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Mycorrhizal Root Exudates Induce Changes in the Growth and Fumonisin Gene (*FUM1*) Expression of *Fusarium proliferatum*

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Abstract: In this study, root exudates from mycorrhizal and non-mycorrhizal plants growing at low or high nutrient supply were used in vitro to examine their effects on the growth and fumonisin B1 gene (*FUM1*) expression of *Fusarium proliferatum* (Hypocreales: Nectriaceae). After one day of exposure to root exudates originating from non-mycorrhizal and low nutrient supply treatment, a significant change in the growth of *F. proliferatum* was measured, which then equalized after 5 days of incubation. Aside from the fumonisin gene (*FUM1*) gene, the expression of the mitogen-activated protein kinase gene (*HOG1*) was also studied using quantitative real-time polymerase chain reaction (qRT-PCR). After 5 days of incubation, mycorrhizal root exudates significantly reduced the expression of the *FUM1* gene, irrespective of the extent of the nutrient supplement and colonization level of the target plant. Similar trends in the expressions of *FUM1* and *HOG1* genes found in our experiment suggest that arbuscular mycorrhizal fungal colonization did not only affect directly the growth and mycotoxin production of *F. proliferatum*, but also modulated indirectly a number of other mechanisms. Mycorrhizal inoculation showed potential as a biological control agent in the suppression of fumonisin production by *F. proliferatum*.

Keywords: arbuscular mycorrhizal fungi; gene expression; *Fusarium proliferatum*; fumonisin B1 gene; mitogen-activated protein kinase gene

1. Introduction

Wheat and maize, together with rice, are the main cereal crops of the world. The Food and Agriculture Organization (FAO) of the United Nations estimates global cereal production at 2.609 million tonnes in 2018 [1]. Under field conditions, crops are often exposed to different stress factors, negatively influencing plant productivity, of which plant pathogens such as *Fusarium proliferatum* (teleomorph: *Gibberella intermedia*; Hypocreales: Nectriaceae) is included. Genetic and phenotypic diversity of *F. proliferatum* isolates occur globally and associate with a diverse range of agriculturally important plants as a parasite or secondary invader [2–6]. Moreover, *F. proliferatum* secretes a wide range of secondary metabolites, including mycotoxins such as fumonisins (FB), moniliformin (MON), beauverine (BEA), fuzaric acid (FA) and fusaproliferin (FUP), which pose a high risk to human and animal health, and to food safety [7,8]. The fumonisin analogs (FB1, FB2, FB3) are the most abundant types of fumonisins, with FB1 predominating, and usually being found at the highest level. Various biotic and abiotic factors, such as chitosan, water capacity [9], different plant extracts [10], temperature [11,12] and carbon sources [13] influence the growth of *F. proliferatum* and its mycotoxin production.

Besides soil and postharvest management [14–21], there is an increased demand to use an environmentally-friendly way to control mycotoxin producing organisms. Among these strategies, there has been an increasing interest in beneficial microbes, such as arbuscular mycorrhiza.

A symbiotic relationship between plant and arbuscular mycorrhizal fungi (AMF) has been established more than 400 million years ago to offer some benefits to the target plant, including enhanced nutrients, mostly phosphorus and water uptake [22]. Moreover, AMF may induce a systemic defense mechanism called mycorrhiza-induced resistance (MIR) in a host plant [23]. The meta-analysis of Veresoglou et al. [24] showed that mycorrhizal inoculation of crops reduces fungal infections by 30%–42%. This beneficial effect of AMF can be attributed to the dramatically altered plant primary and secondary metabolism in affected roots, thereby influencing microorganisms living in the rhizosphere [23,25–28]. The decreased growth of filamentous fungi in the presence of AMF has already been investigated [29–32] but how its mycotoxin producing ability works is not well known. Ismail et al. [33] covered the opportunities of using mycorrhizal fungi to control fumonisin production, but there is no data regarding *Fusarium proliferatum*.

Hence, the aims of this work were to study the effects of root exudates originated from mycorrhizal plants growing under different nutrient levels on the growth and fumonisin production of *F. proliferatum*. Mycotoxin production is followed by measuring the expression of the fumonisin B1 gene (*FUM1*) and HOG-type mitogen-activated protein (MAP) kinase gene (*HOG1*) playing important roles in fungal adaptation to a wide range of stress conditions, moreover in secondary metabolite production [34–36].

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The plant growth experiment was carried out in climatic chamber EKOCHL 1500 (Angelantoni, Massa Martana, Italy) (24/26 °C, 60% RH, 16 h light/8 h dark) consisting of two combined treatments: mycorrhizal inoculation and two levels of nutrient supplies for target plant growth. Maize (*Zea mays* L. 'Golda F1') seeds were surface sterilized in 1% NaOCl, then washed with sterilized water three times and placed on wet filter paper for germination. Three days after germination, the seedlings were transferred to plastic pots containing 750 g of three times sterilized (121 °C for 30 min) peat and sand 1:4 (*v/v*) substrate. Half of the pots were inoculated with the mixture of mycorrhizal fungi (*Funneliformis mosseae* BEG12 (Glomerales: Glomeraceae); *Rhizophagus intraradices* BEG53 (Glomerales: Glomeraceae)) propagated on maize (*Zea mays* L. 'Golda F1') for three successive propagation cycles, each for 5 months. The most probable number (MPN) of infective propagules were determined following the method of Feldmann and Idczack [37]. For control treatments, sterilized mycorrhizal inoculum was prepared at the same rate (16 g plot^{-1}, about 35 infective propagules g^{-1}) as used in mycorrhizal treatment. Every second day, half of both mycorrhizal and non-mycorrhizal plants were irrigated with 50 mL tap water or 5× Long Ashton (0.75 mM $MgSO_4$ $7H_2O$, 1 mM $NaNO_3$, 1 mM K_2SO_4, 2 mM $CaCl_2$ $2H_2O$, 32 mM Na_2HPO_4 $12H_2O$, 0.25 mM FeNa-EDTA, 0.005 mM $MnSO_4$ H_2O, 0.00025 mM $CuSO_4$ $5H_2O$, 0.0005 mM $ZnSO_4$ $7H_2O$, 0.025 mM H_3BO_3, 0.001 mM Na_2MoO_4 $2H_2O$) nutrient solution, representing low (LN) or high nutrient supply (HN), respectively. Altogether, four treatments were replicated five times: mycorrhizal inoculated plants at low nutrient supply (+AM LN), non-mycorrhizal plants at low nutrient supply (−AM LN), mycorrhizal inoculated plants at high nutrient supply (+AM HN) and non-mycorrhizal plants at high nutrient supply (−AM HN). After 42 days of growth, the plants were removed carefully from the pots to collect different types of root exudates. Fresh shoot and root weights were determined for each treatment.

2.2. Production of Root Exudates

Root exudates were collected from maize plants treated, as described previously, by removing all substrate particles from the roots and then submerging them into 50 mL of 0.01 M L^{-1} KOH according to da Silva Lima et al. [38]. After 5 min, the root system was washed with tap water, then with

distilled water and were incubated in Erlenmeyer flasks filled with 50 mL sterilized distilled water for 24 h. Solutions were sterilized by filtration through 0.22 µm Ø nitrocellulose filters (Millipore, Burlington, MA, USA). Concentrations of root exudates were adjusted to a ratio of 1 g of root fresh weight based on the method of Lioussanne et al. [39]. In this way, the effects of various root exudates remained comparable, however, roots from different treatments showed significant differences in weights. The root exudates were kept at −20 °C until use.

2.3. Culture Conditions and Expression Analyses of FUM1 and HOG1 Genes

Fusarium proliferatum ITEM 2287 (Institute of Sciences of Food Production, CNR, Bari, Italy) was used in the study. Mycelium for fumonisin production was prepared in 50 mL DM (22 mM KH_2PO_4, 2.5 mM $MgSO_4$, 85 mM NaCl and 117 mM sucrose, pH 5.9) according to Shim and Woloshuk [40], supplemented with 30 mM ammonium dihydrogen phosphate and inoculated with 10^6 mL^{-1} conidia. Cultures were incubated at 26 °C with shaking (150 rpm) for 48 h then filtrated and washed with sterilized distilled water and mycelium, then transferred directly to 50 mL of new DM medium. The co-cultures were inoculated separately with 5 mL (concentrations adjusted to a ratio of 1 g of root fresh weight) of each of root exudate (+AM LN, −AM LN, +AM HN, −AM HN). The control treatment was prepared by adding 5 mL of sterilized distilled water. All treatments, with six replicates each, were incubated at 26 °C using a shaker (150 rpm). After 24 h and 5 days of incubation, three cultures from each treatment were filtered and total RNA was extracted using the E.Z.N.A.™ Fungal RNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions. For quantitative real-time polymerase chain reaction (qRT-PCR) analysis, first-strand cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was performed on a Stratagene Mx3000P QPCR System (Agilent Technologies, Santa Clara, CA, USA). Each reaction was performed in a final volume of 25 µL containing 12.5 µL SYBR Green Master Mix reagent (Thermo Fisher Scientific, Waltham, MA, USA), 1 µL of diluted cDNA sample, 70 nM of gene-specific primers and 8 µL of nuclease-free water. Gene-specific primer sequences used for qRT-PCR are as follows: *FUM1* gene (amplified fragment 107 bp: forward 5′-CAAACGGCTATGCAAGAGGC-3′, reverse 5′-AGATGTTGCCCTGACCACAG-3′); *HOG1* gene (amplified fragment 137 bp: forward 5′-CACACGATACTACCGAGCCC-3′, reverse 5′-TGACGTGATCTTTTCCGGGG-3′). The PCR cycle procedure was 95 °C for 15 min; followed by 40 cycles at 95 °C for 15 s, 57 °C for 30 s and 72 °C for 16 s; finally 1 cycle at 95 °C for 60 s, 57 °C for 30 s and 95 °C for 30 s. The qRT-PCR experiment was carried out under identical conditions with three replications. Amplification of the *histone H3* gene was used as internal reference (amplified fragment 135 bp: forward 5′-ATCTCCGCTTCCAGTCTTCC-3′, reverse 5′-GCTGGATGTCCTTGGATTGGA-3′). The gene expression of *FUM1* and *HOG1* was calculated using the $2^{-\Delta\Delta CT}$ method.

2.4. Growth Assessment of Fusarium Proliferatum under Different Root Exudates

The growth of *Fusarium proliferatum* ITEM 2287 due to various root exudates was followed on PDA (potato dextrose agar). Spread on the surface of PDA plates were 100 µL of different types of concentrated root exudates, and 10^6 mL^{-1} conidia of stock culture was placed in the center of the plate. Plates without root exudates had 100 µL sterilized distilled water spread over their surface and were prepared as described above, which served as the control. Each treatment was replicated five times.

2.5. Assessment of Mycorrhizal Colonization of AM Fungi

Root colonization was estimated in five plants from each treatment. Fine roots from each plant were stained with trypan blue [41]. Internal fungal structures (hyphae, arbuscules) were examined under a stereomicroscope (Olympus, Tokyo, Japan) at ×400 magnification and the percentage of root colonization was calculated using the gridline intersect method [42].

2.6. Statistical Analysis

Values were expressed as means ± standard error (SE). All data were statistically analyzed with the R Statistical Software 3.3.1 [43]. Two-way analysis of variance (ANOVA) was used considering mycorrhizae inoculation and nutrient supply as factors. When the interaction of factors was statistically significant, simple main effects were interpreted and Tukey's post-hoc test was used to compare treatment groups. For analysis of the growth of *Fusarium proliferatum* under different root exudates, one-way ANOVA and Dunnett's post-hoc test were used to compare various treatments to the control (no root exudates added). A p-value < 0.05 was considered to indicate statistical significance.

3. Results

3.1. The Effect of Different Nutrient Levels and AM Fungi Colonisation on Plant Growth

The percentage of mycorrhizal root colonization assessed by trypan blue staining showed significant differences ($p < 0.05$) between samples collected from +AM LN (54% ± 4.02) and +AM HN (39% ± 1.79), and no infection was observed in −AM treatments. Changes in plant growth due to mycorrhizal inoculation under different treatments are shown in Figure 1. Plants colonized by mycorrhiza in low nutrient solution had significantly ($p < 0.05$) higher shoots and higher root growth than non-mycorrhizal plants. The main effects in fresh weight of plant shoots were all significant, except between mycorrhizal and non-mycorrhizal treatments at high nutrient supply ($p = 0.07$). Both nutrient and mycorrhizal inoculation effects were significant, as well as their interaction ($F = 168.32$, $df = 1$, $p = 6.59 \times 10^{-10}$; $F = 71.13$, $df = 1$, $p = 2.78 \times 10^{-7}$; $F = 21.54$, $df = 1$, $p = 0.000272$; for nutrient, mycorrhizal treatment and interaction; respectively, $p < 0.05$). The mycorrhizal colonization resulted in enhanced growth at the rate of 166% in shoot fresh weight and 234% in root fresh weight under LN treatment compared with non-mycorrhizal plants. The opposite tendency was found in root weights at HN treatment, and significantly ($p < 0.05$) higher root weights were measured in −AM plants than +AM ones. In general, nutrient supply and mycorrhizal inoculation and their interaction were significant regarding the fresh root weight of plants (for nutrients: $F = 13.58$, $df = 1$, $p = 0.002$; for mycorrhizal treatment: $F = 29.08$, $df = 1$, $p = 5.98 \times 10^{-5}$; for interaction: $F = 198.90$, $df = 1$, $p = 1.92 \times 10^{-10}$, respectively; $p < 0.05$). The effect of nutrient supply on root fresh weights were all significant ($p < 0.05$) except for +AM LN and −AM HN ($p = 0.63$) treatments (Figure 1).

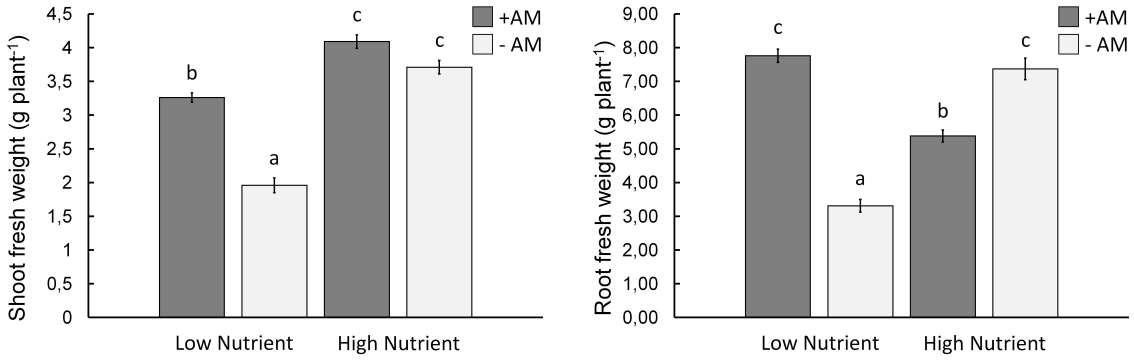

Figure 1. Effects of arbuscular mycorrhizal inoculation on (**a**) shoot and (**b**) root fresh weight of maize growing at two levels of nutrient (+AM: mycorrhizal plants; −AM: non-mycorrhizal plants); mean values ± standard error (SE) with small letters indicating statistical differences between treatments, according to two-way ANOVA combined with Tukey's post-hoc test at $p < 0.05$.

3.2. The Effect of Different Root Exudates on Fusarium proliferatum Growth

To study the influence of mycorrhizal inoculation upon on changes in root exudation on *Fusarium proliferatum* growth, an in vitro experiment was carried out. Spreading different root exudates before inoculating *F. proliferatum* on the surface of PDA agar, significant differences in growth rate were observed among the treatments after 1 day of incubation. After 5 days of incubation, the colony diameter values equalized under different treatments (Table 1).

Table 1. Growth of *Fusarium proliferatum* after 1 and 5 days of incubation under different origins of root exudates. +AM: mycorrhizal plants; −AM: non-mycorrhizal plants; LN: low nutrient supply; HN: high nutrient supply.

Incubation Time	1 Day	5 Days
Root Exudates Treatments	**Colony Diameter (mm) [1]**	**Colony Diameter (mm) [1]**
+AM LN	19.78 ± 0.32	51.00 ± 0.29
−AM LN	23.11 ± 0.31 *	51.00 ± 1.42
+AM HN	20.11 ± 0.31	51.31 ± 1.49
−AM HN	20.44 ± 0.34	54.11 ± 1.01
Control (C)	20.11 ± 0.31	51.12 ± 1.42
One-way ANOVA	$F = 18.33$, $df = 4$, $p = 1.26 \times 10^{-8}$ **	$F = 1.27$, $df = 4$, $p = 0.298$ n.s.
Dunnett's post-hoc test	$p < 0.001$ **	

[1] Values are presented as the mean ± SE; * significant at $p < 0.05$, ** significant at $p < 0.001$, n.s. = contrast is non-significant, according to one-way ANOVA and Dunnett's post-hoc test ($p < 0.05$).

3.3. The Effect of Different Root Exudates on FUM1 and HOG1 Gene Expression

After 24 h incubation, definite induction of *FUM1* gene expression was observed using a fold change unit for measuring (Figure 2). Exudates from low colonized roots (+AM HN) increased the expression of the *FUM1* gene ($p = 0.0001479$) compared with root-exudates at a higher mycorrhizal colonization level (+AM LN). The effects of inoculation in the *FUM1* gene expression at a low nutrient level were not significant ($p = 0.09$), nevertheless, in general both nutrient and mycorrhizal treatments and their interaction were significant ($F = 166.7$, $df = 1$, $p = 1.22 \times 10^{-6}$; $F = 434.7$, $df = 1$, $p = 2.94 \times 10^{-8}$; $F = 611.2$, $df = 1$, $p = 7.66 \times 10^{-9}$ for nutrient treatments, mycorrhizal treatment and interaction, respectively at $p < 0.05$).

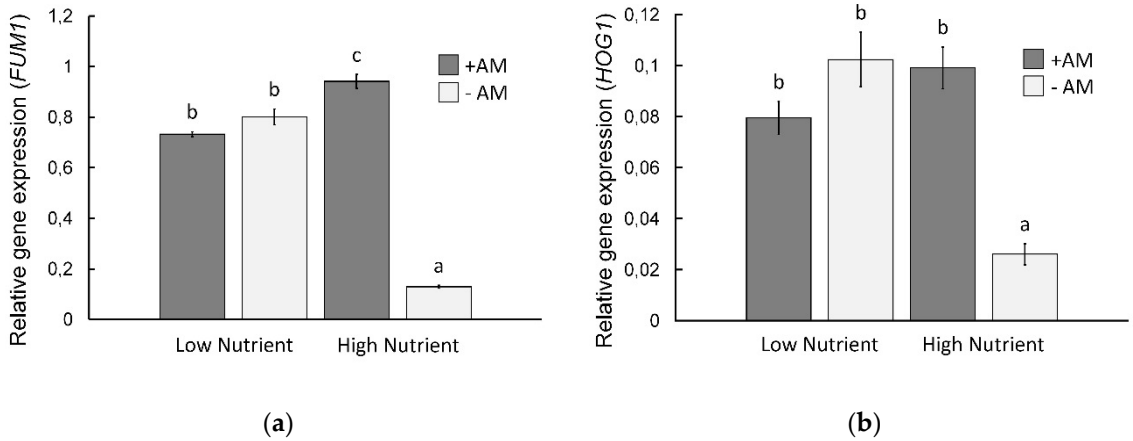

Figure 2. (**a**) Fumonisin (*FUM1*) and (**b**) HOG-type mitogen-activated protein (MAP) kinase (*HOG1*) gene expression after 1 day of incubation under different origins of root exudates (+AM: mycorrhizal plants; −AM: non-mycorrhizal plants); mean values ± SE with small letters indicating statistical differences between treatments according to two-way ANOVA combined with Tukey's post-hoc test at $p < 0.05$.

After 1 day of incubation, *HOG1* gene expression (Figure 2) followed the expression of the *FUM1* gene, and no significant differences ($p = 0.37$) were recognized among the mycorrhizal treatments as well as treatments at low nutrient supply ($p = 0.22$). Under −AM HN treatment, the gene expression of *HOG1* was significantly lower than other treatment groups ($p < 0.05$). Both nutrient and mycorrhiza inoculation effects were significant, as well as interaction based on two-way ANOVA at $p < 0.05$—nutrient supply ($F = 14.07$, $df = 1$, $p = 0.005612$), mycorrhizal treatment ($F = 10.0$, $df = 1$, $p = 0.013339$) and interaction ($F = 38.16$, $df = 1$, $p = 0.000266$).

After 5 days, the influence of mycorrhizal root exudates significantly reduced the relative expression of the *FUM1* gene (LN treatment: $p = 0.0000042$; HN treatment: $p = 0.0015556$), irrespective of the extent of the nutrient supplement and colonization of the target plant (Figure 3). Parallel with this decreasing, increased *FUM1* gene expression was measured in the occurrence of −AM root exudates. The main effects were significant, except for +AM HN and +AM LN ($p = 0.85$) treatment. Both nutrient level and mycorrhizal inoculation effect, as well as their interaction, caused significant differences: nutrient supply ($F = 41.58$, $df = 1$, $p = 0.000199$), mycorrhizal treatment ($F = 188.09$, $df = 1$, $p = 7.7 \times 10^{-7}$) and interaction ($F = 28.26$, $df = 1$, $p = 0.000714$). *HOG1* gene expression was significantly ($p = 0.0000002$) lower after 5 days of incubation at +AM LN plants root exudates compared with the effect of −AM plant root exudates (Figure 3). There was no significant difference ($p = 0.3661917$) in *HOG1* gene expression between the root exudates of plants at a high nutrient supply. Gene expression of *HOG1* was only significantly higher under −AM LN treatment compared with the other treatments ($p < 0.05$). Nutrient levels, mycorrhizal colonization and interaction of both treatments had a significant effect on *HOG1* transcript levels ($F = 123.5$, $df = 1$, $p = 3.84 \times 10^{-6}$; $F = 153.7$, $df = 1$, $p = 1.67 \times 10^{-6}$; $F = 220.7$, $df = 1$, $p = 4.16 \times 10^{-7}$ for nutrients, mycorrhizal treatment and interaction, respectively at $p < 0.05$).

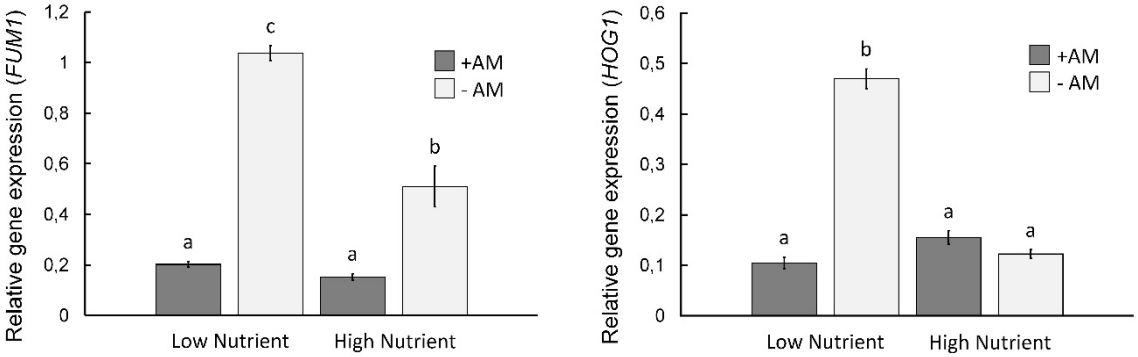

Figure 3. (**a**) Fumonisin (*FUM1*) and (**b**) HOG-type mitogen-activated protein (MAP) kinase (*HOG1*) gene expression after 5 days of incubation under different origins of root exudates (+AM: mycorrhizal plants; −AM: non-mycorrhizal plants); mean values ± SE with small letters indicating statistical differences between treatments, according to two-way ANOVA combined with Tukey's post-hoc test at $p < 0.05$.

4. Discussion

In agreement with previous studies, our results confirmed that mycorrhizal dependency was higher at a low nutrient supply than at a high nutrient level [44,45]. Aside from the growth parameters of target plants, mycorrhizal colonization could be realized which also shows the efficacy of the inoculation (Figure 1). The higher mycorrhizal infection at low nutrient level observed in this study has been shown in other research [46,47] but opposite results have also been reported [48].

Arbuscular mycorrhizal symbiosis alters plant metabolism, including low molecular organic acids (glutamic, aspartic, asparagine, palmitic and oleic acid), secondary metabolites (phenyl alcohols,

a-linolenic acid, apocarotenoids and isoflavonoids), plant hormones (oxylipin, cytokinins and jasmonic acid) and others, which affect soil microorganisms in direct or indirect ways [49–51]. The production of these substances are highly variable within and between different types of mycorrhizal fungi and are influenced by environmental conditions [52].

The in vitro growth of *Fusarium proliferatum* increased when exposed to root exudates originating from −AM LN treatment (t = 6.67, $p < 0.05$) compared with the control. In a nutrient deficient environment, plants evolved different mechanisms to overcome nutrient shortages, including the improved production of attractants for rhizosphere microorganisms [53]. Moreover, some studies have reported that mycorrhizal inoculation can boost plant defenses against soil pathogens [54–56]. The negative effect of mycorrhizal fungi on the growth of filamentous fungi has already been described in different studies [29,30] while only moderate, not significant inhibitory effects were found in our experiment (Table 1). This was most likely due to the short duration of exudate-collection and moderate mycorrhizal colonization of plants compared with other studies [57,58]. Moreover, the published results based on high hyphae density prepared in vitro AMF cultures, as Filion et al. [57] reported, reduced germination of *Fusarium oxysporum* (Hypocreales: Nectriaceae) conidials in the presence of *Rhizophagus intraradices*.

In general, suppressing the growth of pathogen fungi by mycorrhizae is most likely based on complex processes, where root and hyphae exudates may play a crucial role [29,31]. Lioussanne et al. [26] showed that at an early stage of mycorrhizal colonization attractively effects on zoospore germination of *Phytopthora nicotianae* (Peronosporales: Peronosporaceae) while only root exudates from plant roots that are extensively colonized by AM fungi show inhibitory effects. This tendency correlates well with the higher level of proline found in well-colonized roots compared with non-mycorrhizal ones, suggesting the importance of the mycorrhizal colonization level [26]. A study by Ismail et al. [58] stated that *Rhizophagus irregularis* (Glomerales: Glomeraceae) decreased not only the growth of *Fusarium sambucinum* (teleomorph: *Gibberella pulicaris*; Hypocreales: Nectriaceae) but also the expression of the trichothecene gene together with its inhibited production. Following this line of reasoning, beside the effects of different root exudates on the growth of *Fusarium proliferatum*, change in the fumonisin B1 gene (*FUM1*) expression was studied using quantitative real-time PCR (Figures 2 and 3). It is well known that mycotoxin production is regulated by different factors, such as carbon sources, nitrogen starving and the oxylipins group [12,13,59,60], but data is missing regarding mycorrhizal root exudates. Increased expression of the *FUM1* gene under low AM fungi colonized root exudates (+AM HN) for 24 h compared with a higher mycorrhizal level in our work is similar to the tendency found by Lioussanne et al. [26] for fungal growth. The initial increase (after 24h) in gene expression of *FUM1* can be attributed to the root exudates as a stress factor described by Zheng et al. [61] measuring the HOG-type mitogen-activated protein (MAP) kinase gene (*HOG1*) expression. Therefore, parallel with the regulation of the *FUM1* gene, the expression of *HOG1* gene was also analyzed (Figures 2 and 3). Increased *HOG1* and *FUM1* gene expression is documented under nitrogen starvation [62], but their changes under mycorrhizae influences have not been tested until now. Mitogen-activated protein kinase (MAPK) cascades are important in stress-responsive signaling pathways for both plants and fungi, which may result in an accumulation of induced reactive oxygen species (ROS) in fungal cells [35,63]. Low nutrient availability, together with the occurrence of mycorrhizal fungi, may act as stress factors altering both *HOG1* and *FUM1* gene expressions (Figure 2) at a higher rate, 24 h after incubation. However, the influence of mycorrhizal inoculation through root exudates resulted in a moderate amount of stress on tested genes after 5 days of incubation (Figure 3). The root exudates originated from mycorrhizal plants significantly reduced the expression of the *FUM1* gene, irrespective of the extent of the nutrient supplement and colonization level of the target plant, confirming the beneficial effects of AMF.

Our results indicate that root exudates both from −AM and +AM plants, depending on environmental circumstances, contain mycotoxin production regulator and/or stronger mycotoxin production inducer substrates. Aside from this, the similar tendency in the expression of *HOG1*

and *FUM1* genes suggests that AM fungal colonization did not only directly affect the mycotoxin production, but also modulated other mechanisms, including mitogen-activated protein (MAP) kinase indirectly influencing the mycotoxin production.

5. Conclusions

To study the influence of mycorrhizal inoculation upon changes in root exudation on *Fusarium proliferatum* growth and its mycotoxin producing ability, an in vitro experiment was carried out. We conclude that AMF can modulate the growth and mycotoxin gene expression (*FUM1*) of plant pathogens. The growth of *F. proliferatum* and its fumonisin B1 production resulted in a complex interaction, wherein MAP kinases could have an important role in their regulation. Additional experiments are required to clarify the mechanisms of fumonisin production under mycorrhizal influence, and will have fascinating implications for advancing our knowledge of plant-microbe interactions and controlling plant pathogens.

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