control) and negative control: water treatment.

Fig. 5. Investigation of Fe^{3+} production in wheat leaves with different treatments at various hours post inoculation (hpi) by *Fusarium graminearum.* Fe^{3+} levels were detected by potassium ferrocyanide stainings (A), and the staining intensities of Fe^{3+} using Image J software (B). Scale bar = 50 μm. Error bars correspond to the standard error (SE) of three experiments Fg: *Fusarium graminearum*, FgMe: *F. graminearum* and methionine, FgSNP: *F. graminearum* and sodium nitroprusside, FgAj: *F. graminearum* and *Acrophialophora jodhpurensis,* FgMeAj: *F. graminearum,* methionine and *A. jodhpurensis*, FgSNPAj: *F. graminearum*, sodium nitroprusside and *A. jodhpurensis,* FgMeSNPAj: *F. graminearum*, methionine, sodium nitroprusside and *A. jodhpurensis,* FgMeSNP: *F. graminearum*, methionine and sodium nitroprusside, FgSi: *F. graminearum* and *Serendipita indica* (formerly *Piriformospora indica*: as positive control) and negative control: water treatment.

Fig. 6. Investigation of Fe^{2+} production in wheat leaves with different treatments at various hours post inoculation (hpi) by *Fusarium graminearum.* Fe^{2+} levels were detected by potassium ferrocyanide stainings (A), and the staining intensities of Fe^{2+} using Image J software (B). Scale bar = 50 μm. Error bars correspond to the standard error (SE) of three experiments Fg: *Fusarium graminearum*, FgMe: *F. graminearum* and methionine, FgSNP: *F. graminearum* and sodium nitroprusside, FgAj: *F. graminearum* and *Acrophialophora jodhpurensis,* FgMeAj: *F. graminearum,* methionine and *A. jodhpurensis*, FgSNPAj: *F. graminearum*, sodium nitroprusside and *A. jodhpurensis,* FgMeSNPAj: *F. graminearum*, methionine, sodium nitroprusside and *A. jodhpurensis,* FgMeSNP: *F. graminearum*, methionine and sodium nitroprusside, FgSi: *F. graminearum* and *Serendipita indica* (formerly *Piriformospora indica*: as positive control) and negative control: water treatment.

Fig. 7. Cell death (A), total phenolics (B), catalase (CAT; C), guaiacol peroxidase (GPX; D), ascorbate peroxidase (APX; E) superoxide dismutase (SOD; F) activity, membrane stability index (MSI; G) and relative water content (RWC; H) in wheat seedlings with different treatments at various hours post inoculation (hpi) by *Fusarium graminearum*. Fg: *Fusarium graminearum*, FgMe: *F. graminearum* and methionine, FgSNP: *F. graminearum* and sodium nitroprusside, FgAj: *F. graminearum* and *Acrophialophora jodhpurensis*, FgMeAj: *F. graminearum*, methionine and *A. jodhpurensis*, FgSNPAj: *F. graminearum*, sodium nitroprusside and *A. jodhpurensis*, FgMeSNPAj: *F. graminearum*, methionine, sodium nitroprusside and *A. jodhpurensis*, FgMeSNP: *F. graminearum*, methionine and sodium nitroprusside, FgSi: *F. graminearum* and *Serendipita indica* (formerly *Piriformospora indica*: as positive control) and negative control: water treatment.

Fig. 1.

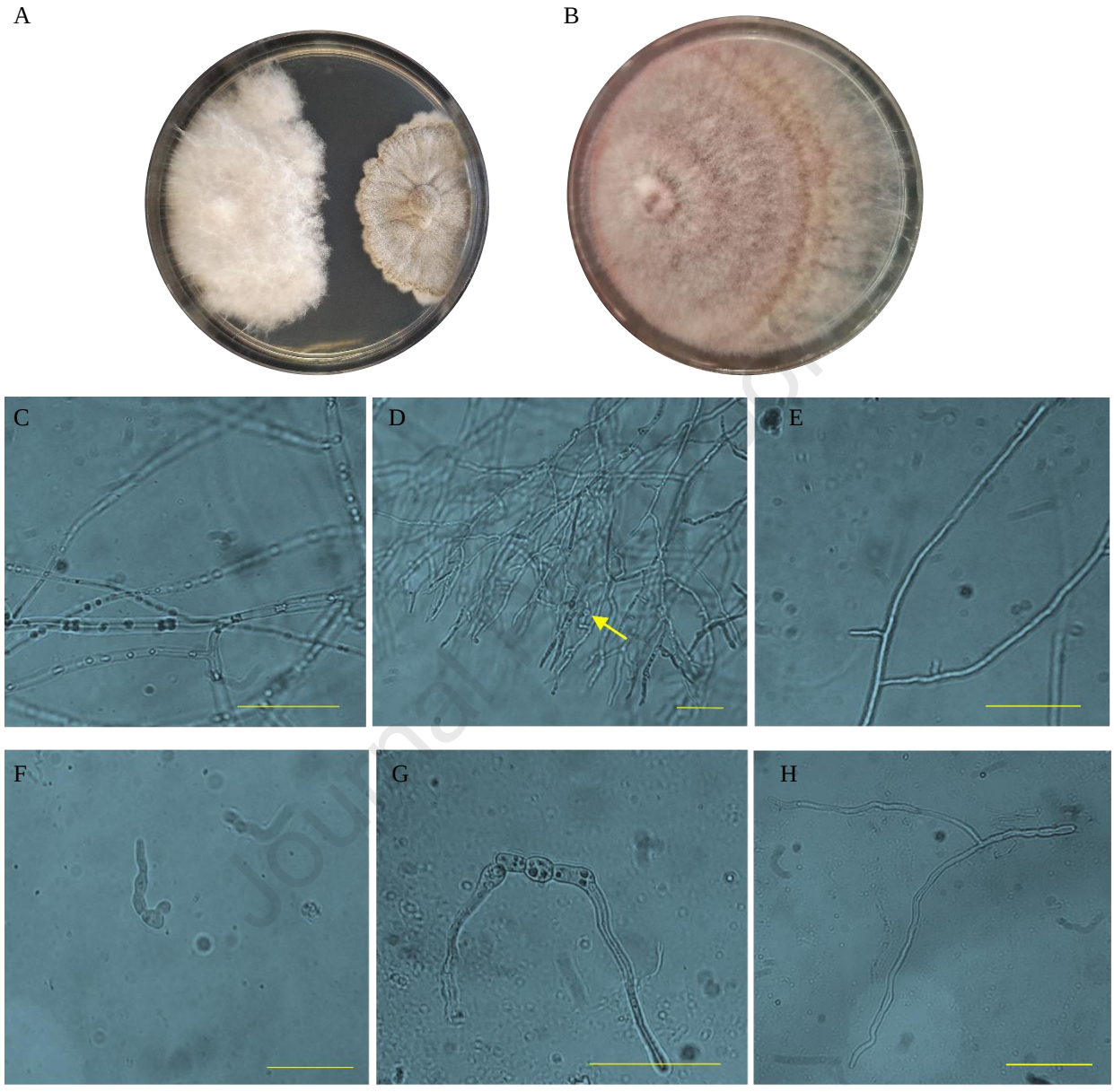


Fig. 2.

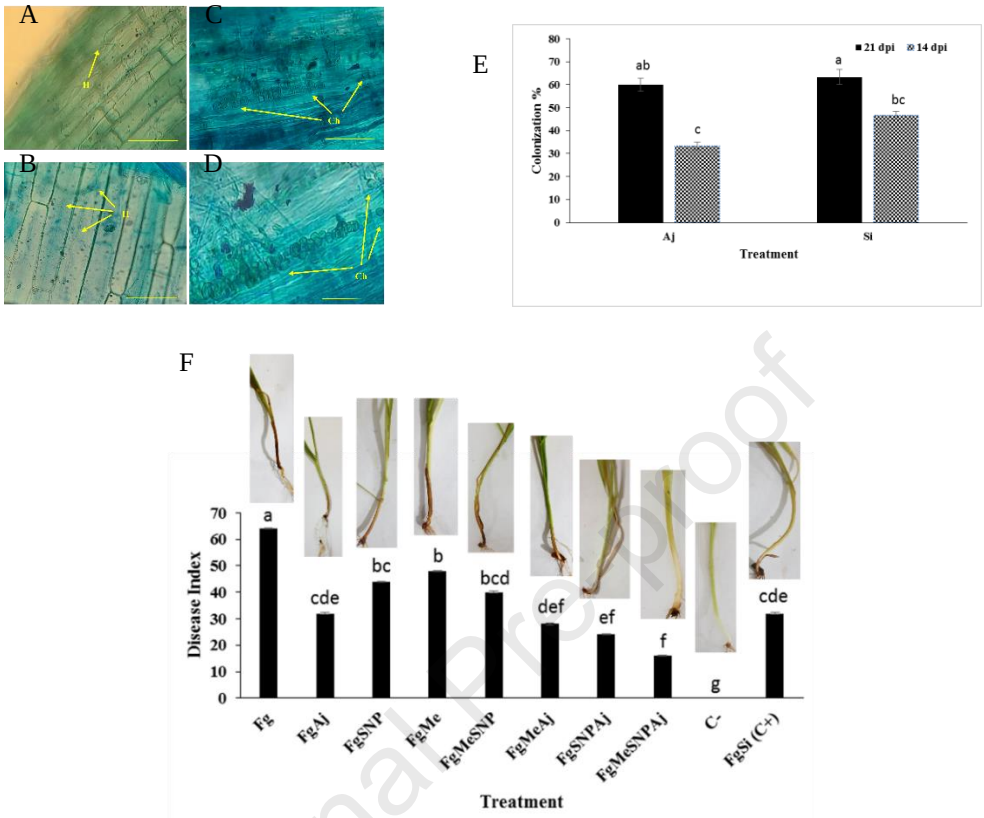
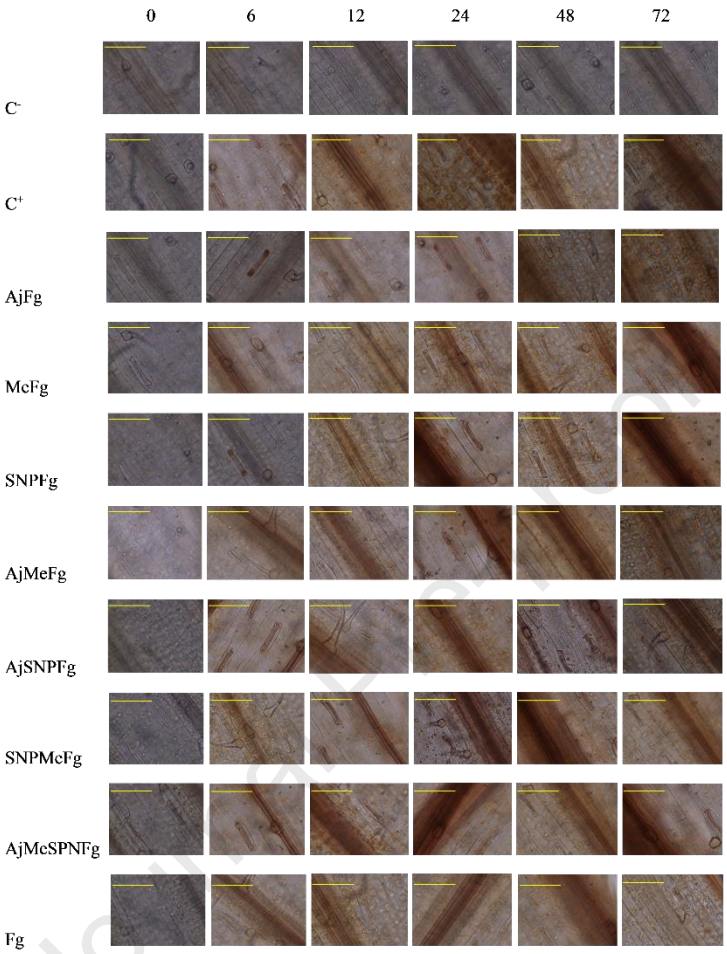


Fig. 3.

A


0
6
12
24
48
72
C-
C+
AjFg
McFg
SNPFg
AjMeFg
AjSNPFg
SNPMcFg
AjMcSPNFg
Fg

B

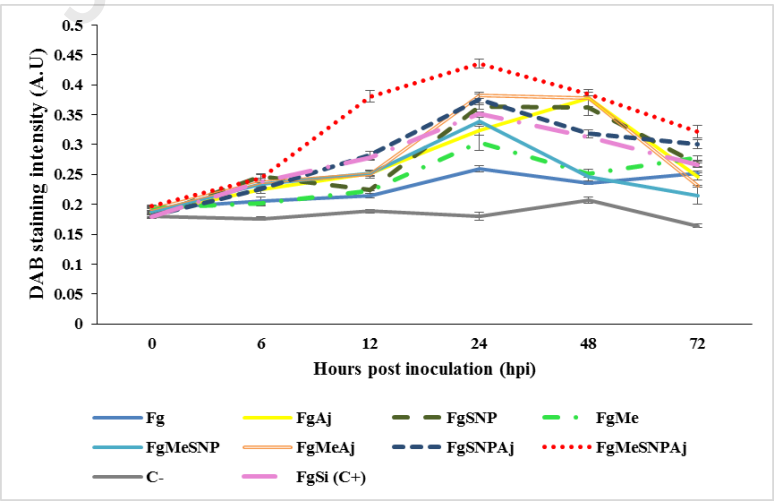
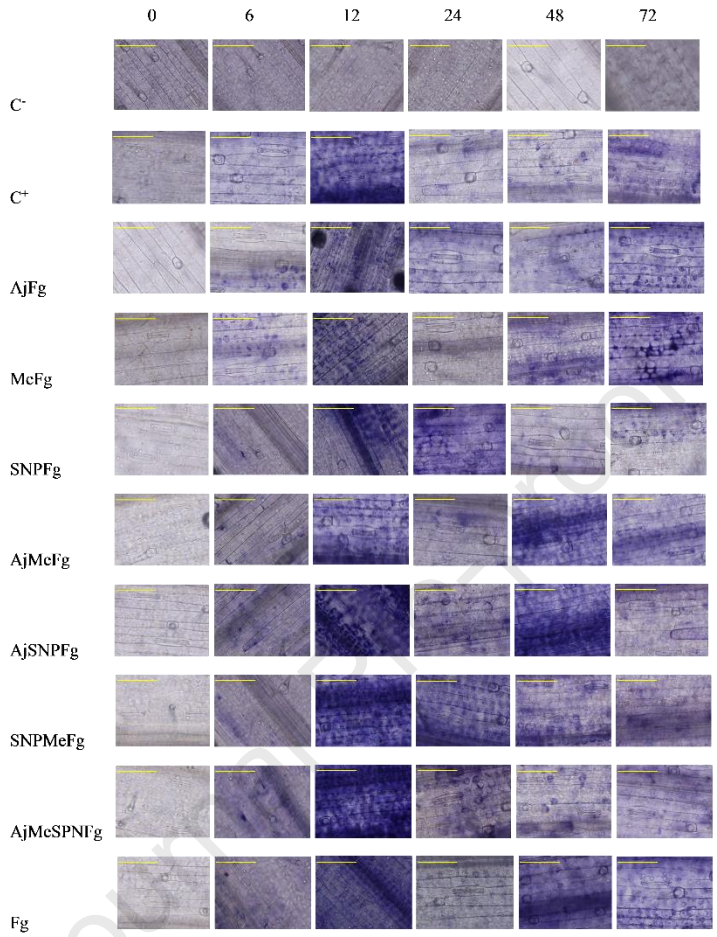

DAB staining intensity (A.U)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
6
12
24
48
72
Hours post inoculation (hpi)
Fg
FgAj
FgSNP
FgMe
FgMeSNP
FgMeAj
FgSNPAj
FgMeSNPAj
C-
FgSi (C+)

Fig. 4.

A


0
6
12
24
48
72
C-
C+
AjFg
McFg
SNPFg
AjMcFg
AjSNPFg
SNPMeFg
AjMcSPNFg
Fg

B

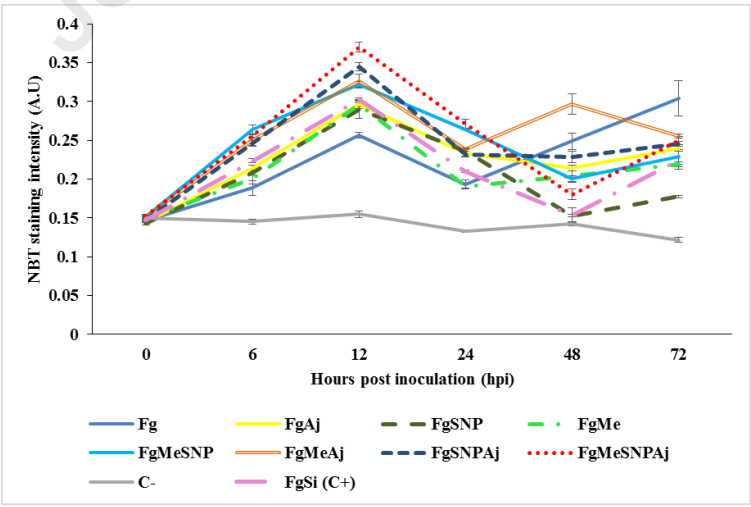

NBT staining intensity (A.U)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
6
12
24
48
72
Hours post inoculation (hpi)
Fg
FgAj
FgSNP
FgMe
FgMeSNP
FgMeAj
FgSNPAj
FgMeSNPAj
C-
FgSi (C+)

Fig. 5.

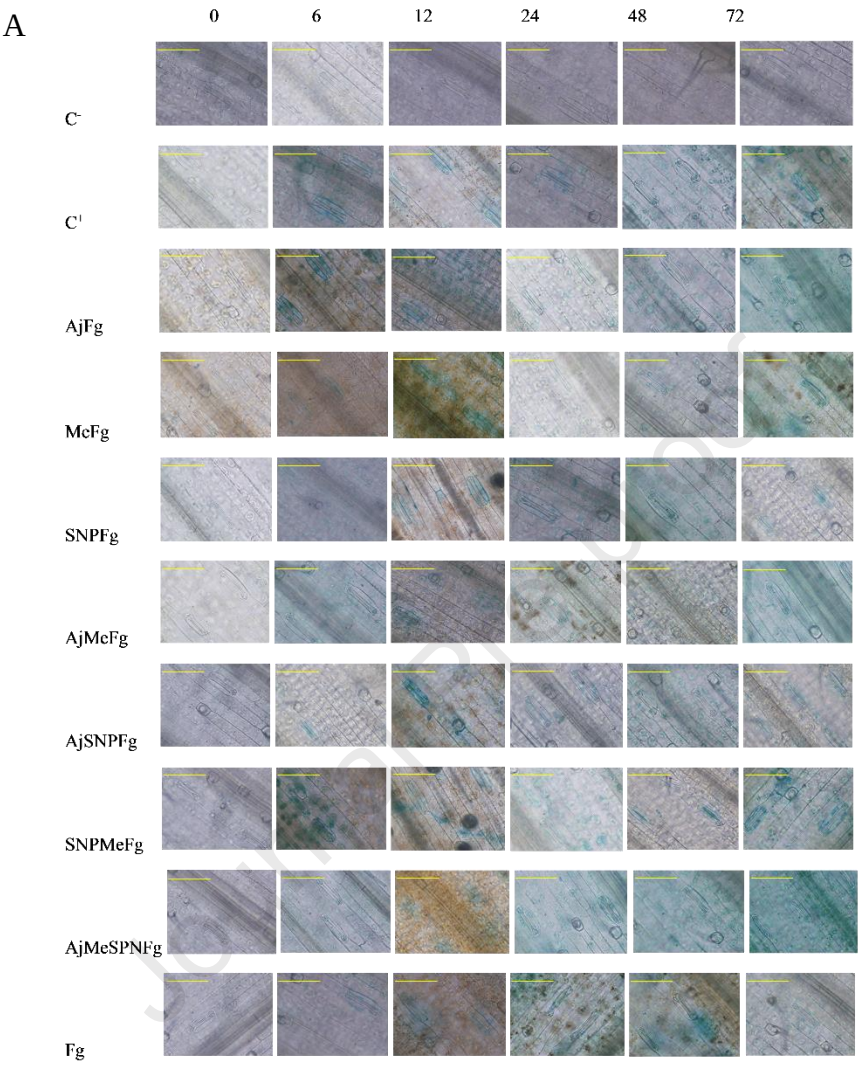

A
0
6
12
24
48
72
C-
C+
AjFg
McFg
SNPFg
AjMcFg
AjSNPFg
SNPMeFg
AjMeSPNFg
Fg
B
Potassium ferrocyanide staining intensity (A.U) for Fe3+
0.3
0.25
0.2
0.15
0.1
0.05
0
0
6
12
24
48
72
Hours post inoculation (hpi)
Fg
FgAj
FgSNP
FgMe
FgMeSNP
FgMeAj
FgSNPAj
FgMeSNPAj
C-
FgSi (C+)

Fig. 6.

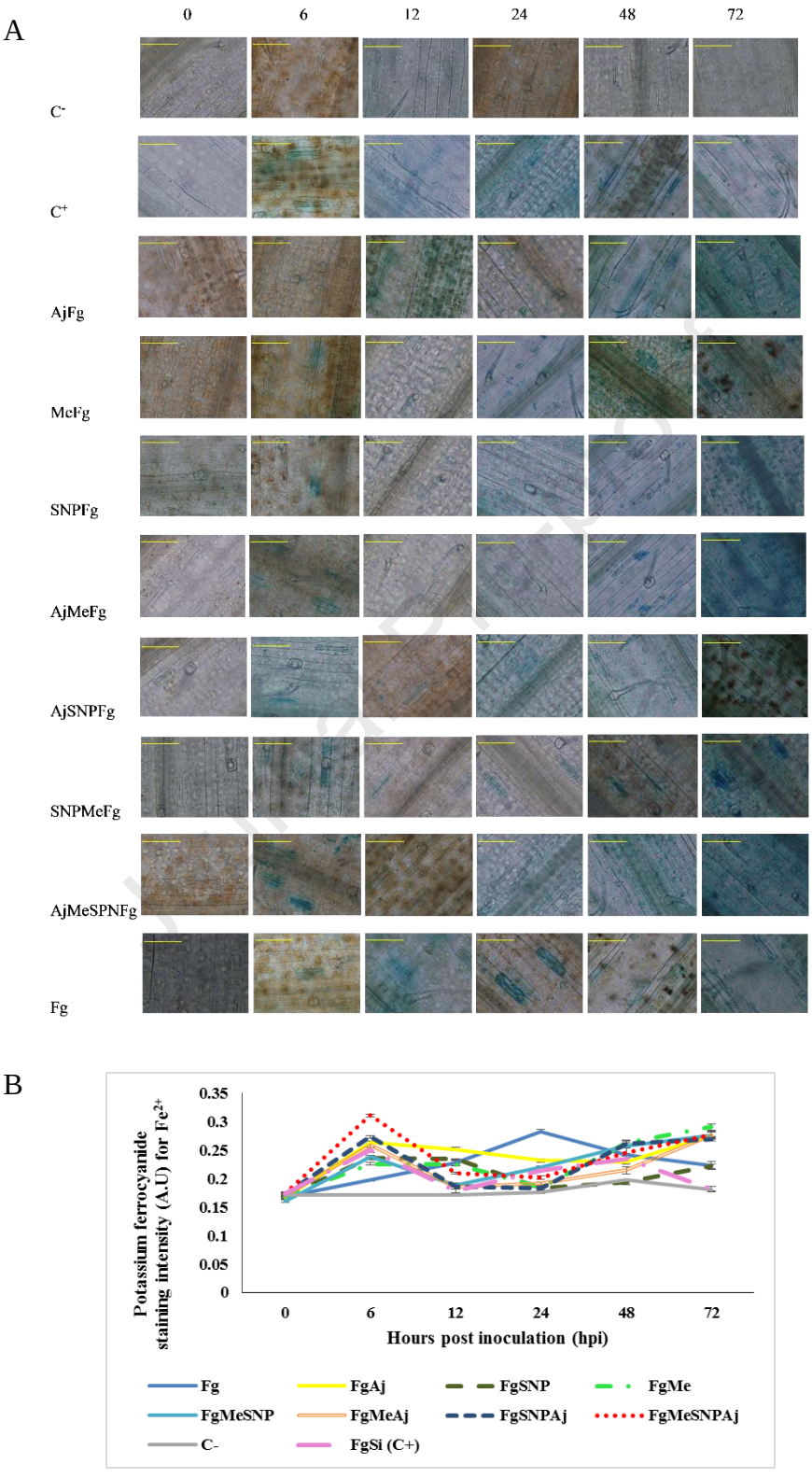
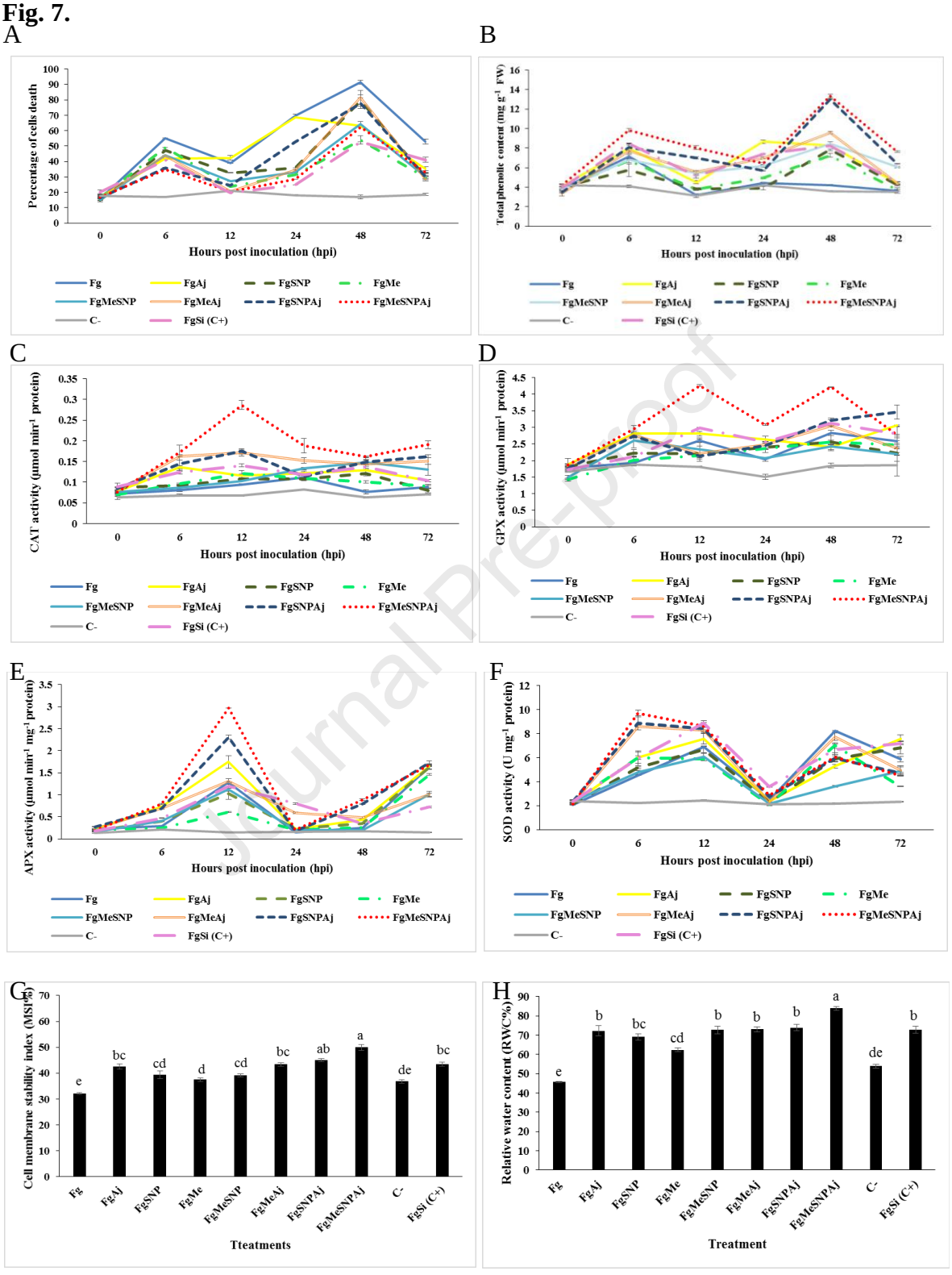

A
0
6
12
24
48
72
C-
C+
AjFg
MeFg
SNPFg
AjMeFg
AjSNPFg
SNPMeFg
AjMeSPNFg
Fg
B
Potassium ferrocyanide staining intensity (A.U) for Fe2+
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
6
12
24
48
72
Hours post inoculation (hpi)
Fg
FgAj
FgSNP
FgMe
FgMeSNP
FgMeAj
FgSNPAj
FgMeSNPAj
C-
FgSi (C+)

Fig. 7.


A
Percentage of cells death
Hours post inoculation (hpi)
B
Total phenolic content (mg g-1 FW)
Hours post inoculation (hpi)
C
CAT activity (µmol min-1 protein)
Hours post inoculation (hpi)
D
GPX activity (µmol min-1 protein)
Hours post inoculation (hpi)
E
APX activity (µmol min-1 mg-1 protein)
Hours post inoculation (hpi)
F
SOD activity (U mg-1 protein)
Hours post inoculation (hpi)
Fg
FgAj
FgSNP
FgMe
FgMeSNP
FgMeAj
FgSNPAj
FgMeSNPAj
C-
FgSi (C+)
G
Cell membrane stability index (MSI%)
Tteatments
H
Relative water content (RWC%)
Treatment

Highlights

• Application of methionine, sodium nitroprusside, and *Acrophialophora jodhpurensis* increased wheat growth parameters.

• The application of methionine, sodium nitroprusside, and *A. jodhpurensis* partially protected wheat plants against *Fusarium graminearum.*

• Wheat defense responses occurred via the application of methionine, sodium nitroprusside, and *A. jodhpurensis.*

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: