Phytopathology Research

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Overexpression of wheat spermidine synthase gene enhances wheat resistance to Fusarium head blight

Jingyi Ren[1], Chengliang Li[1], Qi Xiu[1], Ming Xu[1*] and Huiquan Liu[1*]

Abstract

Polyamines, such as putrescine, spermidine, and spermine, are crucial for plant defense against both abiotic and biotic stresses. Putrescine is also known as a significant inducer of deoxynivalenol (DON) production in *Fusarium graminearum*, the primary causal agent of Fusarium head blight (FHB). However, the impact of other polyamines on DON production and whether modifying polyamine biosynthesis could improve wheat resistance to FHB are currently unknown. In this study, we demonstrate that key precursor components of putrescine synthesis, including arginine, ornithine, and agmatine, can induce DON production, albeit to a lesser extent than putrescine in trichothecene biosynthesis-inducing (TBI) culture under the same total nitrogen conditions. Intriguingly, spermidine and spermine, downstream products of putrescine in the polyamine biosynthesis pathway, do not induce DON production under the same conditions. Additionally, externally applying either spermidine or spermine to wheat heads significantly reduces the diseased spikelet number caused by *F. graminearum*. Furthermore, our results show that overexpression of the wheat spermidine synthase (SPDS) gene *TaSPDS*-7D1 significantly enhances the spermidine content and wheat resistance to FHB. In addition, the *TaSPDS*-7D1-overexpressing line OE3 exhibited a 1000-grain weight and plant height increase compared to the wild type. Our findings reveal that overexpression of the spermidine synthase gene can enhance wheat resistance to FHB without compromising wheat yield.

Keywords Fusarium head blight, SPDS, Polyamines, Deoxynivalenol (DON), Resistance

Background

Polyamines are small organic amine molecules found in almost all living organisms. The primary polyamine species consist of putrescine, spermidine, and spermine, with the latter two also referred to as higher polyamines (Pal and Janda 2017; Gerlin et al. 2021). The biosynthesis pathway of polyamines has been well documented, involving the participation of several key enzymes. Briefly, putrescine is synthesized through the decarboxylation of ornithine, catalyzed by the enzyme ornithine decarboxylase (ODC), or indirectly synthesized by the decarboxylation of arginine via agmatine, catalyzed by the enzyme arginine decarboxylase (ADC). Spermidine and spermine are synthesized by sequentially adding aminopropyl moieties to the putrescine skeleton through enzymatic reactions, which are catalyzed by spermidine synthase (SPDS) and spermine synthase (SPMS), respectively. Decarboxylated S-adenosylmethionine (dcSAM), which is used for the addition of the aminopropyl moiety, is synthesized by S-adenosylmethionine decarboxylase (SAMDC) (Michael 2016; Pal and Janda 2017).

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Due to their amine functions, polyamines possess a polycationic nature at physiological pH. Thus, they can stabilize or destabilize anionic macromolecules or negatively charged ions. The chemical property of polyamines as pleiotropic molecules allows them to contribute to a wide range of molecular and biochemical processes. In plants, polyamines contribute to diverse pathways involved in cell proliferation, plant development, abiotic and biotic stress responses (Moschou et al. 2012; Gerlin et al. 2021). In Arabidopsis (*Arabidopsis thaliana*), *SPDS* genes are required for spermidine biosynthesis and essential for plant survival as the *spds1-1 spds2-1* double mutant are embryo lethal (Imai et al. 2004). In tobacco, silencing of spermidine synthase gene *SPDS* resulted in decreased spermidine levels and a slightly increased putrescine, leading to smaller size of flowers, decreased pollen viability, and a reduced and delayed seed germination, but the RNAi transgenic plant showed increased tolerance to salinity and drought conditions (Choubey and Rajam 2018). Several studies have highlighted the role of polyamines in plant immune responses to pathogens. In Arabidopsis, the expression of *ADC2*, a key enzyme in putrescine biosynthesis, was induced by *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) infection. The *adc2* knock-out mutant was defective in putrescine biosynthesis and was more susceptible to pathogen infection (Kim et al. 2013). Overexpression of the Arabidopsis spermine synthase gene *AtSPMS* led to enhanced spermine levels and exhibited increased resistance to *Pseudomonas viridiflava* as compared with the wild-type plant (Gonzalez et al. 2011). Recent transcriptomics and metabolomics analyses of the interaction between *Aspergillus flavus* and both resistant/susceptible maize lines revealed resistant lines accumulated higher amounts of spermidine and spermine compared to the susceptible line at the earliest time after inoculation, indicating that higher polyamine content in maize genotypes may confer higher resistance to *A. flavus* and reduce aflatoxin production (Majumdar et al. 2019).

Fusarium head blight (FHB), caused by the *Fusarium graminearum* species complex, is a destructive fungal disease that affects wheat and barley crops globally. FHB not only causes substantial yield losses in crops but also leads to grain contamination with various mycotoxins, particularly deoxynivalenol (DON) (Xia et al. 2020). In mammals, DON exerts its effects by binding to the ribosome, leading to the inhibition of protein synthesis, then causing emetic effects, anorexia, immune dysregulation, as well as growth and reproductive inhibition, and teratogenic effects (Pestka 2010). To minimize human and animal exposure to DON, maximum permissible levels for DON in cereals and their products have been established in many countries (Ji et al. 2014; Bianchini et al. 2015). Besides, DON is also an important virulence factor for *F. graminearum*. The *TRI5* gene, which is essential for DON biosynthesis, is expressed in infection cushions during the early stages of infection (Boenisch and Schäfer 2011). Deletion of the *TRI5* gene abolishes DON production. Although the *tri5* mutant can cause the initial infection, it is trapped in the infected floret and fails to spread via the rachis node (Jansen et al. 2005).

Several factors that may induce the production of DON by *F. graminearum* have been proposed, including hydrogen peroxide, carbon and nitrogen sources, and acidic pH (Chen et al. 2019). In particular, in a screen of large-scale nutrient profiling, the most potent inducers of DON production in vitro by *F. graminearum* are revealed as metabolites of the plant polyamine biosynthetic pathway, such as arginine, ornithine, agmatine, citrulline, and putrescine (Gardiner et al. 2009). The core polyamine biosynthetic pathway is activated at the early stage during *F. graminearum* infection on wheat and putrescine accumulation occurs before toxin production by the pathogen (Gardiner et al. 2010). It is hypothesized that the pathogen may absorb putrescine and related amino acids as signals for DON production during infection (Gardiner et al. 2009, 2010). In the presence of putrescine, the transcription factor FgAreA facilitates the enrichment of histone H2B monoubiquitination (H2B ub1) and histone 3 lysine 4 di- and trimethylations (H3K4 me2/3) on trichothecene biosynthesis genes to induce their expression (Ma et al. 2021). However, whether manipulation of wheat polyamine biosynthetic pathways to increase the conversion of putrescine to higher polyamines could reduce DON induction and enhance wheat resistance against FHB is largely unknown.

In this study, we showed that, in addition to putrescine, arginine, ornithine, and agmatine, the primary precursor components of putrescine synthesis, can induce DON production. Conversely, spermidine and spermine, which are downstream products of putrescine in the polyamine biosynthesis pathway, do not induce DON production. Notably, the external application of both spermidine and spermine to wheat heads significantly reduces the diseased spikelet number caused by *F. graminearum*. We performed overexpression of the spermidine synthase (SPDS) gene *TaSPDS*-7D1 in wheat to reduce DON production and enhance wheat resistance to FHB by decreasing the content of putrescine and increasing the content of spermidine and spermine. The *TaSPDS*-7D1-overexpressing wheat lines exhibited significantly enhanced resistance to FHB without compromising wheat yield. However, the same level of DON was accumulated in the inoculated spikelet of *TaSPDS*-7D1-overexpressing wheat lines as in the wild type. Analysis of polyamine contents revealed a simultaneous enhancement of putrescine and

spermidine in the wheat heads of *TaSPDS*-7D1-overexpressing lines infected by *F. graminearum*. Our findings demonstrate that overexpression of the spermidine synthase gene can indeed enhance wheat resistance to FHB.

Results

Putrescine and its precursors stimulate DON production, while spermidine and spermine inhibit *F. graminearum* infection

We investigated the impact of polyamine synthesis pathway components (Fig. 1a) – including arginine, ornithine, agmatine, putrescine, spermidine, and spermine – on DON production using trichothecene biosynthesis-inducing (TBI) medium, with each serving as the sole nitrogen source while maintaining a consistent total nitrogen level (Fig. 1b). As depicted in Fig. 1b, putrescine's synthesis precursors (arginine, ornithine, and agmatine) significantly stimulated DON production. Conversely, putrescine's downstream products (spermidine and spermine) did not induce DON production. The precursor components induced a relatively lower level of DON production compared to putrescine. Notably, the concentration of DON induced by putrescine in the TBI medium exceeded 10,000 μg/g dry-weight tissue (Fig. 1b). These findings indicate that putrescine is the most effective polyamine for inducing DON production.

We further assayed the role of putrescine, spermidine, and spermine in *F. graminearum* infection by spraying them on wheat heads along with *F. graminearum* inoculation. As shown in Fig. 1c, d, the exogenous spray of either spermidine or spermine notably decreased the diseased spikelet number, while

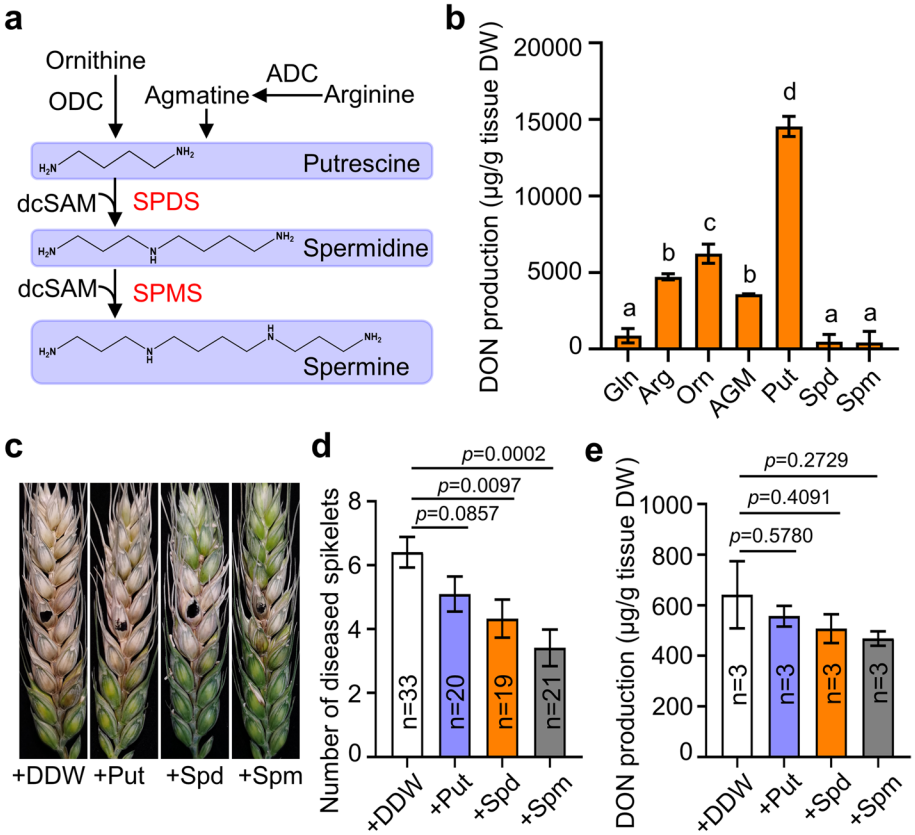

Fig.1 Functional analysis of polyamines in *F. graminearum* infection and Deoxynivalenol (DON) production on wheat. **a** Schematic diagram of putrescine, spermidine, and spermine biosynthesis in plants. SPDS and SPMS are responsible for the transfer of aminopropyl groups to spermidine and spermine, respectively. ADC, arginine decarboxylase; SPDS, spermidine synthase; SPMS, spermine synthase; dc-SAM, decarboxylated S-adenosylmethionine; ADC, ornithine decarboxylase. **b** DON production in trichothecene biosynthesis-inducing (TBI) medium with glutamine (Gln), arginine (Arg), ornithine (Orn), agmatine (AGM), putrescine (Put), spermidine (Spd), and spermine (Spm) as the sole nitrogen source. DW, Dry weight. The error bar represents standard errors (SEs) from three independent replicates ($n=3$). Different letters indicate significant differences based on ANOVA analysis followed by Duncan's multiple range test ($P=0.05$). **c** Representative images of polyamine-treated wheat head with *F. graminearum* inoculation. **d** Diseased spikelet number of polyamine-treated and *F. graminearum* infected wheat. **e** DON levels of polyamine-treated and *F. graminearum* infected wheat. The error bar indicates standard errors of the means (n varies for each column and is shown in each case directly on the graphs). *P*-values are from the Student's *t*-tests

putrescine did not. The result indicates that spermidine and spermine, but not putrescine, enhance FHB resistance in wheat. However, exogenous addition of putrescine, spermidine, and spermine had no effect on DON production (Fig. 1e).

Identification and expression analysis of wheat SPDS genes

Given that SPDS and SPMS facilitate the conversion of putrescine to spermidine and spermine (Fig. 1a), it may be feasible to overexpress the wheat SPDS/SPMS genes to reduce putrescine levels while increasing spermidine and spermine levels. The SPDS/SPMS enzyme possesses a Spermine_synth domain. Genes encoding SPDS/SPMS in Arabidopsis (*A. thaliana*) (Hanzawa et al. 2002), rice (*Oryza sativa*) (Tao et al. 2018), tomato (*Solanum lycopersicum*) (Upadhyay et al. 2021), maize (*Zea mays*) (https://plants.ensembl.org/), tobacco (*Nicotiana benthamiana*) (Choubey and Rajam 2018), figleaf gourd (*Cucurbita ficifolia*) (Kasukabe et al. 2004), pepper (*Capsicum annuum*) (Zhang et al. 2023), and datura (*Datura stramonium*) (Franceschetti et al. 2004) were used as a query against wheat (*Triticum. aestivum*) genome (Additional file 1: Table S1). In the wheat genome, we obtained six sequences related to TaSPDS and six related to TaSPMS. Phylogenetic analysis revealed that a gene duplication event in the wheat ancestor resulted in the emergence of two SPDS paralogs (each occurring in the three subgenomes), while the six TaSPMS sequences originated from an ancestral duplication event, at least in the last common ancestor of the grass family (Fig. 2a). Given that overexpression of *OsSPMS1* in rice resulted in no significant difference in spermine content between the wild-type and overexpressing plants (Tao et al. 2018), we focused on investigating the roles of TaSPDS in wheat. We named TraesCS7A02G265200, TraesCS7B02G163300, and TraesCS7D02G265900 as *TaSPDS*-7A1, *TaSPDS*-7B1, and *TaSPDS*-7D1, respectively. Additionally, TraesCS7A02G265300, TraesCS7B02G163500, and TraesCS7D02G266000 were named *TaSPDS*-7A2, *TaSPDS*-7B2, and *TaSPDS*-7D2, respectively (Additional file 2: Fig. S1).

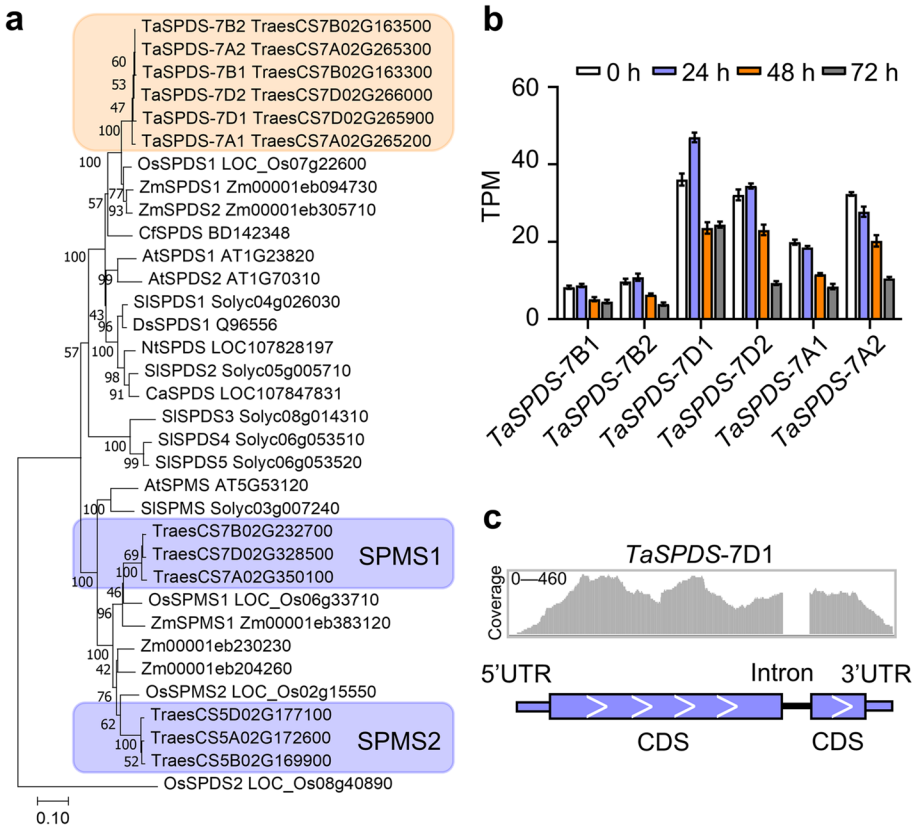

Fig. 2 Phylogenetic analysis of SPDS/SPMS members in wheat and gene expression pattern of *TaSPDS* during *F. graminearum* infection. **a** Phylogenetic tree of SPDS/SPMS proteins from wheat (*Triticum aestivum*), Arabidopsis (*Arabidopsis thaliana*), rice (*Oryza sativa*), tomato (*Solanum lycopersicum*), maize (*Zea mays*), tobacco (*Nicotiana benthamiana*), figleaf gourd (*Cucurbita ficifolia*), pepper (*Capsicum annuum*), and datura (*Datura stramonium*). The phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap repetitions with the MEGA 11.0.9 software. **b** The TPM (transcripts per million) values of 6 *TaSPDS* genes from RNA-seq data of *F. graminearum* inoculated wheat. **c** Gene structure, RNA-seq read coverage of *TaSPDS*-7D1

Based on our previous RNA-seq data of the wheat-*F. graminearum* interaction (Jiang et al. 2019), the transcriptional level of *TaSPDS*-7D1 was slightly elevated at 24 h post inoculation (hpi), followed by a reduction at 48 hpi and 72 hpi (Fig. 2b). Given that *TaSPDS*-7D1 exhibited the highest expression level in wheat both before and after *F. graminearum* infection, it was chosen for further functional analysis in this study. *TaSPDS*-7D1 consists of two exons and encodes 324 amino acids. The genomic and cDNA sequences are 1067 and 975 bp in length, respectively (Fig. 2c).

Overexpression of *TaSPDS*-7D1 in wheat confers resistance to FHB

The overexpression (OE) construct of *TaSPDS*-7D1 was generated under the control of the maize ubiquitin promoter, which was then transformed into the spring wheat cultivar Fielder. After conducting a PCR test with primers 6E-check-F/6E-check-R for the presence of the transgene, 20 positive transgene lines of the first generation (T_0) were identified. T_1 generation of four transgenic lines (OE1, OE3, OE15, and OE21) were chosen for further analysis. According to the segregation analysis based on PCR with primers 6E-check-F/6E-check-R in all the plants investigated, lines OE3 and OE21 were identified as likely homozygous, while lines OE1 and OE15 were verified to be heterozygous (Table 1). We selected the T_3 generation of OE3 and OE21 for further analysis. PCR analysis revealed the presence of a band of the expected size in both transgenic lines (Fig. 3a), indicating the successful insertion of the *TaSPDS*-7D1-overexpressing construct into the wheat genome. The relative expression level of *TaSPDS*-7D1 was significantly increased in OE3 and OE21 lines as compared to the wild-type plant, indicating a stable overexpression of *TaSPDS*-7D1 in the transgenic lines. The means of *TaSPDS*-7D1 relative expression levels were 17.84 in OE3 and 9.81 in OE21 as compared with the wild type (Fig. 3b), respectively. Western blotting analysis showed that the TaSPDS-7D1-HA fusion proteins were accumulated in the OE3 and OE21 lines (Fig. 3c).

Table 1 Segregation analysis of the T_1 generation of four selected transgenic lines

Transgenic Line	Number of Plants Containing the Transgene	Zygosity
Wild type	0/10	Negative control
OE1	7/10	Heterozygous
OE3	10/10	Likely homozygous
OE15	7/10	Heterozygous
OE21	10/10	Likely homozygous

To investigate the potential functions of *TaSPDS*-7D1 in wheat defense against *F. graminearum*, OE3, OE21, and wild-type plants were inoculated with the *F. graminearum* wild-type strain PH-1. The two *TaSPDS*-7D1-overexpressing wheat lines, OE3 and OE21, displayed significantly enhanced resistance to FHB (Fig. 3d). The average diseased spikelet number of the wild-type was 9.0, while those of the two transgenic lines were 6.8 and 6.5 in OE3 and OE21, respectively (Fig. 3e). But compared with the wild-type wheat, the two *TaSPDS*-7D1-overexpressing lines produced the same level of DON in the inoculated spikelet (Fig. 3f). These results indicate that overexpression of *TaSPDS*-7D1 improved wheat resistance to FHB, but had limited effect on reducing DON accumulation.

Overexpression of *TaSPDS*-7D1 do not compromise wheat yield

Several agronomic traits were compared between the wild-type plants and OE lines. At the maturity stage, the two OE lines were significantly taller than the wild type (Fig. 4a, d). Compared with the wild type, the plant height of the OE lines increased by 7.7% and 10.8% in OE3 and OE21, respectively. Whereas there was no significant difference in tiller number (Fig. 4c) and spike length (Fig. 4e), the 1000-grain weight increased by 10.2% in the OE3 line compared with the wild type (Fig. 4b, f). The difference between the two *TaSPDS*-7D1-overexpressing lines may be attributed to variations in the expression levels since OE3 exhibited a 1.8-fold higher than that of OE21.

Overexpression of *TaSPDS*-7D1 leads to increased synthesis of both spermidine and putrescine

To investigate the resistance mechanism, we assayed the level of spermidine, spermine, and putrescine in the OE3 transgenic line in the spikelet and rachis tissues inoculated with *F. graminearum*. As expected, overexpression of *TaSPDS*-7D1 significantly enhanced the spermidine content in both spikelets and rachises as compared to the wild-type plants (Fig. 5a). The increased level was even higher after *F. graminearum* infection. Spermidine content in the rachis of OE line was increased more than twofold as compared with that of the wild-type plant (Fig. 5a). These results suggest that TaSPDS-7D1 functions as a SPDS and promotes the accumulation of spermidine in wheat heads that inhibit *F. graminearum* infection.

While the putrescine content showed a significant increase in both the spikelets and rachises of the OE3 line after *F. graminearum* infection compared to the wild type (Fig. 5b), there were no significant changes in the spermine content (Fig. 5c). These findings suggest that

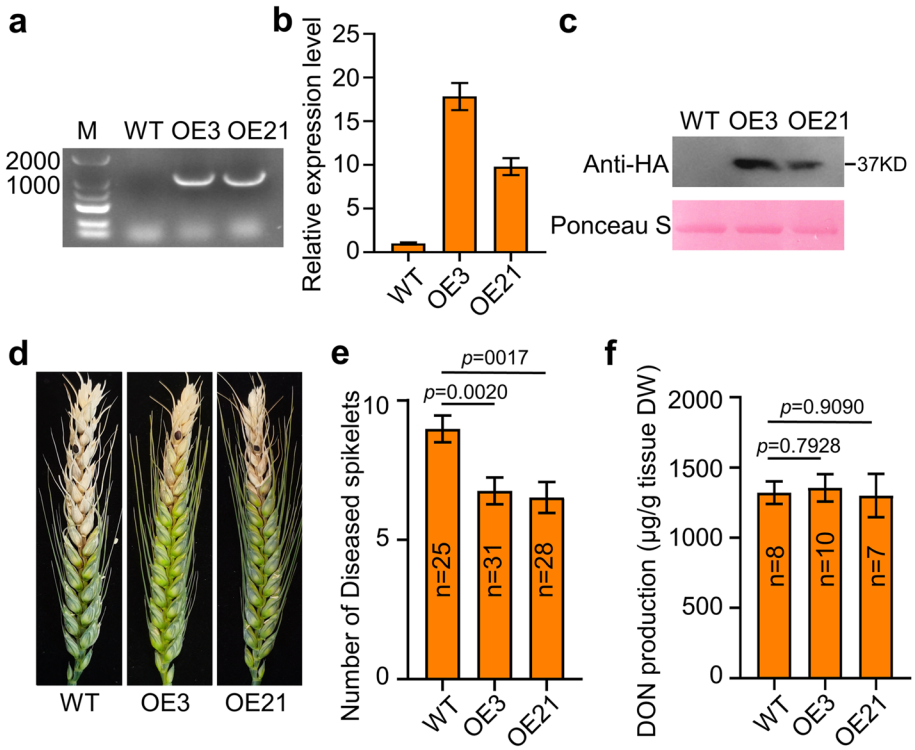

Fig. 3 Overexpression of *TaSPDS*-7D1 in wheat confers resistance to FHB. **a** PCR detection of the wild type (WT) and *TaSPDS*-7D1-overexpressing lines (OE3 and OE21). M, DL2000 DNA marker. **b** Relative expression levels of *TaSPDS*-7D1 in WT and *TaSPDS*-7D1-overexpressing lines. The error bar indicates standard deviation (SD) from three independent replicates ($n = 3$). **c** Western blot of the WT and *TaSPDS*-7D1-overexpressing lines using an anti-HA antibody. **d** Representative images of the WT and *TaSPDS*-7D1-overexpressing lines with *F. graminearum* inoculation. **e** Diseased spikelet number of the WT and *TaSPDS*-7D1-overexpressing lines with *F. graminearum* inoculation. **f** DON levels of WT and *TaSPDS*-7D1-overexpressing lines. The error bar indicates standard errors of the means (n varies for each column and is shown in each case directly on the graphs). *P*-values are from the Student's *t*-tests

the polyamine biosynthesis pathway underwent positive feedback, and the increased levels of spermidine led to an increase in the levels of its synthetic precursor, putrescine. These observations help explain why the overexpression of *TaSPDS*-7D1 enhances resistance to FHB but has a limited effect on reducing DON production.

Discussion

The host stress metabolite putrescine is one of the most potent DON-inducing factors in culture. Compared to other metabolites in the polyamine biosynthesis pathway, putrescine induces the highest DON level in TBI culture while maintaining a consistent total nitrogen level. Exogenously sprayed both spermidine and spermine on wheat head along with *F. graminearum* inoculation significantly reduced the diseased spikelet number, indicating that spermidine and spermine positively contribute to FHB resistance of wheat. According to phylogenetic analyses, wheat *TaSPDS* genes had been identified and named. Expression pattern analysis of *TaSPDS* genes using RNA-seq data showed that the transcript level of *TaSPDS*-7D1 was slightly elevated at 24 hpi, and then reduced at 48 hpi and 72 hpi. We investigated the impact of overexpression of *TaSPDS*-7D1 on FHB resistance in wheat and regulation of the abundance of polyamines. Our results showed that overexpression of *TaSPDS*-7D1 significantly enhances wheat resistance to FHB, although *TaSPDS*-7D1-overexpressing wheat lines accumulate the same level of DON as the wild type. It may be because of a simultaneous increase of spermidine and putrescine in the wheat head after *F. graminearum* inoculation. Moreover, The *TaSPDS*-7D1-overexpressing line OE3 exhibited a 1000-grain weight and plant height increase compared to the wild type. These findings declare a positive effect of the spermidine synthase gene on enhance wheat resistance to FHB without compromising wheat yield.

During FHB development, the level of putrescine increases at the same time as DON production and with a significant increase in the transcription levels of wheat putrescine biosynthesis genes, suggesting that putrescine may play a role in promoting DON production (Gardiner et al. 2010; Ma et al. 2021). However, *F. graminearum*

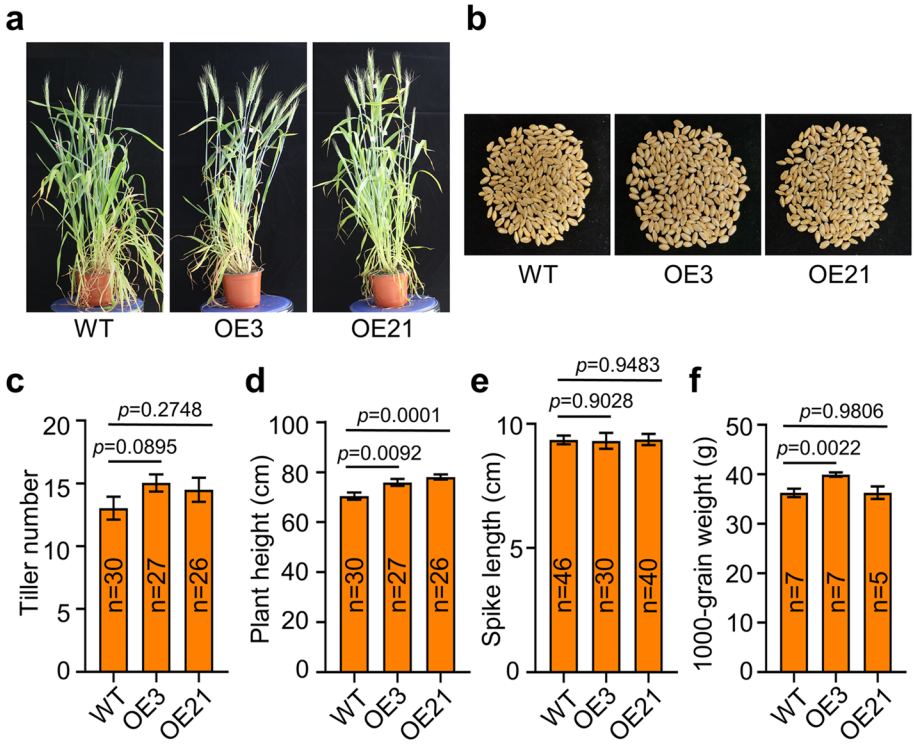

Fig. 4 Overexpression of *TaSPDS*-7D1 affects plant architecture and 1000-grain weight. **a** Plant architecture of WT and *TaSPDS*-7D1-overexpressing lines at the mature stage. **b** Grain shape of WT and *TaSPDS*-7D1-overexpressing lines. **c** Tiller number, **d** Plant height, **e** Spike length, **f** 1000-grain weight of the WT and *TaSPDS*-7D1-overexpressing lines. The error bar indicates standard errors of the means (n varies for each column and is shown in each case directly on the graphs). *P*-values are from the Student's *t*-tests

putrescine synthesis gene *FgODC* is not upregulated during infection on wheat, suggesting unessential role of endogenous putrescine synthesis on DON production during *F. graminearum* infection (Ma et al. 2021). In this study, while maintaining a consistent total nitrogen level, putrescine induced the higher DON level than its precursors in TBI culture. Exogenously application of putrescine had no effect on the diseased spikelet number. On the contrary, spermidine and spermine did not induce DON production, and exogenously application of these two higher polyamines on wheat head significantly reduced the diseased spikelet number, which gives support to the consequence that higher polyamines in the host contribute to FHB resistance.

It is well acknowledged that polyamines contribute to plant resistance against fungal pathogens by multiple functions (KoÇ 2015; Takahashi 2016; Gonzalez et al. 2021). Polyamines have a crucial impact on regulating the production of hydrogen peroxide (H_2O_2), which is recognized as the primary reactive oxygen species (ROS) in plants. Polyamines participate in enhancing H_2O_2 levels through their catabolism by amine oxidases (Gerlin et al. 2021). Generally, putrescine can be oxidized by diamine oxidases (DAO), spermidine and spermine can be oxidized by polyamine oxidases (PAO), these reactions release H_2O_2 (Moschou et al. 2012; Gerlin et al. 2021). In *A. thaliana*, exogenously applied spermine induces the accumulation of H_2O_2, primming resistance against necrotrophic fungus *Botrytis cinerea* (Janse van Rensburg et al. 2021). The increase in H_2O_2 may also be involved in signaling for phytoalexin production (Handa et al. 2018; Gonzalez et al. 2021), and in the initiation of the well-documented hypersensitive response (HR) (Zeiss et al. 2021). In rice, exogenously application of spermidine induces resistance marker genes *OsPR1b* and *PBZ1* expression, as well as phytoalexin biosynthesis genes *CSP4* and *NOMT*, leading to increased resistance against rice blast fungus *Magnaporthe oryzae* (Moselhy et al. 2016). In addition to their free forms, plant polyamines can also occur as conjugated forms. Conjugation of amines to hydroxycinnamic acids (HCAs) generates HCA amides (HCAAs), which are considered as part of the polyamine storage pool. HCAAs have been classified as phytoalexins and their accumulation in infected tissues served as biomarkers of pathogen infection (Zeiss et al. 2021).

Interestingly, the *TaSPDS*-7D1 OE line OE3 exhibited an increase of 1000-grain weight and plant height

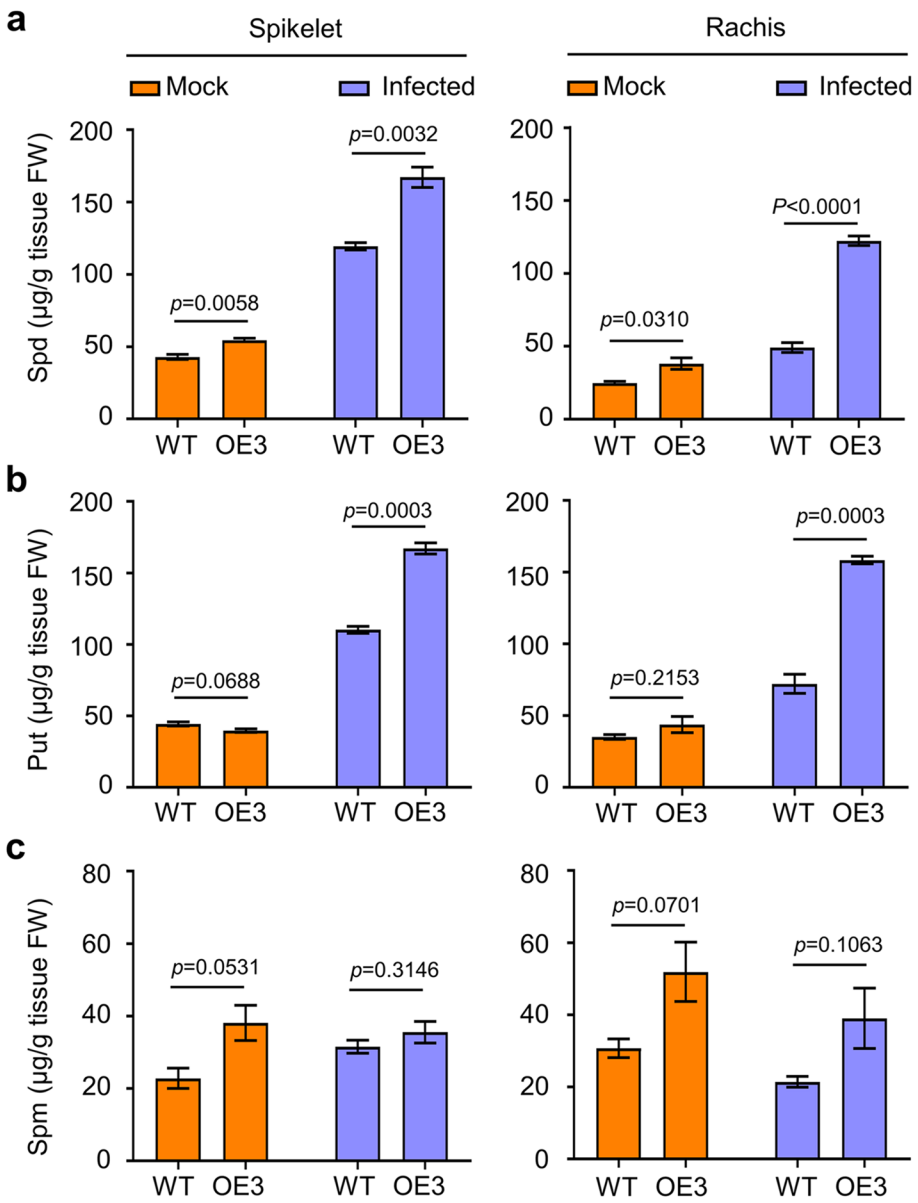

Fig. 5 Overexpression of *TaSPDS*-7D1 affects polyamine synthesis. Contents of **a** spermidine, **b** putrescine, and **c** spermine in the WT and *TaSPDS*-7D1-overexpressing lines with (Infect) or without (Mock) *F. graminearum* inoculation. FW, Fresh weight. The error bar indicates standard errors from three independent experiments ($n = 3$). *P*-values are from the Student's *t*-tests

compared to the wild-type, indicating a positive role of *TaSPDS* in the regulation of wheat growth. It was reported that exogenous spermidine can effectively regulate carbohydrate metabolism in cucumber leaves (Chen et al. 2011). Foliar spray treatment with putrescine and spermine on wheat increases the concentration of soluble, insoluble, and total sugars concentrations of leaves (Ebeed et al. 2017). Such an increase triggered a surge in the flow of sugars into the grains, leading to a significant increase in the grain weight (Kaur-Sawhney et al. 1982). The previous study has suggested that polyamines are notably involved in the grain filling of wheat (Liu et al. 2013). It is reported that a high spermidine level in grain is one of the reasons why superior grain has a higher grain filling rate than inferior grain (Yang et al. 2008). Luo and colleagues reported that exogenous spermidine significantly increased grain filling (Luo et al. 2019). Therefore, the increase in spermidine content may contribute to the enhanced 1000-grain weight of *TaSPDS*-7D1-overexpressing line OE3.

In this study, overexpression of *TaSPDS*-7D1 significantly enhanced the spermidine content but did not affect putrescine and spermine content in the uninfected stage. After *F. graminearum* infection, *TaSPDS*-7D1-overexpressing line showed a significant increase of spermidine and putrescine in the spikelets and rachises compared to the wild type. These findings suggest that the polyamine biosynthesis pathway undergoes positive feedback, and the increased levels of spermidine led to an increase in the levels of its synthetic precursor, putrescine. These observations help explain why the overexpression of *TaSPDS*-7D1 enhances resistance to FHB but has a limited effect on reducing DON production. Overexpression of *SPDS* genes from figleaf gourd (*Cucurbita ficifolia*), pepper (*Capsicum annuum*), and datura (*Datura stramonium*) in Arabidopsis or tobacco has also been conducted. Overexpression of figleaf gourd *SPDS* gene increased the spermidine content (1.3- to twofold), and slightly increased putrescine content (Kasukabe et al. 2004). The transgenic Arabidopsis showed enhanced tolerance to multiple stresses such as freezing, drought, and paraquat toxicity (Kasukabe et al. 2004). Overexpression of pepper (*C. annuum* L.) *SPDS* gene in Arabidopsis resulted in higher spermidine content, and cold tolerance (Zhang et al. 2023). Overexpression of the datura spermidine synthase gene in tobacco results in an increasing activity of SPDS, which elevated the spermidine content, but made no difference to spermine and total polyamine level. The resulting transgenic tobacco plants displayed no observable phenotypic alterations (Franceschetti et al. 2004). In rice, overexpression of *OsSPMS1* led to a decreased level of spermidine and accumulation of putrescine, but there was no significant difference in spermine content between the wild-type and *OsSPMS1*-overexpressing plants (Tao et al. 2018). Therefore, polyamine levels are not only determined by the expression of *SPDS*/*SPMS* or activities of SPDS/SPMS but are also affected by global regulation mechanisms.

Although overexpression of *TaSPDS*-7D1 increases wheat resistance to FHB, it has limited function in reducing DON production on wheat. Since the external application of putrescine did not lead to an increase in DON production (Fig. 1e), a possible explanation for the lack of impact of *TaSPDS*-7D1 overexpression on DON production is that the putrescine levels in wild-type wheat during infection might already be adequate to induce high levels of DON. The heightened putrescine content in *TaSPDS*-7D1 overexpression lines may not further stimulate DON production. Therefore, other approaches need to be explored to generate FHB resistance wheat or reduce FHB severity based on reducing DON accumulation. Transgenic wheat plants silenced for or mutation in *ODC* and/or *ADC* may result in significantly reduced putrescine levels in wheat heads, as it may reduce DON accumulation. However, since polyamines play important roles in many biological processes, it may be difficult to generate such kind of low-polyamine content transgenic wheat without affecting main agronomic traits. Therefore, suppressing the polyamine ingestion pathway by *F. graminearum* would be an effective way. Crespo-Sempere and colleagues studied the effect of polyamine biosynthesis inhibitor DFMO (D, L-α-difluoromethylornithine) and polyamine transport inhibitors on growth, DON production, and *F. graminearum* infection. They found that DFMO can reduce growth and DON production but exogenous putrescine recovers its effects in culture, suggesting an ingestion event of pathogens from outside (Crespo-Sempere et al. 2015). Meanwhile, polyamine transport inhibitors suppress *F. graminearum* growth and DON contamination *in planta*. Therefore, high-affinity and specific polyamine transport inhibitors could be developed into target-specific fungicides for controlling FHB. In addition, reducing host polyamine export by silencing or editing putrescine transporter genes may reduce apoplastic putrescine levels and DON accumulation.

Conclusion

In summary, the present study showed that while maintaining the same amount of total nitrogen in TBI culture, putrescine is the most potent inducer of DON compared to other metabolites in the polyamine biosynthesis pathway. Exogenously application of higher polyamines on wheat head significantly reduced the diseased spikelet number. *TaSPDS*-7D1-overexpressing line confers a higher spermidine level and elevated resistance to *F. graminearum* infection than the wild-type wheat. However, there was no significant difference in DON accumulation. The *TaSPDS*-7D1-overexpressing line OE3 exhibited higher 1000-grain weight and plant height compared to the wild type. Our results showed that overexpression of the spermidine synthase gene enhances wheat resistance to FHB without compromising wheat yield.

Methods

Plant and fungal materials and treatments

Wheat (*T. aestivum* L.) cultivar Fielder was obtained from the State Key Laboratory for Crop Stress Resistance and High-Efficiency Production (Yangling, Shaanxi). All wheat plants were grown in the greenhouse at a relative humidity of 65%, 40,000 Lux with a 16 h light/8 h dark photoperiod, and a temperature of 22°C during the day and then 16°C during the night. Their agronomic traits were recorded and plants were harvested subsequently.

The *F. graminearum* wild-type strain PH-1 was cultured on potato dextrose agar (PDA) plates at 25°C.

Sequence identification and generation of transgenic wheat

The 972-bp full-length coding sequence (CDS) of *TaSPDS*-7D1 was cloned into the pANIC-6E plasmid under the control of the maize ubiquitin promoter using the Gateway system (Invitrogen, USA). Then, the construct was transformed into *Agrobacterium* strain EHA105. The *TaSPDS*-7D1-overexpressing wheat lines were generated in the State Key Laboratory for Crop Stress Resistance and High-Efficiency Production (Yangling, Shaanxi) using *Agrobacterium*-mediated transformation (Ishida et al. 2015) and screened using 10 mg/L glufosinate-ammonium. The 6E-Check-F/6E-Check-R were used for certifying the presence of the pANIC-6E in the transgenic plants. Real-time quantitative PCR (qRT-PCR) was employed to detect the expression levels, *TaSPDS*-7D1 gene expression levels are normalized to that of *TaEF-1α* (Schoonbeek et al. 2015). All primers used in this study are listed in Additional file 1: Table S2. The presence of TaSPDS-7D1-HA fusion protein was detected by western blot analysis with the anti-HA (1:3000 dilution, AE008, ABclonal, USA) antibodies.

F. graminearum inoculation and deoxynivalenol production assay

The wheat heads at the anthesis stage were inoculated with 10 μL of 2×10^5 spores/mL conidium suspensions at the fifth spikelet from the top. Inoculated wheat heads were moisturized for 36 h with plastic bags (Ren et al. 2022). For spraying application of exogenous polyamines, 5 mM of putrescine, spermidine, and spermine were sprayed on the wheat head three times separately along with and every two days after *F. graminearum* inoculation. The number of diseased spikelets per head was examined 14 days post inoculation (dpi). DON production in the inoculated spikelets sampled at 14 dpi was assayed by gas chromatography-mass spectrometry (GCMS-QP2010) system with AOC-20i autoinjector (Shimadzu Co. Japan). TBI medium was used for in vitro induction of DON. 5 mM Glutamine, 2.5 mM arginine, 5 mM ornithine, 2.5 mM agmatine, 5 mM putrescine, 3.33 mM spermidine, and 2.5 mM spermine were used as the sole nitrogen source. A 20 μL aliquot of *F. graminearum* conidial suspension (1×10^6/mL) was mixed with 1.98 mL TBI in 24-well polypropylene plates and incubated in the dark without shaking at 28°C for 7 days.

Polyamines measurement

For polyamines measurement, the inoculation method was the same as introduced before (Ren et al. 2022), the only difference is conidium suspensions of *F. graminearum* were injected into all of the florets on the wheat head during anthesis, while sterile distilled water was inoculated in the same manner as the control. The spikelets and rachises were collected 5 dpi. Polyamines were estimated using high-performance liquid chromatography (HPLC) as described by Flores and Galston (Flores and Galston 1982) with minor modifications. HPLC analysis was performed using a Shimadzu LC-20AD HPLC instrument (Shimadzu Co. Japan). The sample (5 μL) was injected and chromatographed on a WondaSil C18 column (150 mm × 3.0 mm, 5 μm) using an auto-injector with 60% methanol at a flow rate of 0.7 mL/min. Fluorescence was detected at 230 nm. Chromatographic data were analyzed using the Chromatopac system.

Abbreviations

ADC	Arginine decarboxylase
AGM	Agmatine
Arg	Arginine
cDNA	Complementary DNA
CDS	Coding sequence
CMC	Carboxymethyl cellulose
dcSAM	Decarboxylated S-adenosylmethionine
DFMO	D, L-α-difluoromethylornithine
DON	Deoxynivalenol
DPI	Days post inoculation
Fg	*Fusarium graminearum*
FHB	Fusarium head blight
Gln	Glutamine
HCAAs	Hydroxycinnamic acids amides
HCAs	Hydroxycinnamic acids
HPI	Hours post inoculation
HPLC	High-performance liquid chromatography
HR	Hypersensitive response
ODC	Ornithine decarboxylase
Orn	Ornithine
PAO	Polyamine oxidases
PCR	Polymerase Chain Reaction
PDA	Potato dextrose agar
Put	Putrescine
qRT-PCR	Quantitative real-time PCR
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
SAMDC	S-adenosylmethionine decarboxylase
SDS	Sodium dodecyl sulfate
Spd	Spermidine
SPDS	Spermidine synthase
Spm	Spermine
SPMS	Spermine synthase
TBI	Trichothecene biosynthesis-inducing
TPM	Transcripts per million

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00243-y.

Additional file 1: Table S1. SPDS/SPMS protein sequences from different plant species used for phylogenetic tree. **Table S2.** Primers used in this study.

Additional file 2: Figure S1. Alignment of TaSPDS protein sequence. The sequence of the Spermine_synth domain is underlined with a red solid line.

Acknowledgements
We thank Dr. Qinhu Wang at Northwest A&F University for help with the phylogenetic analysis, and Ms. Ping Xiang at Northwest A&F University for help with the measurement of polyamines, and Dr. Cong Jiang and Dr. Guanghui Wang at Northwest A&F University for fruitful discussions.

Authors' contributions
HL, MX, and JR designed experiments; JR, CL, and QX performed the experiments; JR, CL, and QX analyzed the data; JR, MX, and HL wrote the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by grants from the National Key R&D Program of China (2022YFD1400100) and the Chinese Universities Scientific Fund (2452023045).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 28 January 2024 Accepted: 10 April 2024
Published online: 14 May 2024

References

Bianchini A, Horsley R, Jack MM, Kobielush B, Ryu D, Tittlemier S, et al. DON occurrence in grains: a north American perspective. Cereal Foods World. 2015;60:32–56. https://doi.org/10.1094/CFW-60-1-0032.

Boenisch MJ, Schäfer W. *Fusarium graminearum* forms mycotoxin producing infection structures on wheat. BMC Plant Biol. 2011;11:1–14. https://doi.org/10.1186/1471-2229-11-110.

Chen LF, Lu W, Sun J, Guo SR, Zhang ZX, Yang YJ. Effects of exogenous spermidine on photosynthesis and carbohydrate accumulation in roots and leaves of cucumber (*Cucumis sativus* L.) seedlings under salt stress. J Nanjing Agric Uni. 2011;34:31–6. (in Chinese).

Chen Y, Kistler HC, Ma ZH. *Fusarium graminearum* trichothecene mycotoxins: biosynthesis, regulation, and management. Annu Rev Phytopathol. 2019;57:15–39. https://doi.org/10.1146/annurev-phyto-082718-100318.

Choubey A, Rajam MV. RNAi-mediated silencing of spermidine synthase gene results in reduced reproductive potential in tobacco. Physiol Mol Biol Plants. 2018;24:1069–81. https://doi.org/10.1007/s12298-018-0572-x.

Crespo-Sempere A, Estiarte N, Marin S, Sanchis V, Ramos AJ. Targeting *Fusarium graminearum* control via polyamine enzyme inhibitors and polyamine analogs. Food Microbiol. 2015;49:95–103. https://doi.org/10.1016/j.fm.2015.01.020.

Ebeed HT, Hassan NM, Aljarani AM. Exogenous applications of polyamines modulate drought responses in wheat through osmolytes accumulation, increasing free polyamine levels and regulation of polyamine biosynthetic genes. Plant Physiol Biochem. 2017;118:438–48. https://doi.org/10.1016/j.plaphy.2017.07.014.

Flores HE, Galston AW. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 1982;69:701–6. https://doi.org/10.1104/pp.69.3.701.

Franceschetti M, Fornalé S, Tassonia A, Zuccherelli K, Mayer MJ, Bagni N. Effects of spermidine synthase overexpression on polyamine biosynthetic pathway in tobacco plants. J Plant Physiol. 2004;161:989–1001. https://doi.org/10.1016/j.jplph.2004.02.004.

Gardiner DM, Kazan K, Manners JM. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium Graminearum*. Fungal Genet Biol. 2009. https://doi.org/10.1016/j.fgb.2009.04.004. 46:604 – 13.

Gardiner DM, Kazan K, Praud S, Torney FJ, Rusu A, Manners JM. Early activation of wheat polyamine biosynthesis during Fusarium head blight implicates putrescine as an inducer of trichothecene mycotoxin production. BMC Plant Biol. 2010;10:1–13. https://doi.org/10.1186/1471-2229-10-289.

Gerlin L, Baroukh C, Genin S. Polyamines: double agents in disease and plant immunity. Trends Plant Sci. 2021;26:1061–71. https://doi.org/10.1016/j.tplants.2021.05.007.

Gonzalez ME, Marco F, Minguet EG, Carrasco-Sorli P, Blázquez MA, Carbonell J, et al. Perturbation of spermine synthase gene expression and transcript profiling provide new insights on the role of the tetraamine spermine in Arabidopsis defense against *Pseudomonas viridiflava*. Plant Physiol. 2011;156:2266–77. https://doi.org/10.1104/pp.110.171413.

Gonzalez ME, Jasso-Robles FI, Flores-Hernández E, Rodríguez-Kessler M, Pieckenstain FL. Current status and perspectives on the role of polyamines in plant immunity. Ann Appl Biol. 2021;178:244–55. https://doi.org/10.1111/aab.12670.

Handa AK, Fatima T, Mattoo AK, Polyamines. Bio-molecules with diverse functions in plant and human health and disease. Front Chem. 2018;6:10. https://doi.org/10.3389/fchem.2018.00010.

Hanzawa Y, Imai A, Michael AJ, Komeda Y, Takahashi T. Characterization of the spermidine synthase-related gene family in *Arabidopsis thaliana*. FEBS Letters. 2002;527:176 – 80. https://doi.org/10.1016/s0014-5793(02)03217-9.

Imai A, Matsuyama T, Hanzawa Y, Akiyama T, Tamaoki M, Saji H, et al. Spermidine synthase genes are essential for survival of Arabidopsis. Plant Physiol. 2004;135:1565 73. https://doi.org/10.1104/pp.104.041699.

Ishida Y, Tsunashima M, Hiei Y, Komari T. Wheat (*Triticum aestivum* L.) transformation using immature embryos. In: Wang K, editor. Agrobacterium protocols: volume 1. New York, NY: Springer New York; 2015. pp. 189–98. https://doi.org/10.1007/978-1-4939-1695-5_15.

Jansen C, von Wettstein D, Schäfer W, Kogel KH, Felk A, Maier FJ. Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. Proc Natl Acad Sci U S A. 2005;102:16892–7. https://doi.org/10.1073/pnas.0508467102.

Ji F, Xu JH, Liu X, Yin XC, Shi JR. Natural occurrence of deoxynivalenol and zearalenone in wheat from Jiangsu province, China. Food Chem. 2014;157:393–7. https://doi.org/10.1016/j.foodchem.2014.02.058.

Jiang C, Cao SL, Wang ZY, Xu HJ, Liang J, Liu HQ, et al. An expanded subfamily of G-protein-coupled receptor genes in *Fusarium graminearum* required for wheat infection. Nat Microbiol. 2019;4:1582–91. https://doi.org/10.1038/s41564-019-0468-8.

Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol. 2004;45:712–22. https://doi.org/10.1093/pcp/pch083.

Kaur-Sawhney R, Shih L-M, Flores HE, Galston AW. Relation of polyamine synthesis and titer to aging and senescence in oat leaves. Plant Physiol. 1982;69:405–10. https://doi.org/10.1104/pp.69.2.405.

Kim S-H, Kim S-H, Yoo S-J, Min K-H, Nam S-H, Cho BH, et al. Putrescine regulating by stress-responsive MAPK cascade contributes to bacterial pathogen defense in Arabidopsis. Biochem Biophys Res Commun. 2013;437:502–8. https://doi.org/10.1016/j.bbrc.2013.06.080.

KoÇ E. Exogenous application of spermidine enhanced tolerance of pepper against *Phytophthora capsici* stress. Plant Prot Sci. 2015;51:127–35. https://doi.org/10.17221/86/2014-pps.

Liu Y, Gu DD, Wu W, Wen XX, Liao YC. The relationship between polyamines and hormones in the regulation of wheat grain filling. PLoS ONE. 2013;8:e78196. https://doi.org/10.1371/journal.pone.0078196.

Luo J, Wei B, Han J, Liao YC, Liu Y. Spermidine increases the sucrose content in inferior grain of eheat and thereby promotes its grain filling. Front Plant Sci. 2019;10:1309. https://doi.org/10.3389/fpls.2019.01309.

Ma TL, Zhang LX, Wang MH, Li YQ, Jian YQ, Wu L, et al. Plant defense compound triggers mycotoxin synthesis by regulating H2B ub1 and H3K4 me2/3 deposition. New Phytol. 2021;232:2106–23. https://doi.org/10.1111/nph.17718.

Majumdar R, Minocha R, Lebar MD, Rajasekaran K, Long S, Carter-Wientjes C, et al. Contribution of maize polyamine and amino acid metabolism

toward resistance against *Aspergillus flavus* infection and aflatoxin production. Front Plant Sci. 2019;10:692. https://doi.org/10.3389/fpls.2019.00692.
Michael AJ. Biosynthesis of polyamines and polyamine-containing molecules. Biochem J. 2016;473:2315–29. https://doi.org/10.1042/BCJ20160185.
Moschou PN, Wu J, Cona A, Tavladoraki P, Angelini R, Roubelakis-Angelakis KA. The polyamines and their catabolic products are significant players in the turnover of nitrogenous molecules in plants. J Exp Bot. 2012;63:5003–15. https://doi.org/10.1093/jxb/ers202.
Moselhy SS, Asami T, Abualnaja KO, Al-Malki AL, Yamano H, Akiyama T, et al. Spermidine, a polyamine, confers resistance to rice blast. J Pestic Sci. 2016;41:79–82. https://doi.org/10.1584/jpestics.D16-008.
Pal M, Janda T. Role of polyamine metabolism in plant pathogen interactions. J Plant Sci Phytopathol. 2017;1:095–100. https://doi.org/10.29328/journal.jpsp.1001012.
Pestka JJ. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol. 2010. https://doi.org/10.1007/s00204-010-0579-8. 84:663 – 79.
Ren J, Zhang Y, Wang Y, Li C, Bian Z, Zhang X, et al. Deletion of all three MAP kinase genes results in severe defects in stress responses and pathogenesis in *Fusarium graminearum*. Stress Biol. 2022;2. https://doi.org/10.1007/s44154-021-00025-y.
Schoonbeek Hj, Wang HH, Stefanato FL, Craze M, Bowden S, Wallington E, et al. Arabidopsis EF-Tu receptor enhances bacterial disease resistance in transgenic wheat. New Phytol. 2015;206:606–13. https://doi.org/10.1111/nph.13356.
Takahashi Y. The role of polyamines in plant disease resistance. Environ Control Biol. 2016;54:17–21. https://doi.org/10.2525/ecb.54.17.
Tao YJ, Wang J, Miao J, Chen J, Wu SJ, Zhu JY, et al. The spermine synthase OsSPMS1 regulates seed germination, grain size, and yield. Plant Physiol. 2018;178:1522–36. https://doi.org/10.1104/pp.18.00877.
Upadhyay RK, Shao J, Mattoo AK. Genomic analysis of the polyamine biosynthesis pathway in duckweed *Spirodela polyrhiza* L.: presence of the arginine decarboxylase pathway, absence of the ornithine decarboxylase pathway, and response to abiotic stresses. Planta. 2021;254:108. https://doi.org/10.1007/s00425-021-03755-5.
van Janse HC, Limami AM, Van den Ende W. Spermine and spermidine priming against *Botrytis cinerea* modulates ROS dynamics and metabolism in Arabidopsis. Biomolecules. 2021;11:223. https://doi.org/10.3390/biom11020223.
Xia R, Schaafsma A, Wu F, Hooker D. Impact of the improvements in Fusarium head blight and agronomic management on economics of winter wheat. World Mycotoxin J. 2020;13:423–39.
Yang JC, Cao Y, Zhang H, Liu LJ, Zhang JH. Involvement of polyamines in the post-anthesis development of inferior and superior spikelets in rice. Planta. 2008;228:137–49. https://doi.org/10.1007/s00425-008-0725-1.
Zeiss DR, Piater LA, Dubery IA. Hydroxycinnamate amides: intriguing conjugates of plant protective metabolites. Trends Plant Sci. 2021;26:184–95. https://doi.org/10.1016/j.tplants.2020.09.011.
Zhang J, Xie M, Yu G, Wang D, Xu Z, Liang L, et al. *CaSPDS*, a spermidine synthase gene from pepper (*Capsicum annuum* L.), plays an important role in response to cold stress. Int J Mol Sci. 2023;24:5013. https://doi.org/10.3390/ijms24055013.