RESEARCH PAPER

The wheat LLM-domain-containing transcription factor TaGATA1 positively modulates host immune response to Rhizoctonia cerealis

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Abstract

Wheat (Triticum aestivum) is essential for global food security. Rhizoctonia cerealis is the causal pathogen of sharp eyespot, an important disease of wheat. GATA proteins in model plants have been implicated in growth and development; however, little is known about their roles in immunity. Here, we report a defence role for a wheat LLM-domain-containing B-GATA transcription factor, TaGATA1, against R. cerealis infection and explore the underlying mechanism. Through transcriptomic analysis, TaGATA1 was identified to be more highly expressed in resistant wheat genotypes than in susceptible wheat genotypes. TaGATA1 was located on chromosome 3B and had two homoeologous genes on chromosomes 3A and 3D. TaGATA1 was found to be localized in the nucleus, possessed transcriptional activation activity, and bound to GATA-core cis-elements. TaGATA1 overexpression significantly enhanced resistance of transgenic wheat to R. cerealis, whereas silencing of TaGATA1 suppressed the resistance. Quantitative reverse transcription–PCR and ChIP–qPCR results indicated that TaGATA1 directly bound to and activated certain defence genes in host immune response to R. cerealis. Collectively, TaGATA1 positively regulates immune responses to R. cerealis through activating expression of defence genes in wheat. This study reveals a new function of plant GATAs in immunity and provides a candidate gene for improving crop resistance to R. cerealis.

Keywords: B-GATA transcription factor, bread wheat (Triticum aestivum), defence gene, immune response, Rhizoctonia cerealis, transcriptional activation.

Introduction

GATA transcription factors (TFs) are a group of transcriptional regulatory proteins containing a type IV zinc finger CX2CX17–20CX2C (C, cysteine; X, any residue) DNA-binding domain that can recognize GATA core cis-elements in promoters (Reyes et al., 2004). GATA TFs are divided into A-, B-, C-, and D-GATA classes based on conservation of amino
acid sequences and the exon–intron gene structure of the respective gene (Reyes et al., 2004; Behringer et al., 2014; Ranil et al., 2016). In Arabidopsis, B-GATA proteins can be further subdivided into two subfamilies based on the presence of additional conserved domains apart from the GATA domain: GATAs with a conserved LLM (leucine–leucine–methionine) domain at their C-terminus, and GATAs with a conserved HAN (HANABA TARANU) domain in their N-terminus. Based on the amino acid sequence length between the N-terminus and the GATA domain, LLM-domain–containing GATA proteins were further divided into short B-GATAs with an LLM-domain and long B-GATAs with an LLM-domain. Arabidopsis GATA43 is a long B-GATA member with a degenerate LLM-domain, while GATA29 contains a degenerate HAN-domain (Behringer et al., 2014; Behringer and Schwechheimer, 2015). LLM- and HAN-domain–containing B-GATA proteins have been identified in all the sequenced monocot and dicot species (Reyes et al., 2004; Behringer et al., 2014; Ranil et al., 2016; Chen et al., 2017). For instance, genome-wide surveys of GATA domain–containing sequences identified 30 GATA members in Arabidopsis and 29 in rice (Oryza sativa) (Reyes et al., 2004; Bi et al., 2005; Chen et al., 2017).

In model plants, B-GATA factors have been shown to participate in various plant growth and developmental processes (Wang et al., 2009; Houston et al., 2012; Hudson et al., 2013; Behringer and Schwechheimer, 2015; Klermund et al., 2016; Ranil et al., 2016; Lu et al., 2017). For instance, GNC (GATA, NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED) and its parologue CGA1/GNL (CYTOKININ-INDUCED GATA1/GNC-LIKE), two representative long LLM-domain–containing B-GATA factors, promote greening and chloroplast biogenesis, hypocotyl elongation, stomata development, leaf development, and plant architecture, but suppress germination in Arabidopsis (Richter et al., 2010, 2013a; Chiang et al., 2012; Behringer et al., 2014; Klermund et al., 2016). GNC and GNL also delay flowering time and participate in the response to cold stress (Richter et al., 2013b). In Arabidopsis, loss-of-function mutants of GATA15, GATA16, GATA17, and GATA17L, short LLM-domain B-GATA members, displayed early flowering and similar phenotypes to gnc and gnl mutants (Ranil et al., 2016). Additionally, GATA15, GATA16, GATA17, and GATA17L function in phyllotactic patterning, floral organ initiation, and accessory meristem formation (Ranil et al., 2016). These reports suggest that LLM-domain B-GATA factors share redundant biological roles in development and greening, and chloroplast biogenesis. In Arabidopsis, HAN factor, a HAN-domain–containing B-GATA member, has been shown to regulate embryo development and floral development, and to act as a repressor of cell proliferation (Zhao et al., 2004; Nawy et al., 2010). Three HAN paralogous factors, NLR1 (NECK LEAFI) in rice, TSH1 (TASSEL SHEATH1) in maize, and TRD (THIRD OUTER GLUME) in barley, were found to repress growth and cell cycle activities in the shoot meristem (Wang et al., 2009; Whipple et al., 2010). However, no genetic evidence for the roles of GATAs in immune responses has been reported in plant species.

Bread wheat (Triticum aestivum) is one of the most important staple crops in the world. Numerous diseases, caused by various pathogens, reduce yield and grain quality of wheat. Sharp eyespot, caused mainly by the necrotrophic fungus Rhizoctonia cerealis, is a devastating disease of wheat worldwide (Chen et al., 2008; Hamada et al., 2011; Chen et al., 2013). Since 2005, >6.67 Mha of wheat plants each year have been harmed by sharp eyespot in China, which has become the largest epidemic region in the world (Chen et al., 2013; Zhu et al., 2015). Infection by R. cerealis causes not only sharp eyespot of other cereal crops including barley, oats, and rye but also other diseases in important economic crops and bioenergy plants, such as root rot in sugar beet, cotton, potato, and several legumes (Toda et al., 1999; Tomaso-Peterson and Trevathan, 2007). Breeding crop varieties with resistance is an environmentally friendly and effective method to control the diseases caused by R. cerealis. It is necessary to isolate and characterize the key elements in wheat responses to infection with R. cerealis, and to unravel their underlying mechanisms.

In this study, we identified a short LLM-domain B-GATA TF-encoding gene, TaGATA1, in the wheat response to R. cerealis infection through comparative transcriptomic analysis. Molecular biology assays revealed that TaGATA1 was a GATA domain-binding transcription activator localized in the nucleus. Genetic functional assays demonstrated that TaGATA1 positively regulated the immune response to R. cerealis infection in wheat. Both quantitative reverse transcription–PCR (RT–qPCR) and ChiP–qPCR analyses showed that TaGATA1 could bind to and activate the expression of certain defense genes. This study reveals a novel functional role for plant GATA TFs in innate immunity.

Materials and methods

Plant and fungal materials, primers, and treatments

Six wheat cultivars (cv.), namely CI12633, Shanhongmai, Niavr14, Shannong431, Yangmai16, and Wennai6, showing different extents of resistance to sharp eyespot caused by R. cerealis (Zhu et al., 2015), were used to investigate the expression profile or function of TaGATA1. The wheat cv. Chinese spring nullitetrasomic (NT) lines, namely N3A/T3B, N3A/T3D, N3B/T3A, N3B/T3D, N3D/T3A, and N3D/T3B, were used for chromosomal localization of TaGATA1.

The fungal pathogen R. cerealis isolate R0301, which is dominant in Jiangsu and Anhui provinces of China, and the strain WK207, which is dominant in North China, were used in this study. The wheat growth conditions, R. cerealis inoculation, and sampling were conducted following (Zhu et al., 2015). The sequences of all primers are listed in Supplementary Table S1 at JXB online.

Cloning and sequence analysis of TaGATA1

RNA extraction was performed following Zhang et al. (2007). The primers for 3′-RACE and 5′-RACE were designed based on the sequence of the microarray probe TC415152, and synthesized. Through two rounds of PCRs using 3′-RACE primers and 3′-RACE kit v2.0 (TaKaRa, Japan), the 3′-untranslated region (UTR) sequence of TaGATA1 was amplified from cDNA of CI12633 stems inoculated with R. cerealis R0301 for 4 d. The 5′-UTR sequence of TaGATA1 was amplified through three rounds of PCRs using 5′-RACE primers from the CI12633 cDNA. Based on the assembled sequences of the gene, two pairs of TaGATA1-specific primers were designed and used for nested PCR to amplify the
full-length cDNA and DNA sequences of *TaGATA1* from cDNA and genomic DNA of *CI12633* stems. The *TaGATA1* promoter sequence was cloned from *CI12633* genomic DNA using the primers *TaGATA1*-pro-5'F/R. cis-elements in the *TaGATA1* promoter were analyzed using [https://sogo.dna.afrc.go.jp/cgi-bin/sogo.cgi?sid=4&lang=en&pp=640&action=page&page=newplace](https://sogo.dna.afrc.go.jp/cgi-bin/sogo.cgi?sid=4&lang=en&pp=640&action=page&page=newplace). A phylogenetic tree was constructed using a Neighbor-Joining method implemented in MEGA V 5.0 with 1000 bootstrap replications.

**Subcellular localization of TaGATA1**

The *TaGATA1* coding sequence without a stop codon was subcloned to fuse with the 5' end of the GFP- (green fluorescent protein) coding sequence in a p35S-GFP vector. The *TaGATA1*-GFP transcript was controlled using a Cauliflower mosaic virus (CaMV) 35S promoter. The plasmid DNA of the resulting fusion construct and p35S:GFP control were separately introduced into wheat protoplasts by polyethylene glycol (PEG) 4000 or white onion epidermal cells using biolistic bombardment as described previously (Zhang et al., 2007). After incubation at 25 °C for 20 h, GFP signals were observed and photographed (Qi et al., 2017) using a confocal laser scanning microscope (Zeiss LSM 700, Germany) with a Fluor ×10/0.50 M27 objective lens and SP640 filter.

**EMSA**

*TaGATA1* was fused into a His-Trigger Factor (His-TF) tag of a pCold-TF vector. The resulting pHis-TF-*TaGATA1* recombinant construct was transformed into competent cells of *Escherichia coli* Transetta (DE3) (Transgen, China). Subsequently, the His-TF-*TaGATA1* recombinant protein was expressed after induction with 0.5 mM IPTG (isopropyl-β-D-1-thiogalactopyranoside) at 16 °C, and purified using Ni-NTA resin (Transgen, China). The biotin-labelled DNA oligonucleotides listed in Supplementary Table S1 were synthesized and used as probes, and unlabelled DNA fragments of the same sequences were used as the competitors. dsDNA was obtained by heating oligonucleotides at 95 °C for 15 min and annealing at room temperature. EMSA was performed using the LightShift Chemiluminescent EMSA kit (Thermo, USA) according to the manufacturer’s instructions. The His-TF protein alone was used as the negative control.

**Yeast hybrid assay on transcriptional activation activity of TaGATA1**

The yeast strain (AH109) containing the reporter genes HIS3 and ADE2 was used in the transcription activation assay of TaGATA1. The *TaGATA1* coding sequence was fused with the GAL4 DNA-binding domain (BD) in the pGBK7 vector to generate pBD-*TaGATA1* expressing BD–*TaGATA1* fused protein. The resulting vector DNA was transformed into competent cells of yeast AH109 via a PEG-mediated method. The empty pGBK7 (pBD) vector was used as a negative control. Transcription activation activity of *TaGATA1* was evaluated according to the growth on SD/Try (Yeast Synthetic Drop-out Medium Supplement without tryptophan) (Clontech, USA) and SD/Try/His/Ade (Yeast Synthetic Drop-out medium supplement without tryptophan, histidine, or adenine) (Clontech, USA).

**Virus-induced gene silencing for the defence function of TaGATA1 in wheat**

The defensive role of *TaGATA1* was investigated using a Barley stripe mosaic virus (BSMV)-based virus-induced gene silencing (VIGS) method (Holzberg et al., 2002; Scofield et al., 2005). A 200 bp fragment of *TaGATA1* (nucleotides 756–955 in the *TaGATA1* cDNA sequence) was subcloned in an attB sense orientation into the *NheI* restriction site of RNAI*/*S vector resulting in a *BSMV:TaGATA1* recombinant construct (Supplementary Fig. S1) (Donald and Jackson, 1996). At the three-leaf stage, at least 20 plants of resistant wheat line CI12633 were inoculated with *BSMV:TaGATA1* or *BSMV:GFP* (as a control) following Zhu et al. (2015). At 20 d after virus infection, the fourth leaves were sampled to examine BSMV infection and the transcriptional level of *TaGATA1*. At 22–25 d after BSMV inoculation, these CI12633 plants were further inoculated with *R. cerealis* WK207. Following Chen et al. (2008), their infection types (ITs) and disease indexes were scored at 40 days post-inoculation (dpi) with *R. cerealis* WK207.

**Generation of TaGATA1-overexpressing transgenic wheat**

The full ORF sequence of *TaGATA1* was subcloned into a modified pAHC25 vector (Christensen and Quail, 1996) with a c-myc epitope tag (Christensen and Quail, 1996; Zhu et al., 2015). In the resulting transformation vector pUbi:mmyc-*TaGATA1* (Supplementary Fig. S2), c-myc-*TaGATA1* was driven by the maize ubiquitin (Ubi) promoter and terminated by the 3'-non-transcribed region of the *Agrobacterium tumefaciens* nopaline synthase gene (*Nnos*), pUBL:mmyc-*TaGATA1* plasmid DNA was introduced into immature embryos of the wheat cv.‘Yangmai 16 by biolistic bombardment (von Arnim, 2007).

**PCR and western blotting analyses on TaGATA1 transgenic wheat**

The presence of the introduced *TaGATA1* transgene was monitored by PCR using the transgene-specific primer pair (TaGATA1-TF and TaGATA1-TR) that spans the intron of the *TaGATA1* genomic sequence. PCR was performed in a 20 μl volume containing 1 μl of genomic DNA (200 ng μl-1), 10 μl of 2×PCR Mixture (Transgen, China), 0.5 μl of each primer (10 μM), and 8 μl of ddH2O.

Total proteins were extracted from 1 g of stems from each transgenic wheat line using a tissue protein extraction kit (CW BIO, China). Western blotting was deployed to investigate c-myc-*TaGATA1* fusion protein with 100-fold diluted anti-c-myc antibody. The c-myc-*TaGATA1* protein hybridized with 1000-fold diluted secondary antibody conjugated to horseradish peroxidase (HRP) was visualized using the Pro-light HRP Chemiluminescent Kit (Transgen, China).

**RT-PCR and RT–qPCR**

The transcriptional levels of *TaGATA1*, *BSMV*-CP, and defence-associated genes in wheat were analysed by real-time PCR (RT-PCR) or RT–qPCR. RT–qPCR was done on an ABI 7500 real time PCR system (Applied Biosystems, USA) following Dong et al. (2010). The relative expression of the tested genes was calculated using the 2^(-ΔΔCt) method (Livak and Schmittgen, 2001), where the wheat *Actin* gene *TaActin* was used as the internal reference. Three independent replications were performed.

**Assessment of response in transgenic wheat plants to *R. cerealis***

The disease indexes of wheat plants/lines were scored at the harvest stage following Chen et al. (2008), their

**ChIP–qPCR**

A rapid and efficient ChIP analysis was performed following a modified method (Nelson et al., 2006; Lee et al., 2017). Briefly, wheat protoplasts were isolated from two-stage leaf seedlings. p35S:GFP and p35S:*TaGATA1-GFP* vectors were introduced into protoplasts. Total protein was extracted from 100 μl of protoplast solution using a tissue protein extraction kit (CW BIO, China). Transient expression of GFP and
TaGATA1–GFP was confirmed by western blot using 400-fold diluted monoclonal anti-GFP antibody. The remaining protoplasts expressing GFP or TaGATA1–GFP were cross-linked with 1% formaldehyde for 15 min. The nuclei were isolated, then chromatin was sonicated and sheared to 100–500 bp using a Bioruptor UCD-200. Immunoprecipitation was performed with 400-fold diluted monoclonal anti-GFP antibody (Transgen, China). Immune complexes were collected by Dynabeads protein G (Invitrogen, USA) and then DNA fragments were recovered using the phenol–chloroform method. Subsequently, qPCR was used to investigate the degree of enrichment of the GATA-containing DNA fragment bound by GFP–TaGATA1. ChIP–qPCR comparisons were made between lines carrying the TaGATA1–GFP-expressing protoplasts versus GFP-expressing protoplasts.

Results

Identification and transcriptional profiles of TaGATA1 in wheat response to R. cerealis

To mine resistance response-related genes of wheat to R. cerealis, we compared the microarray data (GEO accession number GSE69245) of R. cerealis-resistant wheat cvs CI12633/Shanhongmai and the susceptible cv. Wenmai 6 at 4, 7, and 21 dpi with R. cerealis isolate R0301. One important regulatory gene occupied by the probe (ID: A_99_P340746 in Agilent Wheat GeneChip) was identified and corresponded to the 3’ sequence of a wheat EST sequence with TIGR number TC415152. Hereafter, this gene sequence was cloned from resistant wheat CI12633 and designated TaGATA1 since it encodes an LLM-domain B-GATA protein. The microarray data showed that at 4, 7, or 21 dpi with R. cerealis R0301, the gene transcriptional levels were higher in both resistant wheat cvs CI12633 and Shanhongmai than in susceptible wheat cv. Wenmai 6 (Fig. 1A).

Further RT–qPCR results showed that at 1, 4, 10, and 21 dpi with R. cerealis R0301, the transcriptional levels of TaGATA1 were higher in resistant wheat cv. CI12633 than in susceptible cv. Wenmai 6, consistent with the trend in microarray data (Fig. 1B). The transcription of TaGATA1 in resistant wheat cv. CI12633 was noticeably increased after pathogen infection, and reached a peak at 10 dpi (Fig. 1B). After inoculation with R. cerealis R0301, TaGATA1 transcription was significantly higher in the partially resistant wheat cvs (Shanhongmai, CI12633, Niavt14, and Shannong0431) than in susceptible cv. Wenmai 6 (Fig. 1C). Transcriptional analyses in different organs at the heading stage showed that at 3 dpi with R. cerealis R0301, TaGATA1 transcription was the highest in stems where sharp eyespot disease primarily occurs (Fig. 1D). These results suggested that TaGATA1 might participate in the wheat immune response to R. cerealis.

Sequence characteristics and chromosomal localization of TaGATA1

The full-length cDNA of TaGATA1 (1096 bp) was cloned from resistant wheat CI12633 stem cDNA and has been deposited in GenBank (accession number MG461317). It contains an ORF that is 546 bp in length, with a 5’-UTR of 249 bp and a 3’-UTR of 301 bp. The cDNA sequence of TaGATA1

Fig. 1. Transcriptional analysis of TaGATA1 in Rhizoctonia cerealis-inoculated wheat. (A) Microarray data for fold transcriptional up-regulation of TaGATA1 between resistant wheat cultivars CI12633/Shanhongmai and susceptible wheat cultivar Wenmai 6 at 4, 7, and 21 dpi with R. cerealis. (B) Transcript levels of TaGATA1 in R. cerealis-resistant wheat line CI12633 and susceptible wheat cultivar Wenmai 6 at 1, 4, 10, and 21 dpi with R. cerealis R0301 and mock treatment. TaGATA1 transcription in mock-treated Wenmai 6 was set to 1. (C) TaGATA1 transcription in five wheat cultivars at 7 dpi with R. cerealis R0301. The expression level of TaGATA1 in Wenmai 6 was set to 1. DI indicates disease index of sharp eyespot. (D) Transcript levels of TaGATA1 in roots, stems, leaves, sheath, and spikes of wheat Yangmai 16 at 3 dpi with R. cerealis R0301. TaGATA1 transcription in roots of Wenmai 6 was set to 1. Statistically significant differences are derived from the results of three independent replications (t-test: **P<0.01). Error bars indicate the SE. (This figure is available in colour at JXB online.)
Brachypodium distachyon shares 92.04% identity with the matched sequence TC415152. Genomic sequence 1251 bp in length was also cloned from CI12633. Comparison of the cDNA and genomic sequences showed that TaGATA1 genomic sequence comprises one intron (155 bp) and two exons (Fig. 2A). Furthermore, the promoter sequence (1793 bp) upstream of the start codon of TaGATA1 was cloned from CI12633 genomic DNA, and includes several biotic stress-responsive cis-elements, phytohormone-responsive cis-elements, and light-responsive elements (Supplementary Table S2). The deduced protein TaGATA1 consisted of 181 amino acid residues with a molecular weight of 19.817 kDa and a theoretical pI of 9.313. TaGATA1 contains an acidic region (amino acids 2–32) that possibly acts as a transcription activation domain, a conserved GATA-motif binding domain (amino acids 29–79), two putative nuclear localization signals (NLS1 and NLS2, located in amino acids 58–79 and 145–157, respectively), and a LLM-domain (amino acids 167–174) (Fig. 2B).

TaGATA1 and some GATA proteins from wheat, Arabidopsis, rice, barley, and Brachypodium distachyon were subjected to phylogenetic analysis. The phylogenetic analysis revealed that these GATA proteins belong to short LLM domain B-GATA subfamily, GATA23 with a degenerate LLM-domain, long LLM domain B-GATA subfamily, long B-GATAs with a HAN-domain subfamily, and GATA29 with a degenerate HAN-domain. TaGATA1 fell into the short LLM-domain B-GATA subfamily, while HvGATA6 (TRD1; Houston et al., 2012), its parologue in wheat named TaGATA1AL, TaGATA18, AtGATA18–20, OsGATA8–9, and OsGATA15 as well BdGATA7–8 fell into the long B-GATAs HAN-domain subfamily (Supplementary Fig. S3). TaGATA1 is closer to TaGATA16, with 76.50% identity, than to HvGATA2 (70.62% identity), whose functions have not been reported yet. The whole amino acid sequence of TaGATA1 shared 64.44, 47.51, 28.72, 32.80, 29.74, and 30.35% identities with those of function-unknown B. distachyon BdGATA4 and function-known OsGATA12, AtGATA15, AtGATA16, AtGATA17, and AtGATA17LIKE, respectively. These data showed that TaGATA1 is a short LLM-domain B-GATA protein in wheat.

A BLAST analysis against the hexaploid wheat genome sequence showed that the TaGATA1 genomic sequence shared 98% identity to the sequence TRIAE_CS42_3B_TGACv1_221429_AA0740590 on wheat chromosome 3B, suggesting that TaGATA1 should be located on wheat chromosome 3B. Using TaGATA1-specific primers and the templates from genomic DNAs of NT lines of the wheat cultivar Chinese Spring, PCR results proved that TaGATA1 was located on wheat chromosome 3B (Fig. 2C). Additionally, two homoeologous genes from chromosomes 3A and 3D, TaGATA1-3A and TaGATA1-3D, respectively, were cloned from CI12633. At the ORF region, TaGATA1 on chromosome 3B (TaGATA1-3B) displayed 83.90% and 87.90% sequence identities with TaGATA1-3A and TaGATA1-3D, respectively (Supplementary Fig. S4). The pairwise comparison indicated that the protein sequence of TaGATA1 on chromosome 3B shares 88.20% and 86.30% identities with the homoeologous proteins TaGATA1-3A and TaGATA1-3D, respectively, and all three homoeologous proteins contain the conserved GATA-binding domain (Supplementary Fig. S5).

TaGATA1 is localized in the nucleus

To investigate the subcellular localization, the full coding sequence of TaGATA1 was fused to the N-terminus of GFP (Fig. 3A). DNAs of the resulting p35S:TaGATA1-GFP and p35S:GFP control constructs were individually introduced into wheat mesophyll protoplasts or onion epidermal cells. These fluorescent proteins were transiently expressed and observed via a confocal microscope. The fluorescent images in wheat mesophyll protoplasts showed that TaGATA1–GFP accumulated in the nucleus but the control GFP was distributed throughout the cell (Fig. 3A). Accordingly, in onion epidermal cells, the fluorescent images of TaGATA1–GFP and the control GFP displayed the same patterns as those in the wheat protoplasts (Fig. 3A). These results showed that the TaGATA1 TF localized in the nucleus.

Fig. 2. Gene structure, amino acid sequence, and chromosomal location of TaGATA1. (A) Genomic structure of TaGATA1. Untranslated regions (UTRs), exons, and introns are indicated. (B) Amino acid sequence of TaGATA1 protein. The transcriptional activation domain and GATA-binding domain are marked by the first shaded block and underlining, respectively. Two nuclear localization signals are marked by the second and third shaded blocks. (C) Chromosome localization of TaGATA1 using nullitetrasomic and double ditelosomic lines derived from wheat cv. Chinese Spring (CS). Marker indicates DL2, 000 DNA marker; N3A/T3B, N3A/T3D, N3B/T3A, N3B/T3D, N3D/T3A, and N3D/T3B indicate six CS nullitetrasomic lines. (This figure is available in colour at JXB online.)
TaGATA1 regulates immunity in wheat

TaGATA1 is a GATA-binding transcription activator

The transcription activation assay was performed in yeast. As shown in Fig. 3B, although all the yeast cells were able to grow well on the SD/Trp medium, only yeast cells expressing BD–TaGATA1 could grow on selective medium (SD/Trp/His/Ade). These results indicated that TaGATA1 could activate the transcription of reporter genes ade2 and his3 in the yeast genome, and suggested that TaGATA1 might possess transcriptional activation activity.

To examine the binding ability of TaGATA1 to GATA cis-elements, the recombinant protein His-TF-TaGATA1 was constructed and expressed in E. coli. The purified His-TF-TaGATA1 protein was mixed with the probe containing a GATA core cis-element (the recognition sequence of GATA TFs) in binding reaction buffer. EMSA results showed that the gel mobility shift was present in the combination of His-TF-TaGATA1 protein with the probe containing the GATA core cis-element (lanes 1–4 in Fig. 3C) but absent in the combination of His-TF protein with the probe (lane 5 in Fig. 3C). Furthermore, the binding was reduced with addition of unlabelled competitors with the same sequences (lanes 2–4 in Fig. 3C). These results proved that TaGATA1 could bind to the GATA core cis-element.

Silencing of TaGATA1 suppresses wheat resistance to R. cerealis

The cDNA fragment specific to TaGATA1 on chromosome 3B, with 67.60% and 79.50% sequence identities to the target regions of homoeologous genes on 3A and 3D, respectively, was used to construct the BSMV-based VIGS vector for specifically silencing TaGATA1 in the resistant wheat cv. CI12633. At 20 d after transfection of BSMV-derived RNAs into leaves of CI12633, symptoms of BSMV infection appeared on these leaves and the transcript of BSMV coat protein (cp) was detected (Fig. 4A), indicating that BSMV infected these wheat plants. The transcriptional levels of TaGATA1 were significantly decreased in BSMV:TaGATA1-infected CI12633 plants compared with BSMV:GFP-infected CI12633 plants (Fig. 4B), suggesting that TaGATA1 was successfully silenced in BSMV:TaGATA1-infected (TaGATA1-silenced) plants.

Subsequently, TaGATA1-silenced and BSMV:GFP-infected plants were further inoculated with R. cerealis isolate WK207. At 21 dpi with R. cerealis, the stems of TaGATA1-silenced CI12633 plants displayed more serious necrosis of sharp eyespot than did BSMV:GFP-infected (control) plants (Fig. 4C). Based on two batches of VIGS and disease scoring at 40 dpi with R. cerealis, the average ITs and disease indexes of TaGATA1-silenced CI12633 plants were 2.33/3.78 and 46.6/75.60, but those of BSMV:GFP-infected CI12633 (control) plants were 1.43/2.53 and 28.60/50.60, respectively (Fig. 4D). These results indicated that silencing of TaGATA1 suppressed resistance of wheat CI12633 to R. cerealis.

TaGATA1 overexpression increases resistance of transgenic wheat to R. cerealis

To generate TaGATA1-overexpressing transgenic wheat plants and further explore the defence role of TaGATA1, the transformation vector pUbi:myc-TaGATA1 (Supplementary Fig. S2) was constructed and bombarded into immature embryos of susceptible wheat cultivar Yangmai 16. The presence of alien TaGATA1 transgene was detected by the PCR product using transgene-specific primers (Supplementary Fig. S6). Based on results of PCR detections in four successive generations (T0–T3), five stable transgenic wheat lines (GO1–GO5) were

Fig. 3. Biochemical characteristic assays of TaGATA1. (A) Subcellular localization of TaGATA1 in wheat protoplasts and onion epidermal cells. The control GFP and fused TaGATA1–GFP are transiently expressed in mesophyll protoplasts and onion epidermal cells. Scale bars=20 μm (wheat protoplasts). (B) Transcriptional activation assay of TaGATA1 in yeast. The yeast AH109 cells containing BD-TaGATA1 or BD grow on SD/Trp, whereas only yeast AH109 cells containing BD-TaGATA1 grow on selective medium (SD/Trp/His/Ade). (C) EMSA for TaGATA1 binding to the GATA core cis-element. Each biotin-labelled probe was incubated with the His-TF-TaGATA1 protein. Competitive binding analysis was performed with increasing amounts of the unlabelled probe. The arrow, triangle, and asterisk indicate the shifted bands, non-specific binding, and free probe, respectively. (This figure is available in colour at JXB online.)
screened. RT–qPCR analyses showed that transcriptional levels of TaGATA1 in these five transgenic wheat lines were significantly elevated compared with the non-transformed [wild-type (WT)] wheat Yangmai 16 (Fig. 5A). Western blotting analysis indicated that the introduced myc-TaGATA1 was translated into myc-TaGATA1 protein in these five overexpressing transgenic lines, but not in WT Yangmai 16 (Fig. 5B).

After *R. cerealis* infection, all five TaGATA1-overexpressing lines in three successive (T1–T3) generations displayed significantly enhanced resistance to sharp eyespot compared with WT Yangmai 16 (Fig. 5C, D; Supplementary Table S3). For example, average infection types of these five TaGATA1-overexpressing lines in the T2 generation were 1.00, 1.37, 1.50, 1.50, and 1.00, whereas an average infection type of WT Yangmai 16 was 3.06. The disease index of WT Yangmai 16 was 63.48, whereas those of these TaGATA1-overexpressing lines in the T2 generation were 20–30 (Fig. 5D; Supplementary Table S3), showing that the degree of resistance was significantly increased in all transgenic lines. These results suggest that TaGATA1 positively regulates the resistance response to *R. cerealis* infection.

**TaGATA1 activates the expression of certain defence genes**

The above transactivation assay revealed that TaGATA1 is a transcriptional activator. To uncover whether defence genes were activated by TaGATA1, RT–qPCR was deployed to analyse the transcriptional patterns of several defence marker genes in wheat, including pathogenesis-related (PR) genes PR10 and PR17, and *Chitinase*3, in TaGATA1-overexpressing and TaGATA1-silenced wheat plants as well as their control plants. RT–qPCR results showed that 7 dpi with *R. cerealis* WK207, transcriptional levels of PR10, PR17, and *Chitinase*3 were significantly elevated in TaGATA1-overexpressing lines compared with WT Yangmai 16, whereas they were significantly decreased in TaGATA1-silenced plants compared with
BSMV:GFP-infected control plants (Fig. 6). These results suggest that TaGATA1 can activate the expression of certain defence genes in the wheat immune response to *R. cerealis*.

**ChIP indicates binding of TaGATA1 to PR10, PR17C, and Chitinase3 in wheat**

The promoter sequences of the wheat defence marker genes, *PR10, PR17C*, and *Chitinase3*, encompass 13, 10, and 5 GATA core cis-elements, respectively (Supplementary Table S4). A rapid ChIP protocol was deployed to examine whether TaGATA1 directly binds to GATA core cis-element–containing regions of the promoters of the above defence marker genes. The transient expression of GFP and TaGATA1–GFP in wheat mesophyll protoplasts was confirmed by western blot with monoclonal GFP antibody (Fig. 7A). Subsequently, ChIP–qPCR was used to amplify GATA–containing regions in promoters of *PR10, PR17C*, and *Chitinase3*. The ChIP–qPCR results showed that the GATA core cis-element fragments in the promoters of *PR10, Chitinase3*, and *PR17C* were enriched 5.6-, 3.7-, and 2.4-fold, respectively, more by TaGATA1–GFP than by GFP.

![Fig. 6. Transcriptional analyses of defence genes (*PR10, PR17c*, and *Chitinase3*) in wheat. (A) The transcript levels of the tested genes in *TaGATA1*-overexpressing (GO1 and GO2) wheat *Yangmai* 16 or *TaGATA1*-silenced (BSMV:TaGATA1) wheat *CI12633* plants are relative to those in the wild-type (WT) *Yangmai* 16 or BSMV:GFP-infected *CI12633* plants, respectively. Statistically significant differences were analysed based on three replications (*t*-test; *P*<0.05, **P**<0.01). Error bars indicate the SE.](image-url)
These data showed that TaGATA1 could directly bind in planta to these GATA core cis-element-containing sites present in PR10, PR17C, and Chitinase3 promoters and directly activate expression of these target genes in wheat.

TaGATA1 and its activated defence genes are regulated by cytokinin and jasmonate

Arabidopsis B-GATA genes and rice CGA1 are responsive to the phytohormone cytokinin (Hudson et al., 2013; Ranftl et al., 2016). Jasmonate (JA) is a primary phytohormone in regulation of plant defence responses to necrotrophic pathogens (Thomma et al., 1998; Pieterse et al., 2009). The TaGATA1 promoter contains both cytokinin- and JA-responsive cis-elements (Supplementary Table S2). To investigate how TaGATA1 responds to application of external cytokinin and JA, we analysed transcriptional profiles of TaGATA1 in wheat cultivar Yangmai 16 leaves after external cytokinin [6-benzylaminopurine (6-BA)] and methyl jasmonate (MeJA, JA analogue) as well as mock treatments for 0.5, 1, 3, 6, and 12 h. After 6-BA treatment, TaGATA1 transcription was significantly elevated, ~2.86-fold at 1 h and ~2.47-fold at 6 h, compared with the mock treatment (Fig. 8A). In response to MeJA stimulus, TaGATA1 transcription was elevated at 1 h, reached a peak at 3 h, and was maintained at a high level at 6 h relative to the mock treatment (Fig. 8B). Furthermore, all the promoter sequences of PR10, PR17C, and Chitinase3 contained both cytokinin- and JA-responsive cis-elements (Supplementary Table S4). Thus, we investigated transcriptional profiles of PR10, PR17C, and Chitinase3 in wheat cv. Yangmai 16 leaves treated with 6-BA (for 1 h and 6 h), MeJA (for 3 h and 6 h), or mock treated. As shown in Fig. 8C, PR10 transcription significantly increased after exogenous 6-BA treatment, whereas PR17C and Chitinase3 were down-regulated by 6-BA treatment. Following MeJA treatment, transcription levels of PR10, PR17C, and Chitinase3 were significantly increased compared with mock treatment (Fig. 8D). These results suggested that TaGATA1 and its activated defence genes (PR10, PR17C, and Chitinase3) were responsive to cytokinin and/or JA stimuli.

Discussion

In Arabidopsis, rice, and barley, some B-GATA TFs play regulatory roles in many aspects of growth and development (Houston et al., 2012; Behringer and Schwechheimer, 2015;
TaGATA1 regulates immunity in wheat

However, GATA TFs in wheat have not been reported. In this study, through comparative transcriptome analyses, the wheat LLM-domain B-GATA gene TaGATA1 was identified in the host immune response to \textit{R. cerealis}. TaGATA1 transcription was higher in resistant wheat cvs Shanghongmai and CI12633 than in susceptible wheat cv. Wenmai 6, and significantly elevated after \textit{R. cerealis} infection. TaGATA1 is expressed at the highest level in stems where sharp eyespot disease primarily occurs. Accordingly, the TaGATA1 promoter contains biotic stress-responsive cis-elements.

Previous studies reported that plant TF genes were induced to a higher extent in resistant lines after infection of pathogens and were demonstrated to regulate resistance responses (McGrath \textit{et al.}, 2005; Zhang \textit{et al.}, 2007, 2012). Here, TaGATA1-silenced wheat plants and TaGATA1-overexpressing transgenic wheat lines were generated and their resistance responses after pathogen inoculation were assessed. The genetic functional assays revealed that overexpression of TaGATA1 significantly increased resistance of the transgenic wheat to \textit{R. cerealis} and silencing of TaGATA1 significantly impaired host resistance to the pathogen. These results show that TaGATA1 acts as a positive regulator and is required for the wheat immune responses to \textit{R. cerealis} infection. This is the first report uncovering a defence role for plant GATAs in response to pathogens. This study broadens our understanding of the biological function of GATA in plant species. Additionally, reports of plant responses to necrotrophic pathogens have been limited. This study extends the current knowledge of plant immune responses against necrotrophic pathogens.

In this report, the phylogenetic analysis showed that TaGATA1 belongs to the short LLM-domain-containing B-GATA class. In this class, TaGATA1 shares quite low identity with function-known GATA factors, such as OsGATA12, AtGATA15, AtGATA16, AtGATA17, and AtGATA17LIKE. OsGATA12, AtGATA15, AtGATA16, AtGATA17, and AtGATA17LIKE have been shown to regulate greening, tillering, senescence, flowering time, hypocotyl elongation, and stomata formation in hypocotyls (Klermund \textit{et al.}, 2016; Ranftl \textit{et al.}, 2016; Lu \textit{et al.}, 2017). Herein, the defensive role of TaGATA1 provides a novel function for plant B-GATAs, which may be due to TaGATA1-specific sequence during wheat evolution. It supports reports of the neofunctionalization of monocot-specific B-GATAs during plant evolution to expand their functional repertoire (Reyes \textit{et al.}, 2004; Behringer \textit{et al.}, 2014; Behringer and Schwechheimer, 2015).

GATA TFs all should include a conserved GATA-motif-binding domain and at least one NLS domain (Lu \textit{et al.}, 2017). Some GATA members showed transcriptional activation activity due to possessing a transcription activation domain (Shikata \textit{et al.}, 2003; Sugimoto \textit{et al.}, 2003; Shaikhali \textit{et al.}, 2012). Here, the protein sequence analysis indicated that TaGATA1 contained a conserved GATA-motif-binding domain, two conserved NLS motifs, and a transcription activation domain. Thus, TaGATA1 was speculated to be an activator-type LLM-domain B-GATA TF. Our subcellular localization results confirmed that TaGATA1 is a protein expressed in the nucleus. EMSA, transcription activation, and ChIP–qPCR results indicated that TaGATA1 is a transcription activator and

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**Fig. 8.** Transcriptional analyses of TaGATA1 and its activated defence genes in wheat after exogenous cytokinin and jasmonate treatments. (A and B) Transcriptional profiles of TaGATA1 in leaves of wheat cv. Yangmai 16 after exogenous applications of 10 μM cytokinin 6-BA (A) or 0.1 mM MeJA (B). (C and D) Expression of defence genes including PR10, PR17c, and Chitinase3 in leaves of wheat Yangmai 16 after exogenous applications of 10 μM 6-BA (C) or 0.1 mM MeJA (D). The transcription level of the tested gene in mock-treated wheat plants is set to 1. Statistically significant differences (*P<0.05, **P<0.01) are analysed based on three replications using Student’s t-test. Error bars indicate the SE. (This figure is available in colour at JXB online.)
can bind to GATA core cis-elements. These molecular biology results prove that TaGATA1 is a GATA-binding transcription activator localized in the nucleus, which is consistent with TaGATA1 sequence analysis and LLM-domain B-GATA protein characteristics.

Previous studies showed that several defence genes, such as chitinases, PR10, and PR17C, contributed to resistance of transgenic wheat to fungal pathogens (Anand et al., 2003; Chen et al., 2008; Li et al., 2011; Zhang et al., 2012; Zhu et al., 2014). Here, the results showed that after R. cerealis inoculation, transcriptional levels of PR10, PR17C, and Chitinase3 were significantly elevated in resistant TaGATA1-overexpressing wheat plants compared with WT Yangmai 16, and were the lowest in susceptible TaGATA1-silenced wheat plants. These data reveal that TaGATA1 activates the expression of PR10, PR17C, and Chitinase3. Moreover, ChIP–qPCR results confirm that TaGATA1 directly binds in wheat to GATA core cis-elements present in the promoters of the defence genes targeted by TaGATA1 and activates expression of these genes. Taken together, these results verify that TaGATA1, acting as an LLM B-GATA transcription activator, can activate expression of defence genes followed by interaction with GATA core cis-elements in the promoters of these target genes in wheat, leading to enhanced resistance against R. cerealis infection.

In Arabidopsis thaliana, GNC and CGA1/GNL control different aspects of cytokinin-regulated development (Ranftl et al., 2016), and modulate crosstalk between auxin and gibberellin signalling (Richter et al., 2010, 2013b). JA is primarily associated with necrotrophic pathogen resistance responses and regulates the expression of certain defence genes (Thomma et al., 1998; McGrath et al., 2005; Pieterse et al., 2009). However, little is known about the effect of JA on GATAs. This study indicated that both external cytokinin and JA stimuli up-regulated the expression of TaGATA1. TaGATA1-activated defence genes, including PR10, PR17C, and Chitinase3, were up-regulated by exogenous JA application, but responded differently to exogenous cytokinin treatment. Upon exogenous cytokinin stimulus, PR10 was up-regulated but PR17C and Chitinase3 were down-regulated. Thus, we speculate that JA signaling might play a major role in the TaGATA1-mediated immune response to R. cerealis. In order to clarify the issue further, it will be interesting to investigate expression of the above genes and the pathogen biomass in the TaGATA1-overexpressing and silenced wheat plants as well their controls treated with exogenous JA in the future.

Conclusions

TaGATA1 was identified through transcriptomic analysis and was verified as a positive regulator in the wheat immune response to R. cerealis infection. TaGATA1 was demonstrated to be a LLM-domain B-GATA transcription activator. It directly bound to the GATA core cis-element-containing sequences in promoters of certain defence genes and activated their expression. TaGATA1 and its activated defence genes were up-regulated by JA stimulus. Thus, TaGATA1 positively regulates the immune response to R. cerealis through activating the expression of certain defence genes. This is the first investigation to reveal a defence role for plant LLM-domain B-GATA TFs. TaGATA1 is a promising gene that can be used to improve resistance of wheat, and other cereal and economic crops against R. cerealis.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Schemata of genomic RNAs of BSMV construct and the construct of the recombinant virus expressing the wheat gene TaGATA1, BSMV:TaGATA1.

Fig. S2. TaGATA1-overexpressing transformation vector pUbimyc-TaGATA1.

Fig. S3. Phylogenetic analysis of the deduced amino acid sequence of TaGATA1 with other plant GATA factors.

Fig. S4. Multiple nucleotide sequences alignment of TaGATA1-3A, TaGATA1-3B, and TaGATA1-3D.

Fig. S5. Multiple amino acid sequences alignment of TaGATA1-3A, TaGATA1-3B, and TaGATA1-3D.

Fig. S6. PCR pattern of the transgene in TaGATA1-overexpressing wheat lines.

Table S1. Primers and their sequences in this study.

Table S2. cis-elements in the promoter of TaGATA1.

Table S3. Responses of TaGATA1-overexpressing transgenic and wild-type wheat (Triticum aestivum) lines after Rhizoctonia cerealis infection.

Table S4. cis-elements in promoters of TaGATA1-activated defence genes.

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