Article

Major Facilitator Superfamily Transporter Gene FgMFS1 Is Essential for Fusarium graminearum to Deal with Salicylic Acid Stress and for Its Pathogenicity towards Wheat

Qing Chen 1,2,†, Lu Lei 2,†, Caihong Liu 2, Yazhou Zhang 2, Qiang Xu 2, Jing Zhu 2, Zhenru Guo 2, Yan Wang 2,‡, Qingcheng Li 2, Yang Li 2, Li Kong 2, Yunfeng Jiang 2, Xiujin Lan 2, Jirui Wang 1,2, Qiantao Jiang 2,‡, Guoyue Chen 1,2, Jian Ma 2, Yuming Wei 1,2, Youliang Zheng 2 and Pengfei Qi 2,*

1 State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Chengdu 611130, China; qingchen83@sicau.edu.cn (Q.C.); jirui.wang@gmail.com (J.W.); gychen@sicau.edu.cn (G.C.); ymwei@sicau.edu.cn (Y.W.)
2 Triticeae Research Institute, Sichuan Agricultural University, Chengdu 611130, China; leilu@stu.sicau.edu.cn (L.L.); rainbow@stu.sicau.edu.cn (C.L.); yazhou1012@gmail.com (Y.Z.); xuqiang1264700418@163.com (Q.X.); sicauZJing@163.com (J.Z.); guozhenru@stu.sicau.edu.cn (Z.G.); wyangy@stu.sicau.edu.cn (Y.W.); lqlq@126.com (Q.L.); liyang1@stu.sicau.edu.cn (Y.L.); kongli@sicau.edu.cn (L.K.); jiangyunfeng2018@163.com (Y.J.); lansxj@163.com (X.L.); qiantaojiang@sicau.edu.cn (Q.J.); jianma@sicau.edu.cn (J.M.); ylzheng@sicau.edu.cn (Y.Z.)
* Correspondence: pengfeiqi@hotmail.com; Tel.: +86-28-82650337; Fax: +86-28-82650350
† Contributed equally to this paper.

Abstract: Wheat is a major staple food crop worldwide, due to its total yield and unique processing quality. Its grain yield and quality are threatened by Fusarium head blight (FHB), which is mainly caused by Fusarium graminearum. Salicylic acid (SA) has a strong and toxic effect on F. graminearum and is a hopeful target for sustainable control of FHB. F. graminearum is capable of efficient dealing with SA stress. However, the underlying mechanisms remain unclear. Here, we characterized FgMFS1 (FGFSG_03725), a major facilitator superfamily (MFS) transporter gene in F. graminearum. FgMFS1 was highly expressed during infection and was upregulated by SA. The predicted three-dimensional structure of the FgMFS1 protein was consistent with the schematic for the antiporter. The subcellular localization experiment indicated that FgMFS1 was usually expressed in the vacuole of hyphae, but was alternatively distributed in the cell membrane under SA treatment, indicating an element of F. graminearum in response to SA. ΔFgMFS1 (loss of function mutant of FgMFS1) showed enhanced sensitivity to SA, less pathogenicity towards wheat, and reduced DON production under SA stress. Re-introduction of a functional FgMFS1 gene into ΔFgMFS1 recovered the mutant phenotypes. Wheat spikes inoculated with ΔFgMFS1 accumulated more SA when compared to those inoculated with the wild-type strain. Ecotopic expression of FgMFS1 in yeast enhanced its tolerance to SA as expected, further demonstrating that FgMFS1 functions as an SA exporter. In conclusion, FgMFS1 encodes an SA exporter in F. graminearum, which is critical for its response to wheat endogenous SA and pathogenicity towards wheat.

Keywords: MFS transporter; fusarium head blight; phytohormone; sustainable disease control

1. Introduction

Wheat (Triticum aestivum) is a major staple food crop due to its total grain yield and unique processing quality. Fusarium graminearum is the major causal agent for Fusarium head blight (FHB), which seriously threatens wheat production [1]. FHB leads to yield loss, poor bread-making quality, and contamination of trichothecene mycotoxins (mainly deoxynivalenol (DON)) in grains [1–3]. The contaminated grains are proven to be unsuitable for human consumption and animal feed. In humans, trichothecene mycotoxins cause weight loss, diarrhea, hemorrhage, immunomodulation, emesis, and even death [4,5].
DON, a major virulence factor of *F. graminearum* during infection, is critical for its spread in spikes [6]. There is a great demand for FHB management to reduce its threat in Food and Agriculture.

Novel and sustainable strategies are strongly required for managing FHB. At present, chemical control, resistive breeding, and biological control are three main strategies to control FHB [7–10]. Nevertheless, these methods cannot control this disease well in the field under current conditions. Salicylic acid (SA) is a key phytohormone in wheat resistance against FHB [11–14]. *F. graminearum* infection in wheat heads can significantly increase the accumulation of SA [14], and SA signaling plays an important role in wheat FHB resistance [12,15]. Besides SA signaling, SA has a direct, strong, and toxic effect on conidial germination, mycelial growth, and DON production [13], partially because SA is capable of destroying the fungal cell membrane by reducing the expression level of *FgLAI12* (a linoleic acid isomerase gene) and the inner cell wall by inhibiting the expression of *FgCHS8* (a chitin synthase gene), which are required for the response of *F. graminearum* to environmental stresses, including SA [16,17]. It seems likely that SA is a hopeful and valuable target for sustainable control of FHB. Nevertheless, *F. graminearum* has an efficient system to deal with SA stress, including the capacity to metabolize and to export SA [13,18–20] and to strengthen the outer cell wall [21] (Figure 1). Considering the potential value of SA in designing a sustainable way of managing FHB, it is necessary to further elucidate the molecular mechanisms of *F. graminearum* in response to SA stress.

Transport systems play a critical role in the export of secondary metabolites and waste compounds during infection [22]. In fungi, ATP-binding cassette (ABC) and the major facilitator superfamily (MFS) transporter proteins are two main classes of transporters [22]. As a class of secondary active transporters, the MFS transporters couple the transportation of substrates to proton motive force generated across the cell membrane, rather than ATP hydrolysis, and play a crucial role in diverse physiological processes [23,24]. MFS transporters target a wide spectrum of substrates including lipids, ions, amino acids and peptides, carbohydrates, and nucleosides [25]. Members of this superfamily have been divided into 17 distinct families by phylogenic analysis [26] and into antiporters, uniporters, and symporters, which move substrates across the membranes via exchange, facilitated diffusion, and cotransport, respectively [27]. As one of the 17 families, DHA1 (drug: H+ antiporter-1) has 12-transmembrane-spanner (TMS), and members of this family are antiporters [26]. In *Fusarium*, the MFS transporter TRI12 is attributed to secrete trichothecene mycotoxins [28]; the MFS transporter gene (*FIR1*) is related to siderophore production [29]. It remains unclear whether any MFS transporter contributes *F. graminearum* to reduce the toxicity of SA during infection in wheat spikes.

We previously found that a candidate MFS transporter gene *FGSG_03725* was upregulated by SA [13], which had a high expression level during the infection of *F. graminearum* in wheat spikes [30], indicating that it was involved in the mechanisms alleviating SA. Using the wild-type (WT) strain and the mutants, we determined the effects of *FgMFS1* on mycelial growth, sensitivity to SA, DON production, SA accumulation in spikes, and visual FHB disease symptom. This research deepens our understanding of the mechanisms of *F. graminearum* in response to SA during the wheat/*F. graminearum* interaction and may provide key information for designing an efficient and sustainable strategy to control FHB by relying on wheat endogenous SA.
Figure 1. Schematic of the mechanisms on F. graminearum to deal with the toxicity of SA, based on the reported data. Gene names in red and in black indicate the upregulation and downregulation under SA stress, respectively. The red dotted arrows indicate the export of SA. The blue dotted arrows indicate the proposed alternate non-oxidative decarboxylation of SA to catechol via 2, 3 dihydroxybenzoic acid. The black dotted arrow represents the alternative subcellular localization of FgMFS1 before and after adding SA. FgCWM1 (cell wall mannoprotein 1) is an important component of the outer cell wall and is critical for maintaining the strength of the cell wall. Its expression is up-regulated by SA [21]; FgABCC9 (ATP-binding cassette transporter family C 9) is up-regulated by SA. It is distributed in the cell membrane and cost ATP to export SA [18]; Chitin is a major component of the inner cell wall. FgCHS8 (chitin synthase 8) encodes a chitin synthase (EC 2.1.13.1) that catalyzes polymerization of chitin from UDP-N-acetyl-alpha-D-glucosamine [31,32]. FgCHS8 is an integral membrane protein, and its expression is down-regulated by SA [16]; FgLAI12 (linoleic acid isomerase, EC 5.2.1.5) catalyzes the transformation of linoleic acid to cis-9,trans-11 conjugated linoleic acid. Its expression is down-regulated by SA. FgLAI12 is usually localized in vacuoles even under SA treatment and alternatively expressed in the cell membrane when linoleic acid is added [17]; FgNahG (salicylate hydroxylase, EC 1.14.13.1) catalyzes SA to catechol, which is widely distributed in fungal cells. Its expression is up-regulated by SA [19]; FGSG_03657 (salicylate 1-monoxygenase, EC 1.14.13.1) is up-regulated by SA, which catalyzes SA into catechol [13,20]; FGSG_09061 (2, 3-dihydroxybenzoic acid decarboxylase) is involved in a proposed alternate non-oxidative decarboxylation of SA to catechol via 2, 3 dihydroxybenzoic acid. Its expression is up-regulated by SA [13,20]; FGSG_03667 (catechol 1, 2-dioxygenase) catalyzes catechol into cis, cis-muconate [20]. Its expression is induced by SA.

2. Results
2.1. Sequence Analysis

FgMFS1 (FGSG_03725) is 1926 bp in length with no intron, and its open reading frame (ORF) is 1809 bp. The deduced peptide has 602 residues and 12 transmembrane-spanners (TMS) (Figure 2). Phylogenetic analysis demonstrates that FgMFS1 belongs to the cluster II of the DHA1 (drug: H+ antiporter-1) family (Figure 3). It suggests that when protons flow in, FgMFS1 can export its substrate.
Figure 2. Structure of FgMFS1. (A) Deduced amino acid sequence of FgMFS1. The underlined are the 12 transmembrane spanners (TMS; I-XII). (B) Predicted tertiary structure of FgMFS1. The 12 TMS are colored from the N-terminus in blue to the C-terminus in red. Consistent with [25], TMS I, IV, VII, and X are in the center of the predicted structure; II, V, VIII, and XI are on the sides; III, VI, IX, and XII are placed on the outside. (C) Simplified topology of FgMFS1 in the membrane.

2.2. Deletion and Complementation of FgMFS1 Gene in F. graminearum

The gene sequence of FgMFS1 was removed from the WT strain by homologous recombination (Figure 4A) to create the deletion mutants (ΔFgMFS1). PCR was used to screen the ΔFgMFS1 mutants to make sure that the construct had been recombined into the intended homologous site (Figure 4C). The ORF of FgMFS1 was randomly inserted into the genome of ΔFgMFS1 to create the complementation mutants (C-FgMFS1; Figure 4B). Reverse transcription (RT)-PCR showed that FgMFS1 was normally transcribed in C-FgMFS1 as in the WT strain, and no expression was detected in ΔFgMFS1 as expected (Figure 4D).
Figure 3. Phylogenetic analysis of MFS transporters. (A) MFS transporters can be classified into 17 families: DHA 1 (drug: H⁺ antiporter (12-TMS)) drug efflux family, ACS (anion: cation symporter) family, NNP (nitrate–nitrite porter) family, OPA (organophosphate: inorganic phosphate antiporter) family, AAHS (aromatic acid: H⁺ symporter) family, SP (sugar porter) family, PHS (phosphate: H⁺ symporter) family, MHS (metabolite: H⁺ symporter) family, SHS (sialate: H⁺ symporter) family, OFA (oxalate: formate antiporter) family, MCP (monocarboxylate porter) family, CP (cyanate permease) family, FGHS (fucose–galactose–glucose: H⁺ symporter) family, OHS (oligosaccharide: H⁺ symporter) family, NHS (nucleoside: H⁺ symporter) family, UMF (unknown major facilitator) family, and DHA 2 (drug: H⁺ antiporter (14-TMS)) drug efflux family. (B) Phylogenetic analysis of the members of DHA1 family. “YGR138c (CAA97151.1) Saccharomyces cerevisiae” represents the protein name, accession number, and Latin name, respectively. The numbers under/above each node are the bootstrap values.
2.2. Deletion and Complementation of FgMFS1 Gene in F. graminearum

FgMFS1 is important for fungal response to SA

2.3. FgMFS1 Is Important for Fungal Response to SA

The expression of FgMFS1 was induced by SA (Figure 5D). Moreover, the ΔFgMFS1 strains were more sensitive to SA than the WT and C-FgMFS1 strains on mSNA (modified...
Synthetischer Nährstoffarmer Agar) plates supplemented with 0.9 mM SA (Figure 5A,B), indicating a key role of FgMFS1 in exporting SA in F. graminearum.

Figure 5. FgMFS1 encodes an SA exporter. (A) Mycelial growth on mSNA (modified Synthetischer Nährstoffarmer Agar) plates with and without SA. CK, control. Plates were photographed on the 4th d post inoculation (dpi). (B) Percentages of mycelial growth inhibited by SA ([A1-B1]/A1 for WT, [A2-B2]/A2 for ∆FgMFS1). A1 (A2) and B1 (B2) indicate mycelial diameters of the WT (∆FgMFS1) strain under the CK and SA treatments, respectively. Asterisks indicate significance at $P < 0.01$. (C) Subcellular localization of the FgMFS1 protein, scale bar, 10 µm. (D) SA induces the expression of FgMFS1 in the WT F. graminearum strain. (E) RT-PCR verification of the expression of FgMFS1 in yeast by using primer pair S-FgMFS1-F + S-FgMFS1-R (Table 1). Actin (Actin-F + Actin-R) was used as the reference. (F) Yeast growth on dropout plates without uracil (DO Supplement-Ura) under SA and CK treatments, which were photographed on the 5th dpi. (G) Growth of S-WT and S-FgMFS1 strains in liquid DO Supplement-URA media with (0.5 mM) and without (CK) SA. Optical density was determined at 48 h after initial inoculation. (H) Percentages of growth inhibited by SA ([C1-D1]/C1 for S-WT, [C2-D2]/C2 for S-FgMFS1). C1 (C2) and D1 (D2) indicate the optical density of S-WT (∆FgMFS1) strain under the CK and SA treatments, respectively. Different small letters above each column indicate significance at $P < 0.05$. Asterisks indicate significance at $P < 0.01$.

Subcellular localization experiment indicated that FgMFS1 was usually distributed in the vacuoles and alternatively located in the cell membrane under SA stress (Figure 5C).

To confirm its function, FgMFS1 was expressed in yeast (Saccharomyces cerevisiae) (S-FgMFS1). RT-PCR showed that FgMFS1 was normally expressed in S-FgMFS1 strains, and no expression was found in the WT yeast strain (S-WT) as expected (Figure 5E). The growth of S-WT was more sensitive to SA than that of S-FgMFS1 (Figure 5F–H). These data demonstrate that FgMFS1 plays a key role in the outward transport of SA.

2.4. FgMFS1 Affects Pathogenicity towards Wheat

The spikes point inoculated with ∆FgMFS1 showed fewer visual FHB disease symptoms (Figure 6A,B) and less fungal biomass (Figure 6C) compared to those point inoculated with WT and C-FgMFS1 on the 6th, 8th, and 10th dpi. The expression of FgMFS1 was significantly increased during infection (Figure 6D,E).
The effects of FgMFS1 on DON production were measured both in wheat spikes and in liquid media. ΔFgMFS1 produced much less DON in wheat spikes than WT and C-FgMFS1 (Figure 6F). In liquid media, the accumulation of DON was not affected by the FgMFS1 gene when there was no SA. Consistent with our previous result [13], SA significantly inhibited the production of DON. Notably, this inhibition was much stronger for ΔFgMFS1 than for the WT and C-FgMFS1 strains (Figure 6G).

**Figure 6.** FgMFS1 is essential for full pathogenicity towards wheat. (A) Wheat heads and rachises, respectively, point inoculated with the WT, ΔFgMFS1, and C-FgMFS1 strains on the 8th dpi. The red arrows indicate the inoculated spikelets. (B) Numbers of infected and bleached spikelets in wheat spikes, respectively, point inoculated with the WT, ΔFgMFS1 and C-FgMFS1 strains, on the 6th, 8th, and 10th dpi. (C) Relative expression levels of FgGAPDH in spikes inoculated with the WT and ΔFgMFS1 strains on the 1st, 2nd, and 4th dpi, respectively. (D) Expression of FgMFS1 in spikes inoculated with the WT strain, on the 1st, 2nd, and 4th dpi. (E) Ratio of the expression of FgMFS1 to that of FgGAPDH in wheat spikes inoculated with the WT strain, on the 1st, 2nd, and 4th dpi. (F) Contents of DON in spikes inoculated with WT, ΔFgMFS1, and C-FgMFS1, respectively. (G) Concentrations of DON in liquid media without (CK) and with 1 mM SA. Different letters above each column indicate significance at $P < 0.05$. FW, fresh weight.

### 2.5. Expression of FgMFS1 Affects the Accumulation of Phytohormones in Spikes

SA, jasmonic acid (JA), and indole acetic acid (IAA) participate in wheat resistance against *F. graminearum* [14,33]. To understand whether FgMFS1 affects the accumulation of these hormones, we compared the contents of SA, JA, and IAA in spikes inoculated with water (CK treatment), WT, and ΔFgMFS1, respectively (Figure 7). On the 1st dpi, the contents of SA, JA, and IAA were higher in spikes inoculated with WT and ΔFgMFS1 than those under CK treatment, although most comparisons were insignificant. Inconsistent with the fungal biomass data in Figure 6C, spikes inoculated with ΔFgMFS1 accumulated more SA, JA, and IAA than those inoculated with WT strain. On the 2nd dpi, the contents of these hormones showed similar changes to the 1st dpi.
The manipulation of the expression level or activity of FgMFS1 would be a hopeful way to manage FHB disease. Due to the importance of SA in wheat resistance, F. graminearum has evolved a set of efficient gene tools to make sure that wheat endogenous SA content is below the toxicity threshold during infection (Figure 1). Unsurprisingly, treating wheat spikes with exogenous SA cannot improve wheat FHB resistance [13]. The MFS transporters are one of the largest groups of secondary active transporters conserved from bacteria to mammals, which can transport a broad range of substrates across biological membranes [25]. MFS transporters have been divided into uniporters, symporters, and antiporters, which move substrates across membranes via facilitated diffusion, co-transport, and exchange, respectively [27]. In this study, we characterized the MFS transporter gene FgMFS1 (FGSG_03725) in F. graminearum, which is induced by the defense hormone SA (Figure 5D) and highly expressed during infection in wheat spikes (Figure 6E). As one of the gene tools mentioned above, FgMFS1 functions as an antiporter (DHA1 family; Figure 3). Disruption of the FgMFS1 gene resulted in enhanced sensitivity to SA (Figure 5A), increased accumulation of SA (Figure 7A), less fungal biomass (Figure 6C), and fewer visual disease symptoms in wheat spikes (Figure 6A,B). The presence of FgMFS1 was critical for the outward transport of SA by F. graminearum under SA stress (Figure 5). Our results suggest that the manipulation of the expression level or activity of FgMFS1 would be a hopeful way to manage FHB disease.

FgABCC9 (FG05_07325) encodes an ABC-C (ATP-binding cassette transporter family C) transporter in F. graminearum, which is highly expressed during infection in wheat and is up-regulated by SA as well. FgABCC9 functions as an SA exporter and is localized in the cell membrane [18]. Microscopic observation indicated that FgMFS1 protein was usually expressed in the vacuoles of hyphae, and alternatively expressed in the cell membrane under SA stress (Figure 5C). It reflects an element of F. graminearum in response to SA synthesized by the host plant. FgMFS1 may usually transport other substrates from vacuoles to cytoplasm. When wheat spikes synthesize and release more SA after infection,
*F. graminearum* begins to alter the expression level and the subcellular localization of FgMFS1 protein. Consequently, FgMFS1 and FgABCC9 [18] synergistically export SA to minimize the toxicity of SA.

We previously demonstrated that point inoculation of solution containing $1 \times 10^3$ conidia and 400 µM SA was sufficient to prevent disease in the susceptible common wheat cultivar Roblin [13]. Moreover, no mycelial growth was observed on mSNA plates supplemented with 3 mM SA. Therefore, SA is a valuable chemical target for obtaining wheat germplasms immune/highly resistant to FHB. Considering the redundancy and complicacy of the gene tools of *F. graminearum* in response to SA (Figure 1), host-induced silencing of multiple genes in Figure 1 is a candidate and ongoing strategy for managing FHB [36].

4. Materials and Methods

4.1. Materials and Growth Conditions

The *F. graminearum* virulent strain DAOM180378 (Canadian Fungal Culture Collection, AAFC, Ottawa, ON, Canada) that was highly virulent in wheat was used throughout. The common wheat cultivar “Roblin” that is susceptible to Fusarium head blight (FHB) was used for inoculation. Plants of Roblin were grown in a climate-controlled glasshouse under 12/12 h day/night cycles at 23/18 °C. Plants were watered as needed and fertilized before sowing with 15-15-15 (N-P-K).

Conidia were produced in carboxymethyl cellulose liquid medium [37] at 28 °C, with shaking (180 rpm) for 5 d. The conidial concentration was measured with a hemocytometer by a microscope (BX63, Olympus, Tokyo, Japan). The effects of SA (sigma) on mycelial growth of *F. graminearum* were elevated on mSNA (modified Synthetischer Nährstoffarmer Agar; 1 g KH$_2$PO$_4$, 1 g KNO$_3$, 0.5 g MgSO$_4$, 0.5 g KCl, 1 g glucose, 1 g sucrose, and 20 g agar per L) plates. SA was supplemented after autoclaving. Mycelia were inoculated on each mSNA plate, which were maintained in darkness at 28 °C. There were five replicates for each treatment. The growing mycelia on mSNA plates were recorded by using the EPSON Perfection V700 Photo (Seiko Epson, Bekasi, Indonesia) on the 4th dpi. The diameters of mycelia was measured as [13].

4.2. Nucleic Acid Extraction and Sequence Analysis

Genomic DNA of *F. graminearum* was extracted from fresh mycelia cultured on potato dextrose agar plates at 28 °C for 5 d by the DNA extraction kit (Biofit, Chengdu, China). Total RNA was extracted from fresh mycelia and wheat samples by using the Total RNA extraction Kit (Biofit). RNA was reversely transcribed using PrimeScript™ RT Reagent Kit with genomic DNA Eraser (Takara, Dalian, China). The experiments were performed according to the manufacturer’s instructions.

Gene sequence of FgMFS1 (FGSG_03725) was downloaded from the Ensembl database (http://fungi.ensembl.org/index.html, accessed on 10/October/2019). The protein sequences of MFS transporters [26,38] were downloaded from the website of National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/, accessed on 29/June/2021). Prediction of transmembrane helices in FgMFS1 proteins were performed in TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/, accessed on 10/December/2019) (Figure 2A). The 3D-protein structure was predicted by using I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/, accessed on 15/December/2019) [39,40] and colored by Chimera software [41]. Neighbor-joining trees for the classification of MFS transporters were constructed by using MEGA version 5 [42] on the basis of a complete deletion of gaps and Poisson correction. A bootstrap test of phylogeny was performed with 10,000 replicates.

4.3. Preparation of ΔFgMFS1 and C-FgMFS1 Mutants

The pRF-HU2 vector [43] was used to remove the FgMFS1 gene from the genome of *F. graminearum* [18]. Transformation of *F. graminearum* by Agrobacterium tumefaciens was carried out as described previously [44].
The gene sequence of \textit{FgMFS1} was amplified by using the cDNA of \textit{F. graminearum} and primer pair R-\textit{FgMFS1}-F + R-\textit{FgMFS1}-R. The cloned sequence of \textit{FgMFS1} was verified by sequencing, ligated into the pCAMBIA2301 vector (with green fluorescent protein gene (\textit{GFP}) tag and 35S promoter), frozen thawed into the \textit{A. tumefaciens} strain AGL-1 (Tiangen, Beijing, China), and transformed into \Delta\textit{FgMFS1} to prepare the complementation mutants (C-\textit{FgMFS1}). Primer Premier 5.0 (Premier Biosoft, Palo Alto, CA, USA) was used for designing PCR primers (Table 1). The restriction enzymes from New England Biolabs (Ipswich, MA, USA) were used for constructing vectors.

4.4. Subcellular Localization of \textit{FgMFS1}

The C-\textit{FgMFS1} strains containing a \textit{GFP} (green fluorescent protein) gene tag were used to determine the subcellular localization of the \textit{FgMFS1} protein in hyphae (Figure 5C). A Nikon-80i fluorescence microscope (Nikon, Tokyo, Japan) was used to detect the \textit{GFP} fluorescence.

| Primer | Sequence (5'-3') | Source |
|--------|-----------------|--------|
| \textit{FgMFS1-LB-F} | GCGGGCCCAAGATGCCCTCCACTG | This study |
| \textit{FgMFS1-LB-R} | GCGAGCTCTGCAAGCTCCTTAAT | This study |
| \textit{FgMFS1-RB-F} | GGAAGCTTAAAGGCGAGCCAGAG | This study |
| \textit{FgMFS1-RB-R} | GGAAGCTTCGACACGCAGTTAAT | This study |
| P5 | GAGTGTTACGCCGTTG | This study |
| P6 | CTTACACTGGGCTGTTT | This study |
| P7 | GCCCTGAACAGAACCC | This study |
| P8 | TTCAGAACCTGTGCCC | This study |
| R- \textit{FgMFS1}-F | CGAGCTTCCGGCTCTCTATATAACCTCC | This study |
| R- \textit{FgMFS1}-R | CCGGAGACTCTGTAATGATGCTTGTGTT | This study |
| RJ- \textit{FgMFS1}-F | CTTACACTGGGCTGTTT | This study |
| RJ- \textit{FgMFS1}-R | AAGCCACCGTTCCTCCT | This study |
| QPCR-\textit{FgMFS1}-F | GCCGATGAGCAAGGAC | This study |
| QPCR-\textit{FgMFS1}-R | AAACAGGAAGGACAGAAGG | This study |
| \textit{Fg-GAPDH-F} | TGACTTGAGCTGTCGGCTCGAGAA | [13] |
| \textit{Fg-GAPDH-R} | ATGGAGAGCTGTGTTGCTCCGTTA | [13] |
| \textit{Fg-Factor 1-F} | CCTCAAGAGATCTCAACAAG | [13] |
| \textit{Fg-Factor 1-R} | CTTAAGAGATCTCCTCACAG | [13] |
| S-\textit{FgMFS1-F} | ATGACGTGATGACGCTGAT | This study |
| S-\textit{FgMFS1-R} | TGCTGAATGACGCTCCTTG | This study |
| \textit{Actin-F} | ATTATATGGTGGATGCTGCTTG | [45] |
| \textit{Actin-R} | CAATCGTGGATGATCTGATG | [45] |
| \textit{SY-FgMFS1-F} | CTATTACGGCCAGGCTGATCAGCACTGTAACGAT | This study |
| \textit{SY-FgMFS1-R} | TCGCTCTGTACGATGCTGCTTG | This study |
| \textit{w-GAPDH-F} | AACTGTGTTGTCGTCGTC | [13] |
| \textit{w-GAPDH-R} | AGGACATACGACATGCTGCTG | [13] |
| \textit{hn-RNP-Q-F} | TACGCTTCCGCAAGCTGACACTA | [13] |
| \textit{hn-RNP-Q-R} | ATGTGAATGACGCTGATG | [13] |
| \textit{Aox-F} | GACTGTTGATGTTGATGCTG | [13] |
| \textit{Aox-R} | CAGGACGACGATAACCATC | [13] |

4.5. Gene Expression Analysis

At the mid-anthesis stage, two florets from each fully developed spikelet in a whole spike were inoculated with \(1 \times 10^3\) conidia or water. The plants were placed into a room with a moist plastic wrap for 48 h at 25 °C and then transferred to another greenhouse at 25 °C as well. At least ten plants were used per treatment. At 1, 2 and 4 dpi, the inoculated heads were collected and ground into fine powders in liquid nitrogen, which were used for RNA extraction, for hormone quantification, and for DON measurement. Three biological replicates were conducted with at least 15 heads per treatment.
The primer pair Qpcr-FgMFS1-F + Qpcr-FgMFS1-R was used to measure the expression level of FgMFS1 in *F. graminearum* and in wheat spikes. Two reference genes, i.e., glyceraldehyde 3-phosphate dehydrogenase (FgGAPDH, FG05_06257, Fg-GAPDH-F + Fg-GAPDH-R in Table 1) and elongation factor 1 (Fg-Factor 1, FG05_08811, Fg-Factor 1-F + Fg-Factor 1-R), were used to normalize the expression of FgMFS1 in *F. graminearum* samples. The relative biomass of *F. graminearum* in wheat spikes was estimated by measuring the expression levels of FgGAPDH by using qRT-PCR with normalization to three wheat reference genes (i.e., w-GAPDH [w-GAPDH-F + w-GAPDH-R], Aox [Aox-F + Aox-R], and hn-RNP-Q [hn-RNP-Q-F + hn-RNP-Q-R]). qRT-PCR analyses were carried out as [13] in a MyiQ Real-Time PCR Detection System (Bio-Rad, Hercules, USA).

4.6. Pathogenicity Assay

To determine whether FgMFS1 had an effect on the pathogenicity of *F. graminearum* in wheat heads, two florets of a central spikelet from one head per plant were each inoculated with 1 × 10^3 conidia at the mid-anthesis stage. Five to ten plants were used per treatment. The inoculated plants were treated as above.

To measure whether the FgMFS1 gene was related to DON accumulation in wheat spikes, each powder sample (100 mg; obtained from 4.5) was added with 1 mL sterile water and maintained at 4 °C for 12 h. To make sure whether FgMFS1 gene affected DON production in liquid media, a two-stage protocol was used [13,46]. The DON contents were measured by a DON ELISA kit (Yonghui, Beijing, China) and a Multiskan Spectrum (Thermo Fisher Scientific, Waltham, MA, USA).

4.7. Expression of FgMFS1 in Yeast

The full ORF of FgMFS1 was amplified by the primer pair SY-FgMFS1-F + SY-FgMFS1-R (Table 1), ligated into PYC54 vector and transformed into yeast (*Saccharomyces cerevisiae*) strain Y1H (Oebiotech, Shanghai, China), following the manufacturer’s instructions. Yeast was dropped on the plates after dilution to measure the effect of FgMFS1 on yeast growth under 2 mM SA stress. Three independent experiments were conducted with at least three plates for each treatment.

Optical density (A_{600}) was assessed to determine the effect of FgMFS1 on yeast growth under 0.5 mM SA stress in liquid DO Supplement-URA media by a Genequant Pro nucleic acid protein analyzer (Biochrom, England, UK). There were three biological replications for each treatment.

4.8. Quantification of Phytohormones

The contents of SA, IAA, and JA were quantified from frozen powders (obtained from 4.5) by ultra-performance liquid chromatography electrospray tandem mass spectroscopy utilizing a Waters Acquity UPLC HSS T3 (100 mm × 2.1 mm, 1.8 µm) in Oebiotech as previously described [47].

4.9. Statistical Analysis

Student’s t-test and one-way ANOVA (DPS software version 12.01 [48]) was used to test the significance of differences in fungal growth, hormone contents, gene expression, DON contents, and the level of disease.

Author Contributions: Conceptualization, P.Q.; Data curation, Q.C., L.L., Z.G., Y.Z. (Youliang Zheng) and P.Q.; Formal analysis, L.L., Y.Z. (Yazhou Zhang), Z.G., J.Z., L.W. and G.C.; Funding acquisition, Q.C., Y.W. (Yuming Wei) and P.Q.; Investigation, Q.C., L.L., C.L., Y.Z. (Yazhou Zhang), J.Z., Z.G., Y.W. (Yan Wang), Q.L., Y.L., L.K. and Y.J.; Methodology, Q.C., C.L., Q.X., Y.W. (Yan Wang), Q.I., Y.L., J.W., Q.J., Y.W. (Yan Wang) and P.Q.; Project administration, Q.C. and P.Q.; Resources, L.K., X.L., Q.X., J.W, Q.J., G.C., J.M. and Y.Z. (Youliang Zheng); Validation, Q.L., Y.J., X.L. and J.M.; Writing – original draft, Q.C., L.L. and P.Q.; Writing – review & editing, P.Q. All authors have read and agreed to the published version of the manuscript.
Funding: This research was supported by the National Natural Science Foundation of China (31901961, 31971939 and 32072054) and the Department of Science and Technology of Sichuan (2020YFH0150).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data underlying this article will be shared on reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Goswami, R.S.; Kistler, H. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* 2004, 5, 515–525. [CrossRef]

2. Koga, S.; Rieder, A.; Ballance, S.; Uhlen, A.K.; Veiseth-Kent, E. Gluten-degrading proteases in wheat infected by *Fusarium graminearum*—Protease identification and effects on gluten and dough properties. *J. Agric. Food Chem.* 2019, 67, 11025–11034. [CrossRef]

3. Xu, X.; Berrie, A.M. Epidemiology of mycotoxicogenic fungi associated with *Fusarium* ear blight and apple blue mould: A review. *Food Addit. Contam.* 2005, 22, 290–301. [CrossRef]

4. Kang’Etthe, E.K.; Sirma, A.J.; Murithi, G.; Mburugu-Mosoti, C.K.; Ouko, E.O.; Korhonen, H.J.; Nduhiu, G.J.; Mungatu, J.K.; Joutsjoki, V.; Lindfors, E.; et al. Occurrence of mycotoxins in food, feed, and milk in two counties from different agro-ecological zones and with historical outbreak of aflatoxins and fumonisins poisonings in Kenya. *Food Qual. Saf.* 2017, 1, 161–170. [CrossRef]

5. Sobrova, P.; Adam, V.; Vasatkova, A.; Beklova, M.; Zeman, L.; Kizek, R. Deoxynivalenol and its toxicity. *Interdiscip. Toxicol.* 2010, 3, 94–99. [CrossRef] [PubMed] [CrossRef]

6. Ameye, M.; Audenaert, K.; De Zutter, N.; Steppe, K.; Van Meulebroek, L.; Vanhaecke, L.; De Vleesschauwer, D.; Haesaert, G.; Smagghe, G. Priming of wheat with the green leaf volatile Z-3-hexenyl acetate enhances defense against *Fusarium graminearum* but boosts deoxynivalenol production. *Plant Physiol.* 2015, 167, 1671–1684. [CrossRef]

7. Lopinto, A.J.; Mohammed, H.O.; Ledbetter, E.C. Prevalence and risk factors for isolation of methicillin-resistant *Staphylococcus* in dogs with keratitis. *Vet. Ophthalmol.* 2014, 18, 297–303. [CrossRef]

8. Bai, G.; Shaner, G. Management and resistance in wheat and barley to *Fusarium* head blight. *Annu. Rev. Phytopathol.* 2004, 42, 135–161. [CrossRef]

9. Gilbert, J.; Tekauz, A. Review: Recent developments in research on fusarium head blight of wheat in Canada. *Can. J. Plant Pathol.* 2000, 22, 1–8. [CrossRef]

10. Mesterhazy, A. Types and components of resistance to *Fusarium* head blight of wheat. *Plant Breed.* 1995, 114, 377–386. [CrossRef]

11. Wang, J.R.; Wang, L.; Gulden, S.; Rocheleau, H.; Balcerzak, M.; Hattori, J.; Cao, W.; Han, F.; Zheng, Y-L.; Fedak, G.; et al. RNA profiling of *Fusarium* head blight-resistant wheat addition lines containing the *Thinopyrum elongatum* chromosome 7E. *Can. J. Plant Pathol.* 2010, 32, 188–214. [CrossRef]

12. Makandar, R.; Essig, J.S.; Schapaugh, M.A.; Trick, H.; Shah, J. Genetically engineered resistance to *Fusarium* head blight in wheat by expression of arabidopsis NPR1. *Mol. Plant Microbe Interact.* 2006, 19, 123–129. [CrossRef][PubMed]

13. Qi, P.F.; Johnston, A.; Balcerzak, M.; Rocheleau, H.; Harris, L.J.; Long, X-Y.; Wei, Y-M.; Zheng, Y-L.; Ouellet, T. Effect of salicylic acid on *Fusarium graminearum*, the major causal agent of fusarium head blight in wheat. *Fungal Biol.* 2012, 116, 413–426. [CrossRef]

14. Qi, P.F.; Balcerzak, M.; Rocheleau, H.; Leung, W.; Wei, Y-M.; Zheng, Y-L.; Ouellet, T. 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.* 2016, 93, 39–48. [CrossRef]

15. Sorahinobar, M.; Niknam, V.; Ebrahimzadeh, H.; Soltanlooh, H.; Behmanesh, M.; Enferadi, S.T. Central role of salicylic acid in resistance of wheat against *Fusarium graminearum*. *J. Plant Growth Regul.* 2015, 35, 477–491. [CrossRef]

16. Zhang, Y-Z.; Chen, Q.; Liu, C-H.; Liu, Y-B.; Yi, P.; Niu, K-X.; Wang, Y-Q.; Wang, A-Q.; Yu, H-Y.; Pu, Z-E.; et al. Chitin synthase gene FgCHS8 affects virulence and fungal cell wall sensitivity to environmental stress in *Fusarium graminearum*. *Fungal Biol.* 2016, 120, 764–774. [CrossRef]

17. Zhang, Y-Z.; Wei, Z-Z.; Liu, C-H.; Chen, Q.; Xu, B-J.; Guo, Z-R.; Cao, Y-L.; Wang, Y; Han, Y-N.; Chen, C; et al. Linoleic acid isomerase gene FgLAI12 affects sensitivity to salicylic acid, mycelial growth and virulence of *Fusarium graminearum*. *Sci. Rep.* 2017, 7, 46129. [CrossRef]

18. Qi, P.F.; Zhang, Y-Z.; Liu, C-H.; Zhu, J.; Chen, Q.; Guo, Z-R.; Wang, Y; Xu, B-J.; Zheng, T; Jiang, Y-F.; et al. *Fusarium graminearum* ATP-binding cassette transporter gene FgABC9 is required for its transportation of salicylic acid, fungicide resistance, mycelial growth and pathogenicity towards wheat. *Int. J. Mol. Sci.* 2018, 19, 2351. [CrossRef][PubMed]

19. Qi, P.F.; Zhang, Y-Z.; Liu, C-H.; Chen, Q.; Guo, Z-R.; Wang, Y; Xu, B-J.; Jiang, Y-F.; Zheng, T; Gong, X.; et al. Functional analysis of FgNahG clarifies the contribution of salicylic acid to wheat (*Triticum aestivum*) resistance against *Fusarium* head blight. *Toxins* 2019, 11, 59. [CrossRef]

20. Rocheleau, H.; Al-Harthi, R.; Ouellet, T. Degradation of salicylic acid by *Fusarium graminearum*. *Fungal Biol.* 2019, 123, 77–86. [CrossRef]
21. Zhang, Y.-Z.; Chen, Q.; Liu, C.-H.; Lei, L.; Li, Y.; Zhao, K.; Wei, M.-Q.; Guo, Z.-R.; Wang, Y.; Xu, B.-J.; et al. Transcriptomic analysis of the leaves of two grapevine cultivars under high-temperature stress. *Toxins* **2019**, *11*, 628. [CrossRef]

22. Del Sorbo, G.; Schoonbeek, H.-J.; De Waard, M.A. Fungal transporters involved in efflux of natural toxic compounds and fungicides. *Fungal Genet. Biol.* **2000**, *30*, 1–15. [CrossRef]

23. Poolman, B.; Konings, W.N. Secondary solute transport in bacteria. *Biochim. Biophys. Acta Bioenerg.* **1993**, *1183*, 5–39. [CrossRef]

24. Ward, S.J.; Scopes, D.; Christodoulides, M.; Clarke, I.; Heckels, J.E. Expression of *Neisseria meningitidis* class 1 porin as a fusion protein in *Escherichia coli*. The influence of liposomes and adjuvants on the production of a bactericidal immune reponse. *Microb. Pathog.* **1996**, *21*, 499–512. [CrossRef]

25. Yan, N. Structural advances for the major facilitator superfamily (MFS) transporters. *Trends Biochem. Sci.* **2013**, *38*, 151–159. [CrossRef]

26. Alexander, N.J.; McCormick, S.; Waalwijk, C.; Van Der Lee, T.; Proctor, R.H. The genetic basis for 3-ADON and 15-ADON pathways in *Arabidopsis*.* Sci. Hortic.* **2020**, *265*, 109265. [CrossRef]

27. Yan, N. Structural biology of the major facilitator superfamily transporters. *Annu. Rev. Biochem.* **2015**, *84*, 257–283. [CrossRef]

28. Alexander, N.J.; McCormick, S.; Waalwijk, C.; Van Der Lee, T.; Proctor, R.H. The genetic basis for 3-ADON and 15-ADON pathways in *Arabidopsis*. *Sci. Hortic.* **2020**, 265, 109265. [CrossRef]

29. L...