

Interaction of methyl-jasmonate and *Fusarium poae* in bread wheatM.I. Dinolfo<sup>a,1,\*</sup>, M. Martínez<sup>a,1</sup>, E. Castañares<sup>a</sup>, L.S. Vanzetti<sup>b</sup>, F. Rossi<sup>c</sup>, S.A. Stenglein<sup>a</sup>, A.F. Arata<sup>a,d,\*\*</sup><sup>a</sup> Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CICBA-INBIOTEC-CONICET, Facultad de Agronomía, UNCPBA, Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina<sup>b</sup> Grupo Biotecnología y Recursos Genéticos, EEA INTA Marcos Juárez, Ruta 12 s/n, Marcos Juárez (CP2580), Córdoba, Argentina<sup>c</sup> Instituto Tecnológico Chascomús (INTECH), Universidad Nacional de General San Martín (UNSAM)-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Intendente Marino Km 8.2, CC 164 (7130) Chascomús, Argentina<sup>d</sup> Centro de Investigaciones Integradas sobre Sistemas Agronómicos Sustentables (CIISAS), Facultad de Agronomía, UNCPBA, Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina

## ARTICLE INFO

## Article history:

Received 24 January 2022

Received in revised form

3 October 2022

Accepted 7 October 2022

Available online 12 October 2022

Corresponding Editor: Dr V Valiante

## Keywords:

Fungal presence

Gene expression

Phytohormones

Plant–pathogen interaction

## ABSTRACT

*Fusarium* Head Blight (FHB) is a devastating disease that affects the grain yield and quality of essential crops such as wheat. In the last years, some *Fusarium* species have acquired particular importance as *Fusarium poae*. However, studies to evaluate *F. poae*-wheat interaction are still scarce. The interaction between *F. poae* and two bread wheat cultivars with different resistance levels against FHB was evaluated. Moreover, the application of methyl-jasmonate (MeJA) was evaluated as a possible tool to reduce the fungal presence. Our results showed that the MeJA treatment is isolate-dependent, reducing *F. poae* fungal growth. A decrease in fungal biomass was observed in the susceptible cultivar after MeJA application; however, no differences between inoculated and inoculated-MeJA treatments were observed in the resistant cultivar. Finally, the *F. poae* inoculation induces the expression of *PR1-1* and *PDF1.2*, being early in the resistant cultivar compared to the susceptible ones. The application of MeJA combined with the *F. poae* inoculation increased *PR1-1* and *PDF1.2* expressions in resistant cultivars. To our knowledge, this is the first study that evaluates the interaction between *F. poae* and wheat and the MeJA treatment as a possible management strategy against this important pathogen.

© 2022 British Mycological Society. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

*Fusarium* Head Blight (FHB) is a disease that mainly affects winter cereal crops, causing economic losses by decreasing grain yield and quality by the presence of mycotoxins. Among the primary species capable of developing this disease worldwide, *Fusarium graminearum* is the most prevalent, nevertheless, other species have also been isolated (de Chaves et al., 2022). *Fusarium poae* is another species frequently isolated from infected host

tissues whose occurrence has been reported in winter crops in Argentina (Nogueira et al., 2018), Brazil (Pereira et al., 2021), Czech Republic (Chrpová et al., 2016), Finland (Hietaniemi et al., 2016), Italy (Covarelli et al., 2015), Switzerland (Drakopoulos et al., 2021), among others. This species is one of the most important trichothecene type B producers, especially nivalenol (NIV), the most toxic among this type of mycotoxins. Moreover, this can also produce type A trichothecenes such as deacetoxy-scirpenol (DAS) and neosolaniol (NEO). Furthermore, *F. poae* can produce enniatins (ENNs) and beauvericin (BEA), currently named “emerging mycotoxins” (Nazari et al., 2018).

Plants are developed multiple ways of resistance against pathogens depending on several factors as the pathogen lifecycle. For instance, salicylic acid (SA) is the primary phytohormone involved in the response against biotrophic pathogens, while jasmonic acid (JA) and ethylene (ET) play an important role against necrotrophic pathogens and herbivores. In addition, other plant hormones are involved in the plant immune system against different pathogens, such as gibberellins (GA), auxins (AUX), abscisic acid (ABA), and

\* Corresponding author. Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CICBA-INBIOTEC-CONICET, Facultad de Agronomía, UNCPBA, Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina.

\*\* Corresponding author. Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CICBA-INBIOTEC-CONICET, Facultad de Agronomía, UNCPBA, Av. República de Italia 780, Azul (7300), Buenos Aires, Argentina.

E-mail addresses: [inesdinolfo@gmail.com](mailto:inesdinolfo@gmail.com), [inesdinolfo@faa.unicen.edu.ar](mailto:inesdinolfo@faa.unicen.edu.ar) (M.I. Dinolfo), [arataa@faa.unicen.edu.ar](mailto:arataa@faa.unicen.edu.ar) (A.F. Arata).

<sup>1</sup> Both authors contributed equally to this work.

brassinosteroids (BR) (Delplace et al., 2022). Regarding *Fusarium* species, Ding et al. (2011) concluded that in the first stage of *F. graminearum*-wheat infection (within 6 h after infection), an activation of SA related to hypersensitive response and cell death program was observed. However, between 6 h after inoculation (hai) and before 24 hai, JA/ET signaling pathway was activated, corresponding to necrotrophic plant resistance. The interplay among SA and JA/ET signaling pathways will be expected by a hemibiotrophic pathogen as *F. graminearum*. Also, the role of JA and its derivatives, such as methyl-jasmonate (MeJA) in *F. graminearum*-wheat interactions, was revealed by Makandar et al. (2012). Their results demonstrated that JA prevents SA activation in the early stage of infection and promotes resistance mechanisms during later infection stages. Thus, the crucial role of JA as major phytohormones involved in *F. graminearum*-wheat defense has been well documented (Qi et al., 2016; Sun et al., 2016; Jia et al., 2018).

Regarding *F. poae*, studies carried out in *Arabidopsis thaliana* have demonstrated the activation of ET and JA signaling pathways in *Arabidopsis* leaves against *F. poae* (Dinolfo et al., 2017). Considering the phytohormones involved, this pathogen responds to a necrotrophic life cycle. Nowadays, a vertical resistance against *Fusarium* has not been found. Thus, horizontal resistance through quantitative trait loci (QTL) is the current defense against these pathogens. Moreover, no fungicides treatment completely controlled FHB. The role of phytohormones in plant defense could be a valuable tool to reduce the impact of *Fusarium* spp. against crops. Therefore, our study aimed to evaluate the interaction between *F. poae* and wheat and elucidate whether the MeJA treatment could induce a response against *F. poae* in two wheat cultivars with contrasting *Fusarium* resistance. To our knowledge, no previous studies have evaluated the interaction between *F. poae* and wheat regarding genes signaling defense pathways.

## 2. Materials and methods

### 2.1. In vitro plate assay

The effect of MeJA on the *F. poae* growth was evaluated *in vitro*. Potato dextrose agar 2% (PDA) was amended with 0.2 mM MeJA (as used for inoculation) (Sigma–Aldrich, Missouri, US) and inoculated with a plug of four monospore *F. poae* (encoded as 37, 40, 43, and 47 isolates) previously selected based on its mycotoxin production (Dinolfo et al., 2012) used as inoculum, harvested from seven days old PDA plates. Plates amended with water were used as control and incubated at 25 °C in darkness. The colony diameter (cm) was measured after 72 h. The experiment was carried out twice, each containing three technical replicates per treatment. ANOVA was performed using InfoStat software (Di Rienzo et al., 2012), and the significance levels were calculated using Tukey's test at  $P < 0.05$ .

### 2.2. Plant material

For the present study, two hexaploid wheat (*Triticum aestivum* L.) with contrasting levels of *Fusarium* resistance were chosen. Apogee is a full-dwarf hard red spring wheat cultivar (pedigree: Parula/Super dwarf) released by the Utah Agric. Exp. Station in cooperation with NASA in 1996 (Li et al., 2017) with high susceptibility to *F. poae* (Stenglein et al., 2014). On the other hand, MS INTA 416 is a hard red winter wheat (pedigree [PROAS\*6/Pavon 76 T7AS-7S]\*2//SUMAI3) carrying *Fhb1* and *Lr47* resistance genes, conferring FHB and leaf rust resistance, respectively (Bainotti et al., 2017) and released by the Instituto Nacional de Tecnología Agropecuaria (INTA) in 2016. The experiment was carried out at the greenhouse of the Facultad de Agronomía, Universidad Nacional del Centro de la Provincia de Buenos Aires (36° 41 'S, 59° 48' W). Ten seeds were

placed in 20 L pots filled with clay loam soil resulting in a total of 24 pots for each cultivar. Plants were irrigated as needed to maintain humidity and fertilized twice with: 1.4 g of diammonium phosphate (DAP, 18-46-0), 1.4 g of urea (46-0-0), and 1 g of  $\text{Ca}_2\text{SO}_4$  (23.4% Ca, 18.6% S) in split doses at emergence and tillering.

### 2.3. Chemical application and fungal inoculation

Four treatments were applied: control/control (0/0), inoculated/control (1/0), control/MeJA treated (0/1), and inoculated/MeJA treated (1/1). To avoid different flowering times among wheat cultivars, MS INTA 416 was sown 18 days before to Apogee that allowed treating plants simultaneously. For MeJA treatment, wheat spikes were sprayed with 0.2 mM MeJA (Sigma–Aldrich, Missouri, US) or distilled water once a day for three consecutive days before *F. poae* inoculation, according to Sun et al. (2016). After chemical treatment, each wheat plant was covered with a polypropylene bag along with the treatment. A combination of the four *F. poae* isolates previously described were used for inoculation, as described by Dinolfo et al. (2017). Inoculation was performed by depositing 10  $\mu\text{l}$  of  $10^5$  conidia/ml with Tween 20 (0.01%) into a central spikelet of the spike. Inoculated plants were covered with clear plastic bags sprayed with distilled water during the assay. For the first 24 h, inoculated plants were kept in dark, and then under greenhouse conditions with controlled minimum temperatures around 14 °C. Control plants were treated by depositing 10  $\mu\text{l}$  of Tween 20 (0.01%) and covered as described for inoculated plants.

### 2.4. Quantification of fungal sporulation

A total of five spikes at 7, 10, 14, and 21 days after inoculation (dai) were pooled in sterile Erlenmeyer flasks containing 15 ml of sterile water and shaken for 30 min at 180 rpm. Then, the resulting suspension was filtered and quantified (conidia/ml) using a Neubauer haemocytometer and a binocular microscope (Olympus CX 31®). ANOVA was performed using InfoStat software (Di Rienzo et al. 2012), and the significance levels were calculated using Tukey's test at  $P < 0.05$ .

### 2.5. Quantification of *F. poae* genomic DNA

Five spikes were collected at 24, 72, and 168 hai in liquid nitrogen in the 1/0 and 1/1 treatments. Total genomic DNA from 100 mg of ground spikes was extracted using the cetyltrimethylammonium bromide (CTAB) method, according to Stenglein and Balatti (2006). The DNA quality was examined by electrophoresis in 0.8% (w/v) agarose gels containing GelRed™ (Biotium, California, US) at 80 V in 1X Trisborate-EDTA buffer for 3 h at room temperature, and the visualization was made under UV light. The DNA concentration was calculated using a Fluorometer (Qubit Fluorometer, Invitrogen) and was diluted to 10 ng/ $\mu\text{l}$ . A species-specific qPCR was made to quantify *F. poae* genomic DNA using the primer developed by Nicolaisen et al. (2009). Real-time PCR was carried out in a total of 10  $\mu\text{l}$  consisting of 5  $\mu\text{l}$  2 x SsoAdvanced™ Universal SYBR® Green Supermix (BIO-RAD), 0.1  $\mu\text{M}$  of each primer, and 2  $\mu\text{l}$  template DNA. PCR reactions were performed twice on an Applied Biosystems 7500 real-time PCR system (Thermo Fisher Scientific) using the following cycling protocol: 2 min at 50 °C; 95 °C 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 1 min followed by dissociation curve analysis at 60–95 °C. A standard curve was run with a pure *F. poae* DNA using six different dilutions: from 10 ng/ $\mu\text{l}$  to 0.0001 ng/ $\mu\text{l}$ . ANOVA was performed using InfoStat software (Di Rienzo et al. 2012), and the significance levels were calculated using Tukey's test at  $P < 0.05$ .

## 2.6. Real-time PCR gene expression

The expression of several genes was analyzed at 0, 24, and 72 hai for all the treatments.

For RNA extraction, TRI® reagent (Sigma–Aldrich, Missouri, US) was used following the manufacturer's instructions. The dry pellet was dissolved in 20 µl of distilled water and was stored at –80 °C. The RNA concentration was calculated before cDNA synthesis using a fluorometer (Qubit Fluorometer; Invitrogen). Total RNA (1 µg) was used to synthesize cDNA using M-MuLV reverse transcriptase (New England Biolabs, Hitchin, Hertfordshire, UK) in a final volume of 30 µl. The cDNA was used as a template. The expression of marker genes linked with JA and SA signaling pathways, *PDF1.2* (*PLANT DEFENSIN 1.2*) and *PR1-1* (*PATHOGENESIS-RELATED 1*), respectively, were determined by RT-qPCR using specific primers (Qi et al., 2016; Desmond et al., 2006). The expression of these genes was normalized with *elongation factor-1α* (*ef1α*) gene expression chosen by its stability under biotic stresses (Beccari et al., 2011). The final threshold cycle (Ct) values were the mean of biological triplicates with technical duplicates. The comparative  $\Delta\Delta C_t$  method was used to evaluate the relative quantities of each amplified product in the samples. The Ct was automatically determined by the Applied Biosystems 7500 real-time PCR system (Thermo Fisher Scientific). ANOVA was performed using InfoStat software (Di Rienzo et al. 2012), and the significance levels were calculated using Tukey's test at  $P < 0.05$ .

## 3. Results

### 3.1. In vitro plate assay

The growth of the four *F. poae* isolates, evaluated separately, showed different behavior against the treatment with MeJA. The most affected *F. poae* isolate was 47 with a decrease of 19.36% compared to control, followed by the 43 isolate that decreased by 15.32% this parameter. The 40 and 37 *F. poae* isolates were the less affected by MeJA treatment (4.69 and 1.36%, respectively) (Fig. 1).

### 3.2. Quantification of fungal sporulation

The results showed no statistical differences between both cultivars, although MS INTA 416 showed a low number of conidia compared to Apogee ( $4.63 \pm 4.97$  vs.  $5.01 \pm 3.15$  conidia/ml). The MeJA treatment showed a decrease in the conidial number in both cultivars. In Apogee, the MeJA treatment reduces the conidial number by 40.29% compared to the control ( $6.28 \pm 5.39$  vs.  $3.75 \pm 4.28$  conidia/ml). In MS INTA 416, the conidial number

showed a reduction by 37.72% ( $5.70 \pm 3.65$  vs.  $3.55 \pm 2.13$  conidia/ml). However, these differences were not statistically significant (Fig. 2A). As regards the days after inoculation, at 7 and 14 dai did not show differences. At 14 dai, MS INTA 416 showed differences statistically significant among the treatments, observing differences in Apogee only at 21 dai (Fig. 2B).

### 3.3. Quantification of *F. poae* genomic DNA

The results of fungal quantification were made for each wheat cultivar separately. In Apogee, the only variable that was statistically significant was the treatment. The inoculation with *F. poae* + MeJA showed a reduction in the amount of fungal DNA with a mean of  $0.72 \pm 1.02$  compared to the inoculation with *F. poae* with a mean of  $3.09 \pm 2.52$ . The remaining variables as hai and the interaction hai\*treatment were not statistically significant (Fig. 3A; Table 1).

In MS INTA 416, none of the variables analyzed were statistically significant (Fig. 3B; Table 1).

### 3.4. Real-time PCR gene expression

The transcript levels of *PR1-1* and *PDF1.2* were quantified to evaluate the role of hormones that contribute to the defense against pathogens. The expression of these genes was evaluated in control (0/0) and MeJA treatment plants (0/1) to analyze if the pretreatment induces gene expression before *F. poae* inoculation. The results showed that *PR1-1* and *PDF1.2* were expressed after the MeJA treatment compared to control only for Apogee. In MS INTA 416, the changes registered in the expression of these genes were not significant compared to the control (Fig. 4). Moreover, the application of MeJA produced the down-regulation of *PR1-1* and *PDF1.2* in both cultivars at 24 and 72 hai. Regarding *F. poae* treatments, gene expression results were obtained from each treatment (1/0 and 1/1) compared to the control (0/0). The presence of *F. poae* only and the presence of *F. poae* with a MeJA treatment in Apogee produced the down-regulation of *PR1-1* and *PDF1.2* gene expression at 24 hai, but an up-regulation of both genes was observed at 72 hai. In MS INTA 416, the up-regulation of both genes was observed at 24 hai in the *F. poae* treatment, and this expression was kept at 72 hai. In the *F. poae*-MeJA treatment, the *PR1-1* gene was up-regulated, increasing with the hours of inoculation. Regarding *PDF1.2*, a down-regulation was observed at 24 hai, but the expression increased at 72 hai (Fig. 4).

## 4. Discussion

*F. poae* is a pathogen considered as a “minor” *Fusarium* species, but its prevalence has increased in the last years worldwide (Okorski et al., 2022). Moreover, this species acquired particular importance considering its high capacity to produce NIV and a wide range of mycotoxins. However, no studies have been developed to understand how important crops, such as wheat, activate the plant defense against *F. poae*. To our knowledge, this study represents the first evaluation of wheat phytohormones in the presence of *F. poae*. Our results showed that the presence of MeJA reduced the diameter colony of *F. poae* isolates. However, the differences were only statistically significant for two isolates tested. Other studies have evaluated the growth of *F. graminearum* in liquid and solid media amended with increasing concentration of SA (Qi et al., 2012). The results revealed that *F. graminearum* could degrade SA. However, in higher concentrations (400 µM), SA inhibited the growth and germination of *F. graminearum*, showing an effect of phytohormones in the pathogen behavior. The impact of epibrassinolide (epiBL) on *Fusarium culmorum* growth was also evaluated (Ali et al., 2013). In

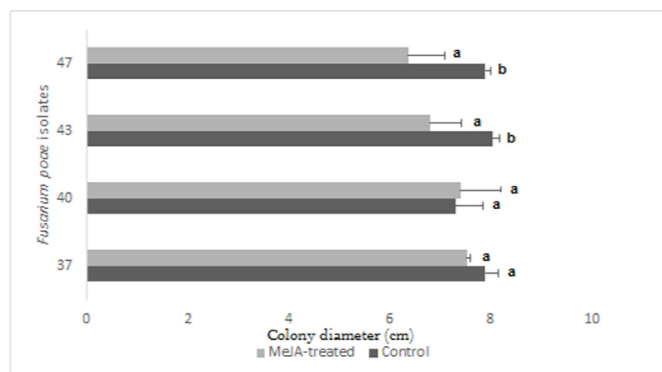
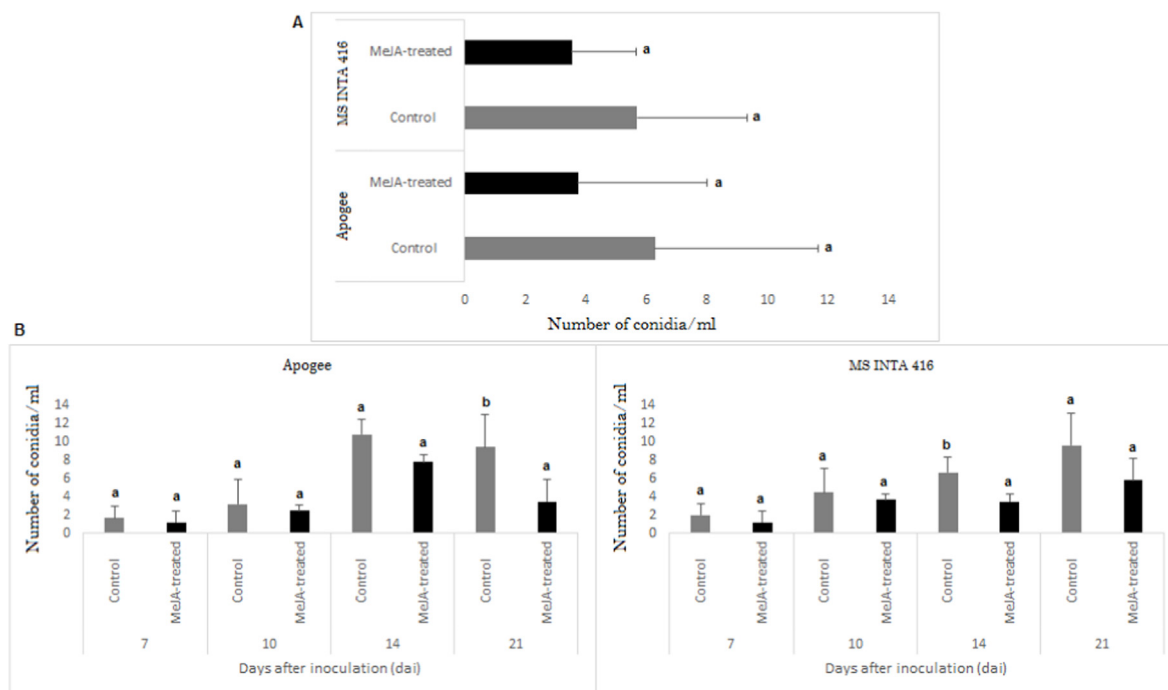
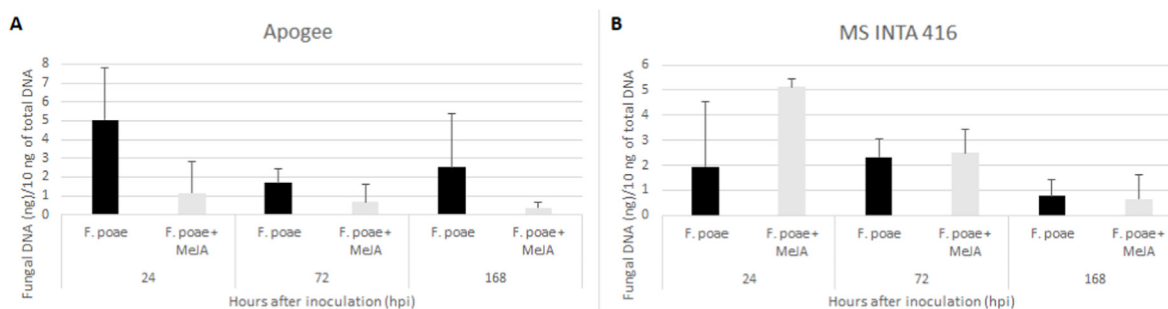


Fig. 1. Growth (in cm) of four *F. poae* isolates in PDA plates amended with 0.2 mM methyl-jasmonate (MeJA) and control.



**Fig. 2.** (A) Average of number of *Fusarium poae* conidia/ml for the total of days after inoculation evaluated in Apogee and MS INTA 416 inoculated with *F. poae* and treated with 0.2 mM MeJA (B) Number of *F. poae* conidia in both cultivars at 7, 10, 14, and 21 days after inoculation (dai).



**Fig. 3.** *Fusarium poae* biomass quantification at 24, 72, and 168 hpi in inoculated/control (1/0) and inoculated/MeJA pretreatment (1/1) in (A) Apogee and (B) MS INTA 416.

**Table 1**

Analysis of variance for fungal DNA (ng)/10 ng of total DNA for Apogee (A) and MS INTA 416 (B). S.V.: source of variation; d.f.: degree of freedom.

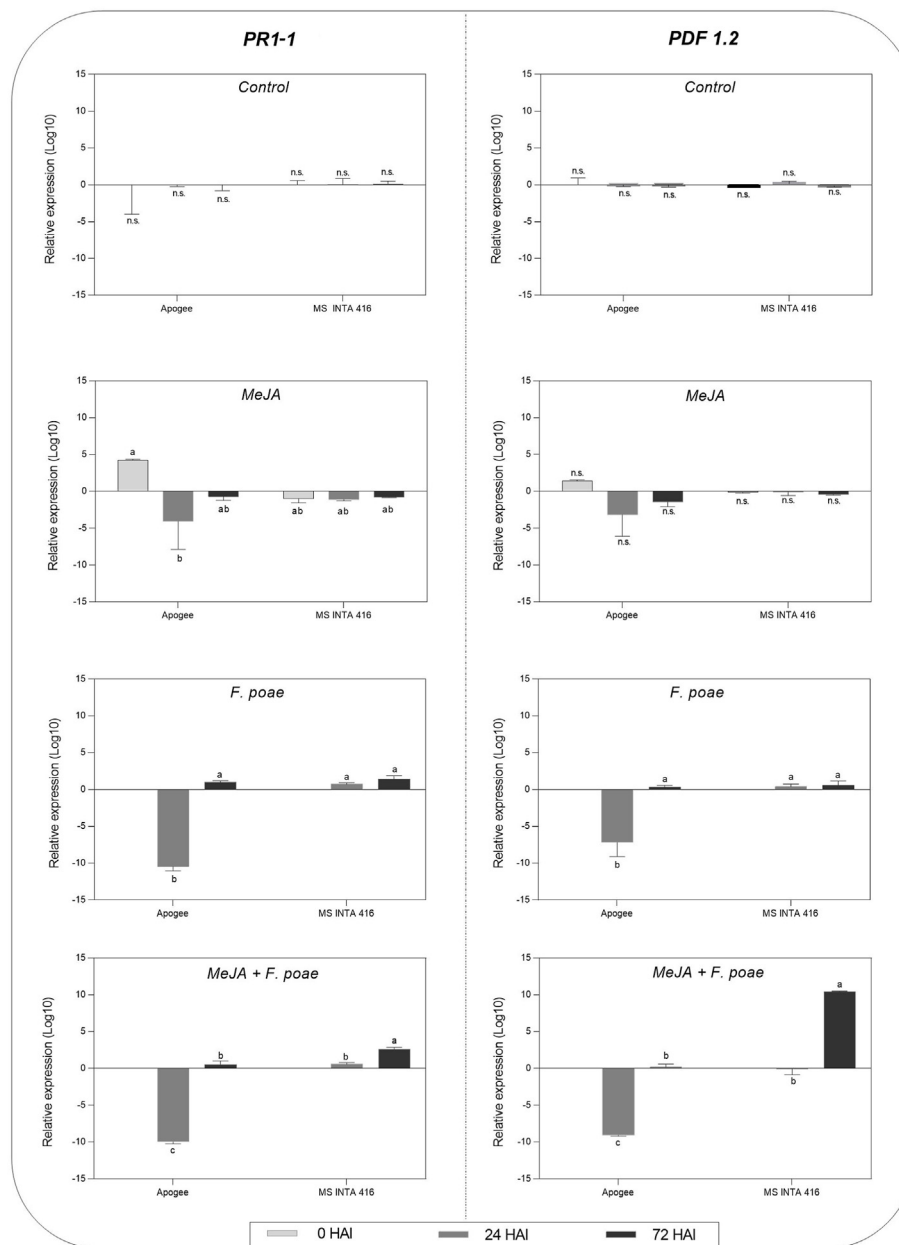
Apogee			
S.V.	d.f.	F-value	P-value
HAI (H)	2	1.96	0.1829
Treatments (T)	1	7.60	<b>0.0174*</b>
H x T	2	0.95	0.4146
MS INTA 416			
S.V.	d.f.	F-value	P-value
HAI (H)	2	2.69	0.1086
Treatments (T)	1	1.15	0.3048
H x T	2	1.11	0.3606

this study, the epiBL treatment did not reduce the fungal growth in any concentration evaluated with a maximum of 200  $\mu$ M. Moreover, no effect of AUX and ABA on the *F. culmorum* growth, irrespective of the hormone concentration, has been found (Petti et al., 2012). Thus, the effect of phytohormones on fungal growth could depend on the type of phytohormone and the fungus evaluated.

The exogenous application of phytohormones improves plant resistance as observed in different pathosystems as *Blumeria graminis* f. sp. *tritici*-wheat (Duan et al., 2014), *F. culmorum*-wheat (Motallebi et al., 2017), *Botrytis cinerea*-grape berries (Wang et al., 2015), *Erysiphe graminis* f. sp. *hordei*-barley (Schweizer et al., 1993), *Tilletia laevis*-wheat (Lu et al., 2006), among others. In our study, the exogenous application of MeJA before *F. poae* inoculation was evaluated as a possible tool to reduce the fungal presence on two wheat cultivars with a contrasting level of *Fusarium* resistance. Our results demonstrated that the MeJA treatment reduces the number of conidia at 7, 10, 14, and 21 dai in both cultivars evaluated. Regarding fungal quantification, the MeJA treatment reduces significantly the *F. poae* DNA quantity compared to the inoculated ones in the Apogee susceptible cultivar. In the MS INTA 416 resistant cultivar, the MeJA treatment increased the *F. poae* DNA quantity, but the differences were not statistically significant.

The presence of *F. poae* could be related to the presence of important mycotoxins such as NIV. Studies carried out by Yli-Mattila et al. (2008) have found a significant correlation among the presence of *F. poae* and NIV production. For future works, it





**Fig. 4.** The *PR1-1* and *PDF1.2* gene expression in the following treatments: control/control (0/0); control/MeJA pretreatment (0/1) inoculated/control (1/0); and inoculated/MeJA pretreatment (1/1). The results were obtained by comparing of the changes in relative gene expression of *PR1-1* and *PDF1.2* between each treatment (0/1, 1/0, and 1/1) with the control (0/0).

would be interesting to evaluate the response of MeJA against not only the pathogen but also the mycotoxin produced. Sun et al. (2016) evaluated the exogenous application of JA and ET in wheat defense against *F. graminearum*, the main agent-causal of FHB. Using a Wangshuibai resistant cultivar and two susceptibles cultivars, NAUH117 and Alondra, the percentage of scabbed spikelets (PSS) was measured after the MeJA and ethephon (an ethylene-releasing compound) application and *F. graminearum* inoculation. The results demonstrate that the MeJA treatment in the resistant cultivar did not change the PSS values. However, the treatment showed an effect on PSS in the susceptible cultivars decreasing these values in both cultivars. The ethephon treatment did not show any impact on any of the cultivars treated. Motallebi et al. (2017) evaluated the disease severity (DS) in two different bread wheat cultivars (Sumai 3 resistant vs. Falat susceptible) pretreated

with MeJA against *F. culmorum*. The cv. Falat treated with MeJA + *F. culmorum* showed a decrease in DS compared with *F. culmorum* alone. Interestingly, Sumai 3 did not show statistical differences among both treatments. As observed in our results, these works demonstrated that the treatment with MeJA could improve the behavior of susceptible cultivars against *Fusarium* spp., not showing significant differences in resistance ones.

Regarding gene expression, our results showed that the *PR1-1* and *PDF1.2* genes were induced later in Apogee than in MS INTA 416 after being inoculated with *F. poae*. Moreover, the induction of these genes at 72 hpi could be related to the decrease in DNA fungal biomass observed at this time in Apogee compared to 24 hpi. Then, at 168 hpi, an increase of *F. poae* DNA quantity was observed, which could indicate that the later expression of the genes would not be efficient to reduce effectively the fungal growth. Therefore, the

expression level of both genes in MS INTA 416 did not show statistical differences between 24 and 72 hai. Desmond et al. (2006) observed the induction of some genes related to SA signaling pathways in two wheat cultivars with contrasting crown rot disease levels (Sunco partially-field resistant cultivar and Kennedy susceptible cultivar) after inoculation with *Fusarium pseudograminearum*. Some genes, such as *PR1-1* was induced more rapidly in Sunco than in Kennedy. Moreover, this gene was strongly induced by MeJA pretreatment in both cultivars.

As the results observed with the *F. poae* inoculation alone, in our study, the application of MeJA followed by *F. poae* inoculation showed that *PR1-1* and *PDF1.2* genes were also induced later in Apogee compared to MS INTA. In MS INTA 416, the expression level of *PR1-1* and *PDF1.2* was noticeably increased at 72 hai compared to *F. poae* inoculation alone. Our results demonstrated that in the susceptible cultivars, the induction of some genes occurred later than in the resistant ones. Other authors have observed these differences in gene induction. For instance, Gottwald et al. (2012) found that the inoculation of *F. graminearum* in two resistant cultivars (Dream and Sumai 3) produced an earlier induction of several genes related to FHB resistance, while those gene inductions were late and temporary in susceptible cultivar (Lynx cultivar). Therefore, the responses of wheat cultivars with different behavior against *Fusarium* spp. could be due to the time of induction of defense-related genes in the pathogen presence.

Our results demonstrated that: i) the MeJA treatment have negative effects on the *F. poae* fungal growth being isolates dependent; ii) the MeJA treatment reduces the growth of *F. poae*; iii) the early expression of *PR1-1* and *PDF1.2* in resistant cultivar could explain its behavior compared to the susceptible cultivar. The application of MeJA combined with the *F. poae* inoculation increased *PR1-1* and *PDF1.2* expressions in resistant cultivars.

This study represents the first work that evaluates the interaction of two different bread wheat with contrasting FHB resistance against *F. poae* and MeJA application as a possible tool to reduce the fungal presence according to the behavior of the different wheat responses against *Fusarium*.

## Declaration of competing interest

None.

## Acknowledgements

We kindly thank the Laboratorio Azul S.A for allowing the use of their qPCR equipment to perform gene expression.

## References

- Ali, S.S., Sunil Kumar, G.B., Khan, M., Doohan, F.M., 2013. Brassinosteroid enhances resistance to *Fusarium* diseases of barley. *Phytopathology* 103, 1260–1267.
- Bainotti, C.T., Lewis, S., Campos, P., Alberione, E., Salines, N., Gomez, D., Frascina, J., Salines, J., Formica, M.B., Donaire, G., Vanzetti, L.S., Lombardo, L., Nisi, M.S., Cuniberti, M.B., Mir, L., Conde, M.B., Helguera, M., 2017. MS INTA 416: a new Argentinean wheat cultivar carrying *Fhb1* and *Lr47* resistance genes. *Crop Breed Appl Biot* 17, 274–280.
- Beccari, G., Covarelli, L., Nicholson, P., 2011. Infection processes and soft wheat response to root rot and crown rot caused by *Fusarium culmorum*. *Plant Pathol.* 60, 671–684.
- Chrpová, J., Šíp, V., Šumíková, T., Salava, J., Palicová, J., Štočková, L., Džuman, Z., Hajslová, J., 2016. Occurrence of *Fusarium* species and mycotoxins in wheat grain collected in the Czech Republic. *World Mycotoxin J.* 9, 317–327.
- Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C., Ferrer, E., Manes, J., 2015. *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *J. Sci. Food Agric.* 95, 540–551.
- de Chaves, M.A., Reginatto, P., da Costa, B.S., de Paschoal, R.I., Teixeira, M.L., Fuentefria, A.M., 2022. Fungicide resistance in *Fusarium graminearum* species complex. *Curr. Microbiol.* 79, 62.

- Delplace, F., Huard-Chauveau, C., Berthomé, R., Roby, D., 2022. Network organization of the plant immune system: from pathogen perception to robust defense induction. *Plant J.* 109, 447–470.
- Desmond, O., Edgar, C.I., Manners, J.M., Maclean, D.J., Schenk, P.M., Kazan, K., 2006. Methyl jasmonate induced gene expression in wheat delays symptom development by the crown rot pathogen *Fusarium pseudograminearum*. *Physiol. Mol. Plant Pathol.* 67, 171–179.
- Ding, L., Xu, H., Yi, H., Yang, L., Kong, Z., Zhang, L., Xue, S., Jia, H., Ma, Z., 2011. Resistance to hemi-biotrophic *F. graminearum* infection is associated with co-ordinated and ordered expression of diverse defense signaling pathways. *PLoS One* 6, e19008.
- Dinolfo, M.I., Barros, G.G., Stenglein, S.A., 2012. Development of a PCR assay to detect *Fusarium poae* isolates with the potential to produce nivalenol. *FEMS Microbiol. Lett.* 332, 99–104.
- Dinolfo, M.I., Castañares, E., Stenglein, S.A., 2017. Resistance of *Fusarium poae* in *Arabidopsis* leaves requires mainly functional JA and ET signaling pathways. *Fungal Biol* 121, 841–848.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., González, L., Tablada, M., Robledo, C.W., 2012. InfoStat. version. Universidad Nacional de Córdoba, Argentina: Grupo InfoStat.
- Drakopoulos, D., Sulyok, M., Jenny, E., Kägi, A., Bänziger, L., Logrieco, A.F., Krska, R., Vogelgsang, S., 2021. *Fusarium* head blight and associated mycotoxins in grains and straw of barley: influence of agricultural practices. *Agronomy* 11, 801.
- Duan, Z., Lv, G., Shen, C., Li, Q., Qin, Z., Niu, J., 2014. The role of jasmonic acid signaling in wheat (*Triticum aestivum* L.) powdery mildew resistance reaction. *Eur. J. Plant Pathol.* 140, 169–183.
- Gottwald, S., Samans, B., Lück, S., Friedt, W., 2012. Jasmonate and ethylene dependent defence gene expression and suppression of fungal virulence factors: two essential mechanisms of *Fusarium* head blight resistance in wheat? *BMC Genom.* 13, 369.
- Hietaniemi, V., Ramo, S., Yli-Mattila, T., Jestoi, M., Peltonen, S., Kartio, M., Sieviläinen, E., Koivisto, T., Parikka, P., 2016. Updated survey of the *Fusarium* species and toxins in Finnish cereal grains. *Food Addit. Contam.* 33, 831–848.
- Jia, H., Zhou, J., Xue, S., Li, G., Yan, H., Ran, C., Zhang, Y., Shi, J., Jia, L., Wang, X., Luo, J., Ma, Z., 2018. A journey to understand wheat *Fusarium* head blight resistance in Chinese wheat landrace Wangshuibai. *Crop J* 6, 48–59.
- Li, G., Boontang, R., Powers, C., Belamkar, V., Huang, T., Miao, F., Yan, L., 2017. Genetic basis of the very short life cycle of ‘Apogee’ wheat. *BMC Genom.* 18, 1–12.
- Lu, Z.X., Gaudet, E., Puchalski, B., Despina, T., Frick, M., Laroche, A., 2006. Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defence-gene expression. *Physiol. Mol. Plant Pathol.* 67, 138–148.
- Makandar, R., Nalam, V.J., Lee, H., Trick, H.N., Don, Y., Shah, J., 2012. Salicylic acid regulates basal resistance to *Fusarium* head blight in wheat. *Mol. Plant Microbe Interact.* 25, 431–439.
- Motallebi, P., Tonti, S., Niknam, V., Ebrahimzadeh, H., Pisi, A., Nipoti, P., Hashemi, M., Prodi, A., 2017. Induction of basal resistance by methyl jasmonate against *Fusarium culmorum* in bread wheat. *Cereal Res. Commun.* 45, 248–259.
- Nazari, L., Patteri, E., Manstretta, V., Terzi, V., Morcia, C., Somma, S., Miretti, A., Ritieni, A., Rossi, V., 2018. Effect of temperature on growth, wheat head infection, and nivalenol production by *Fusarium poae*. *Food Microbiol.* 76, 83–90.
- Nicolaisen, M., Supronienė, S., Nielsen, L.K., Lazzaro, I., Spliid, N.H., Justesen, A.F., 2009. Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. *J. Microbiol. Methods* 76, 234–240.
- Nogueira, M.S., Decundo, J., Martinez, M., Dieguez, S.N., Moreyra, F., Moreno, M.V., Stenglein, S.A., 2018. Natural contamination with mycotoxins produced by *Fusarium graminearum* and *Fusarium poae* in malting barley in Argentina. *Toxins* 10, 78.
- Okorski, A., Milewska, A., Pszczółkowska, A., Karpiesiuk, K., Kozera, W., Dąbrowska, J.A., Radwińska, J., 2022. Prevalence of *Fusarium* fungi and deoxynivalenol levels in winter wheat grain in different climatic regions of Poland. *Toxins* 14, 102.
- Pereira, C.B., Ward, T.J., Del Ponte, E.M., Moreira, G.M., Busmam, M., McCormick, S.M., Feksa, H.R., De Almeida, J.L., Tessmann, D.J., 2021. Five-year survey uncovers extensive diversity and temporal fluctuations among *Fusarium* head blight pathogens of wheat and barley in Brazil. *Plant Pathol.* 70, 426–435.
- Petti, C., Reiber, K., Ali, S.S., Berney, M., Doohan, F.M., 2012. Auxin as a player in the biocontrol of *Fusarium* head blight disease of barley and its potential as a disease control agent. *BMC Plant Biol.* 12, 224.
- Qi, P.F., Johnston, A., Balcerzak, M., Rocheleau, H., Harris, L.J., Long, X.Y., Wei, Y.L., Zheng, Y.L., Ouellet, T., 2012. Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of *Fusarium* head blight in wheat. *Fungal Biol* 116, 413–426.
- Qi, P.F., Balcerzak, M., Rocheleau, H., Leung, W., Wei, Y.M., Zheng, Y.L., Ouellet, T., 2016. Jasmonic acid and abscisic acid play important roles in host-pathogen interaction between *Fusarium graminearum* and wheat during the early stages of *Fusarium* head blight. *Physiol. Mol. Plant Pathol.* 93, 39–48.
- Schweizer, P., Gees, R., Mosinger, E., 1993. Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f. sp. *Hordei*. *Plant Physiol.* 102, 503–511.
- Stenglein, S.A., Balatti, P.A., 2006. Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. *Physiol. Mol. Plant Pathol.* 68, 158–167.
- Stenglein, S.A., Dinolfo, M.I., Barros, G.G., Bongiorno, F., Chulze, S.N., Moreno, M.V., 2014. *Fusarium poae* pathogenicity and mycotoxin accumulation on wheat and barley. *Plant Dis.* 98, 1733–1738.

- Sun, Y., Xiao, J., Jia, X., Ke, P., He, L., Cao, A., Wang, H., Wu, Y., Gao, X., Wang, X., 2016. The role of wheat jasmonic acid and ethylene signaling pathways in response to *Fusarium graminearum* infection. *Plant Growth Regul.* 80, 69–77.
- Wang, K., Liao, Y., Kan, J., Han, L., Zheng, Y., 2015. Response of direct or priming defense against *Botrytis cinerea* to methyl jasmonate treatment at different concentrations in grape berries. *Int. J. Food Microbiol.* 194, 32–39.
- Yli-Mattila, T., Paavanen-Huhtala, S., Jestoi, M., Parikka, P., Hietaniemi, V., Gagkaeva, T., Sarlin, T., Haikara, A., Lakaksonen, S., Rizzo, A., 2008. Real-time PCR detection and quantification of *Fusarium poae*, *F. graminearum*, *F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. *Arch. Phytopathol. PFL* 41, 243–260.