Research Paper

Transgenic wheat expressing Thinopyrum intermedium MYB transcription factor TiMYB2R-1 shows enhanced resistance to the take-all disease

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Abstract

The disease take-all, caused by the fungus Gaeumannomyces graminis, is one of the most destructive root diseases of wheat worldwide. Breeding resistant cultivars is an effective way to protect wheat from take-all. However, little progress has been made in improving the disease resistance level in commercial wheat cultivars. MYB transcription factors play important roles in plant responses to environmental stresses. In this study, an R2R3-MYB gene in Thinopyrum intermedium, TiMYB2R-1, was cloned and characterized. The gene sequence includes two exons and an intron. The expression of TiMYB2R-1 was significantly induced following G. graminis infection. An in vitro DNA binding assay proved that TiMYB2R-1 protein could bind to the MYB-binding site cis-element ACI. Subcellular localization assays revealed that TiMYB2R-1 was localized in the nucleus. TiMYB2R-1 transgenic wheat plants were generated, characterized molecularly, and evaluated for take-all resistance. PCR and Southern blot analyses confirmed that TiMYB2R-1 was integrated into the genomes of three independent transgenic wheat lines by distinct patterns and the transgene was heritable. Reverse transcription–PCR and western blot analyses revealed that TiMYB2R-1 was highly expressed in the transgenic wheat lines. Based on disease response assessments for three successive generations, the significantly enhanced resistance to take-all was observed in the three TiMYB2R-1-overexpressing transgenic wheat lines. Furthermore, the transcript levels of at least six wheat defence-related genes were significantly elevated in the TiMYB2R-1 transgenic wheat lines. These results suggest that engineering and overexpression of TiMYB2R-1 may be used for improving take-all resistance of wheat and other cereal crops.

Key words: Gaeumannomyces graminis var. tritici, MYB transcription factor, take-all resistance, Thinopyrum intermedium, transformation, Triticum aestivum.

Introduction

Wheat (Triticum aestivum) is one of the most important food crops in the world. Root diseases have considerable economic impacts on wheat production. The disease known as take-all, caused by the necrotrophic fungus Gaeumannomyces graminis var. tritici (Ggt), is one of the most destructive root diseases of wheat worldwide (Gutteridge et al., 2003; Daval et al., 2011). The disease begins by Ggt hyphae penetrating the cortical cells of the root and progresses upwards into the base of the stem, and even leads to premature death of the infected plant. The symptoms of the disease are manifested as black lesions on
the roots. Symptoms on above-ground parts of the infected plant include stunting, premature ripening, and white heads (bleached white and empty spikes) (Cook, 2003; Guilleroux and Osbourn, 2004). Take-all can affect the quality and yield of wheat [i.e. yield losses of 40–60% (Gutteridge et al., 2003)]. Take-all also impacts the production of barley (Hordeum vulgare) and triticale (Secale × Triticum) (Gutteridge et al., 2003; Bithell et al., 2011).

Breeding wheat varieties with resistance is the most promising and reliable way to protect wheat from take-all. Since no effective resistance has been identified in wheat accessions (Gutteridge et al., 2003; Yang et al., 2011), to breed take-all-resistant wheat varieties using traditional methods does not appear to be feasible. The advent of genetic engineering and its application to the production of crops make it possible to generate wheat materials with resistance to this disease.

Plants have evolved sophisticated defence mechanisms to cope with pathogens. Many transcription factor (TF) families have been shown to play important roles in defence responses through regulating the expression of defence-related genes. MYB TFs are characterized by a MYB domain conferring the cis-acting elements of targeted genes (Dubos et al., 2010). Based on the numbers of adjacent repeats in the MYB domain, MYB proteins are classified into four subfamilies, namely R1MYB, R2R3-MYB, 3R-MYB, and 4R-MYB (Dubos et al., 2010). Since the first plant MYB gene required for synthesis of anthocyanins, CI, was isolated from maize (Zea mays) (Paz-Ares et al., 1987), a large number of MYB proteins have been identified in various plant species, namely Arabidopsis thaliana, rice (Oryza sativa), maize (Zea mays), cotton (Gossypium hirsutum), grapevine (Vitis vinifera L.), poplar (Populus tremuloides), apple (Malus domestica), wheat, and Avicennia marina (Rabinowicz et al., 1999; Cedroni et al., 2003; Dias et al., 2003; Chen et al., 2006; Dubos et al., 2010; Zhang et al., 2011; Ganesan et al., 2012). MYB proteins perform diverse biological functions in the cell cycle and in development, regulation of primary and secondary metabolism, and abiotic stress response (Ma et al., 2009; Seo et al., 2009; Dubos et al., 2010; Seo and Park, 2010; He et al., 2011; Xue et al., 2011; Zhang et al., 2011; Qin et al., 2012; Romano et al., 2012; Shen et al., 2012). Some plant MYB proteins are involved in defence responses to pathogens. For instance, Arabidopsis R2R3-MYB proteins, including AtMYB108 and AtMYB06, participate in disease resistance (Mengiste et al., 2003; Seo and Park, 2010). Our previous study showed that overexpression of a wheat MYB gene TaPIMPI enhanced resistance to biotic and abiotic stresses in transgenic tobacco and wheat (Liu et al., 2011; Zhang et al., 2012).

Thinopyrum intermedium (Agropyron intermedium, intermediate wheatgrass; 2n=42), a wild relative of wheat, is naturally resistant to wheat diseases, such as leaf rust, yellow rust, and stem rust (Cauderon et al., 1973), Wheat streak mosaic virus (WSMV; Sharma et al., 1984), Barley yellow dwarf virus (BYDV; Sharma et al., 1984), Fusarium head blight (FHB; Fedak and Han, 2005), and eyespot (Li et al., 2005). The resistance to wheat rusts, BYDV, WSMV, FHB, and eye-spot was introgressed into the wheat background and characterized by different groups (Cauderon et al., 1973; Xin et al., 1991; Banks et al., 1995; Sharma et al., 1995; Fedak and Han, 2005; Li et al., 2005). However, it is not clear whether R2R3 MYB TFs in T. intermedium are involved in defence responses. Therefore, a study of the species-specific MYB genes may provide insights into T. intermedium defence mechanisms.

In this study, the first R2R3-MYB gene isolated from T. intermedium, TiMYB2R-1, was cloned. Its R2R3-MYB activity was confirmed by subcellular localization and cis-element binding activity assays. The functional characteristics of TiMYB2R-1 in defense responses to take-all pathogen Ggt were also explored through its expression in generated transgenic wheat lines. The results showed that the ectopic expression of TiMYB2R-1 significantly increased resistance to take-all in transgenic wheat.

Materials and methods

Plant and fungal materials and treatments

Thinopyrum intermedium cultivar (cv.) Z1146 was provided by Dr Liuhui Li, Institute of Crop Science, CAAS. The wheat cv. Yangmai 12, provided by Lixiahe Agricultural Institute of Jiangsu, China, was used as the recipient of TiMYB2R-1 transformation. Yangmai 12 is a Chinese commercial wheat variety with susceptibility to Ggt and is a good material for this study.

The fungal pathogen Ggt XNQS-2 was isolated, identified, and provided by Dr Yang Wang, College of Plant Protection, Northwest A&F University, China.

For inoculation, the Ggt fungus was cultured on potato dextrose agar (PDA) at 25 °C for ~10 d, then 1 cm² plugs from the edge of Ggt colonies were placed onto the surface of sand in pots. One seed germinated for 2 d was put on the top of each Ggt plug, and covered with 2 cm of sand. The plants were cultured in a growth chamber at a 23 °C, 14 h light/15 °C, 10 h dark regime at 70% relative humidity. The roots were collected at 0, 4, 7, 14, and 21 days post-inoculation (dpi) for RNA extraction.

DNA and RNA extraction and first-strand cDNA synthesis

Genomic DNA was extracted from leaf tissues of T. intermedium Z1146 or wheat as described by Sharp et al. (1988). Total RNA was extracted from roots of T. intermedium or wheat using TRIZOL reagent (Invitrogen), and then subjected to RNase-free DNase I (TaKaRa) treatment and purification.

A 5 µg aliquot of RNA per sample was used to synthesize the first-strand cDNA using a Superscript II First-Strand Synthesis Kit for RT-PCR (Invitrogen).

Cloning and sequence analysis of the TiMYB2R-1 gene

Based on the sequence of the wheat MYB gene TaPIMPI (accession no. EF587267), a pair of primers (MYB-OF, 5′-ACTCGC GTACGTCTTCCCTGA-3′; and MYB-OR, 5′-GCAGCTCTAGTTA AGTTCATCGTC-3′) was designed and used to amplify the full-length cDNA sequence of the MYB gene TiMYB2R-1 from cDNA of T. intermedium Z1146 roots at 4 d post-challenge with Ggt. The PCR fragment corresponding to TiMYB2R-1 was excised, cloned, and its sequence was analysed. The cDNA sequence of TiMYB2R-1 of 1038 bp in length was deposited in the National Center for Biotechnology Information (NCBI) with accession number JX683795. TiMYB2R-1 contains an open reading frame (ORF) of 972 bp (NCBI accession no. JQ663861). The genomic sequence of TiMYB2R-1 was amplified from genomic DNA of Z1146 using the primers MYB-OF and MYB-OR, then cloned and sequenced.
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The genomic sequence was deposited in the NCBI with accession no. JX683794. DNA and protein sequences were analysed using DNAMAN software, DNASTAR software, and BLAST online (http://www.ncbi.nlm.gov/blast).

Subcellular localization of TiMYB2R-1

The coding region of TiMYB2R-1 without the stop codon was amplified using gene-specific primers with HindIII and XhoI restriction sites (TIM-NLFI, 5’-GaaGACATGACGACATAGGACAAGGAGTA-3’; and TIM-NLRI, 5’-CCTAGATCGCAGAATGTCTCCTAG-3’). TiMYB2R-1 was fused in-frame to the 3’ terminus of the green fluorescent protein (GFP) gene in the phi6318 vector (Dr Daowen Wang, CAS), and controlled by the Cauliflower mosaic virus (CaMV) 35S promoter. The resulting TiMYB2R-1-GFP fusion construct or GFP alone were transformed separately into white onion epidermal cells using a PDS-1000/He gene gun (Bio-Rad, USA) at 1100 psi. After incubation for 40 h at 25 °C, GFP fluorescence in the transformed onion cells was observed under 488 nm excitation using a confocal laser scanning microscope (Zeiss LSM 700, Germany) with a Fluor 10×/0.52 M27 objective lens and a SP640 filter.

The cis-element binding assay of TiMYB2R-1

Previously R2R3-MYB proteins were shown to bind to MYB-binding site (MBS) AC cis-elements including ACI (core sequence: cis-AC). The cis-element binding assay of TiMYB2R-1 laser scanning microscope (Zeiss LSM 700, Germany) with a Fluar 10×/0.52 M27 objective lens and a SP640 filter.

Southern blot analysis

Southern blotting was conducted following a modified protocol of Sharp et al. (1988). Genomic DNAs (~20 µg each) of the transgenic wheat plants and non-transformed Yangmai 12 were digested separately by the restriction enzyme EcoRV, and then blotted onto a Hybond N+ nylon membrane (GE Amersham). The amplified fragment (533 bp, Fig. 4A) specific to the chimeric Ubi::TiMYB2R-1 was labelled by [α-32P]dCTP and then used as the probe. Hybridization was performed at 65 °C for 20 h. The hybridized membrane was washed twice in 1× SSC, 0.1% SDS, twice in 0.5× SSC, 0.1% SDS, and once in 0.2× SSC, 0.1% SDS at 65 °C for 10 min each time.

Transcription analyses of TiMYB2R-1 and wheat defence-related genes in transgenic wheat

In a previous microarray analysis, wheat TaPIMP1 overexpression activated transcription of defence-related genes (Zhang et al., 2012), including PR1a (TC384382), PR1c (TC398605), Chitinase 2 (Chit2; TC384382), class III chitinase (Chit3; TC386649), nsLTP1 (TC415106) encoding a non-specific lipid transfer protein1, and GST22 (TC372250). These TC numbers and sequences can be found at the website: http://compbio.dfci.harvard.edu/cgi-bin/tgi/tc_ann.pl?oud=weat.

Reverse transcription–PCR (RT–PCR) and real-time quantitative RT–PCR (Q-RT–PCR) were used to analyse transcription levels of TiMYB2R-1 and the above-mentioned wheat defence-related genes in TiMYB2R-1 transgenic and control wheat lines. The RT–PCRs for TiMYB2R-1 transcription were set up with 31 cycles (95 °C for 1 min, 56 °C for 40 s, 72 °C for 35 s) to illustrate high expression of TiMYB2R-1 in the transgenics with primers (MRT-F and MRT-R) specific to the TiMYB2R-1 sequence (Table 1); the third from last nucleotide of the reverse primer MRT-R is different from that of TaPIMP1 (Supplementary Fig. S1 available at JXB online). Q-RT–PCR was performed using SYBR Green I Master Mix (TaKaRa, Japan) in a volume of 25 µl on an ABI 7300 RT–PCR system (Applied Biosystems). Reactions were set up with the following thermal profile: 95 °C for 5 min, followed by 41 cycles of 95 °C for 15 s and 60°C for 35 s. The wheat actin gene was used to normalize amounts of cDNAs among the samples. The relative transcript level of a target gene was calculated using the 2–ΔΔCT method (Livak and Schmittgen, 2001). The relative transcript levels of the tested genes in the transgenics were relative to those in the wild-type (WT) recipient since the genetic backgrounds of the transgenics and the WT recipient are the same except for the introduced TiMYB2R-1 and the reactions (including defence genes) caused by TiMYB2R-1 overexpression. In Q-RT–PCR analysis, transcription of TaPIMP1, a wheat endogenous homologue of TiMYB2R-1, may be detected in TiMYB2R-1 transgenics and WT recipient wheat. The relative transcript levels of TiMYB2R-1 were the transcript levels of TiMYB2R-1 and TaPIMP1 in transgenics minus those of TaPIMP1 in WT plants. Three replications for each treatment were performed.

The primer sequences for TiMYB2R-1, and wheat defence-related and actin genes, and related information on (Q)-RT–PCR analyses are listed in Table 1.

Western blotting assay

The expression of the TiMYB2R-1-encoded protein in the transgenic wheat lines was tested by western blotting analysis. Total proteins were extracted from 0.3 g of ground root powder. About 12 µg of total soluble proteins for each line were separated on 12% SDS–polyacrylamide gels and transferred to a polyvinyl difluoride
membrane (Amersham). The western blots were incubated with the polyclonal GST–TiMYB2R-1 antibody (1:100 dilution), which was developed in mice from GST–TiMYB2R-1 protein. TiMYB2R-1 protein in these lines was visualized with the ECL Western Blot Detection and Analysis System (GE Healthcare).

Take-all assessments in transgenic wheat

Take-all responses of TiMYB2R-1 transgenics in the T3–T5 generations and untransformed wheat controls were evaluated following inoculation with Ggt. At 3 weeks post-inoculation, the disease severity in each plant was assessed as the percentage area of take-all lesions covering the root system (Daval et al., 2011). The infection types (ITs) were categorized from 0 to 4 according to Bithell et al. (2011) (i.e. IT 0, no take-all; IT 1, >0% and ≤10%; IT 2, >10% and ≤30%; IT 3, >30% and ≤60%; IT 4, >60%). The take-all index (TAI) was \( \frac{10 \times N_1 + 30 \times N_2 + 60 \times N_3 + 100 \times N_4}{N_1 + N_2 + N_3 + N_4} \), where \( N \) was the number of plants with each infection type (Bithell et al., 2011). At least 70 plants per line were tested.

To investigate the take-all resistance of transgenic wheat lines further, Q-RT–PCR was used to assess the relative abundance of Ggt in transgenic wheat plants based on Ggt 18S rRNA (FJ771002; Gaeumannomyces graminis var. tritici) in reference to wheat 18S rRNA (AY049040). The primer sequences for Ggt 18S rRNA and wheat 18S rRNA in Q-RT–PCR analyses are listed in Table 1.

Results

TiMYB2R-1 is an R2R3-MYB transcription factor in T. intermedium

Both the full-length cDNA and genomic sequences of the TiMYB2R-1 gene were isolated from T. intermedium. The gene sequence includes two exons and an intron of 240 bp (Fig. 1A). The ORF of TiMYB2R-1 encodes a deduced protein, TiMYB2R-1, with 323 amino acids and a mol. wt of 35.4 kDa. The TiMYB2R-1 protein possesses two conserved SANT domains [one (R2) located at amino acids 46–94 and the other (R3) at amino acids 100–145], two acidic transcriptional activation domains (at amino acids 15–60 and 288–307), and two basic nuclear localization signal (NLS) regions (at amino acids 80–89 and 142–150) (Supplementary Fig. S2 at JXB online).

The overall sequence of the TiMYB2R-1 protein displayed various identities with known R2R3-MYB proteins from diverse species, namely wheat MYB proteins, including TaPIMP1 (ABU93236.1, 95.99%), TaMYB73 (AEW23186.1, 30.03%), TaMYB32 (AEV91155.1, 28.4%), TaMYB13-1 (AER38255.1, 27.24%), and TaMYB2 (AAT37168.1, 26.99%), and Arabidopsis MYB TFs, including AtMYB108 (AEE74402.1, 41.26%), AtMYB96 (AED97611.1, 22.6%). Phylogenetic analysis indicated that TiMYB2R-1 clustered with R2R3-MYB proteins (Fig. 1B), among which AtMYB108, AtMYB96, and TaPIMP1 are implicated in responses to biotic and abiotic stresses in Arabidopsis and wheat (Mengiste et al., 2003; Seo and Park, 2010; Zhang et al., 2012). The above results suggest that TiMYB2R-1 is probably an R2R3-MYB protein.

TiMYB2R-1 transcript level increases in T. intermedium after Ggt infection

Following Ggt inoculation, the transcription of TiMYB2R-1 in T. intermedium was significantly induced. The transcription level at 7 dpi reached a peak (8-fold above that of non-treated plants, 0 h), declined afterwards, but remained higher than the non-treatment control (Fig. 2). The results suggested that TiMYB2R-1 is potentially involved in the host defence response to Ggt.

Table 1. Primers used in (Q-)RT–PCR.

| Gene name       | Accession no. | MBS position and sequence | Sequence of gene-specific primer       | Species                      |
|-----------------|---------------|---------------------------|----------------------------------------|------------------------------|
| TiMYB2R-1       | JX683795      | –43 to –39, GGATA         | F: 5'-ACGGACAACGAGGTCAAGAAAC-3'        | Thinopyrum intermedium       |
| PR1a            | TC384382      | –58 to –54 GGATA          | F: 5'-AGACATCAGCGACAAGGAG-3'           | Triticum aestivum            |
| PR17c           | TA65181       | –86 to –81(-), CGGTTG     | F: 5'-AGGATGGGCAAGGACAGGAG-3'          | Triticum aestivum            |
| nsLTP1          | TC411506      | –211 to –206(-), CGGTTG   | F: 5'-AGGATGGGCAAGGACAGGAG-3'          | Triticum aestivum            |
| GST22           | TC372250      | No promoter sequence      | F: 5'-TTCTGGATACGCGCAAGAAG-3'          | Triticum aestivum            |
| Chit2           | TC426538      | No promoter sequence      | F: 5'-TTCTGGATACGCGCAAGAAG-3'          | Triticum aestivum            |
| Chit3           | TC386649      | No promoter sequence      | F: 5'-TTCTGGATACGCGCAAGAAG-3'          | Triticum aestivum            |
| Actin           | BE425627      | –58 to –54 GGATA          | F: 5'-AGGATGGGCAAGGACAGGAG-3'          | Triticum aestivum            |
| 18S rRNA        | AY049040      | –86 to –81(-), CGGTTG     | F: 5'-AGGATGGGCAAGGACAGGAG-3'          | Triticum aestivum            |
| Ggt 18S rRNA    | FJ771002      | No promoter sequence      | F: 5'-AGGATGGGCAAGGACAGGAG-3'          | Gaeumannomyces graminis var. tritici |
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To study the subcellular localization of TiMYB2R-1, a chimeric p35S::TiMYB2R-1-GFP expression vector or control vector p35S::GFP was introduced separately into onion epidermal cells. Confocal imaging of transient expression in the epidermal cells showed that TiMYB2R-1–GFP accumulated only in the nucleus, whereas GFP alone was present throughout the whole cell, indicating that TiMYB2R-1 is a nuclear-localized protein (Fig. 3A). These results were consistent with those of TFs that typically function in the nuclei.

To test whether this TiMYB2R-1 protein bind to MBS ACI cis-elements, purified GST–TiMYB2R-1 recombinant protein was used in EMSAs. The results proved that the GST–TiMYB2R-1 could bind to the ACI cis-element, whereas GST protein alone did not (Fig. 3B). Thus TiMYB2R-1 is indeed an R2R3-MYB TF.

Generation and molecular characterization of TiMYB2R-1 transgenic wheat

To evaluate the role of TiMYB2R-1 in wheat, transgenic wheat lines expressing TiMYB2R-1 were generated via bombarding the TiMYB2R-1 expression vector pA25-TiMYB2R-1 (Fig. 4A) into 1200 immature Yangmai 12 embryos. Four independent transgenic lines containing the Ubi::TiMYB2R-1 transgene were obtained with a transformation efficiency of 0.33%. Using the primers specific for the chimeric Ubi::TiMYB2R-1 (Fig.4A), genomic PCR assays of the T0–T5 generation plants showed that the specific band of the introduced TiMYB2R-1 was detected in progeny derived from three transgenic wheat lines O1, O3, and O5 with enhanced resistance to take-all, but not in non-transformed (WT) Yangmai 12 (recipient) and null-segregants lacking the transgene (Fig. 4B). Southern blot analysis of T4 transgenic wheat lines indicated that the three transgenic lines O1, O3, and O5 had one, one, and two copies, respectively, with different hybridization patterns (Fig. 4C), suggesting that the three lines resulted from independent transformation events. Additionally, a common band was present in these transgenic lines and WT Yangmai 12, suggesting that the transgenic lines and Yangmai 12 contain a sequence (TaPIMP1) homologous to the probe derived from the Ubi::TiMYB2R-1 chimera (Fig.4A, C). Collectively these results proved that the transgene was integrated into the genomes of the three transgenic wheat lines and was stably transmitted to subsequent generations.

The expression of TiMYB2R-1 was examined in roots of three transgenic lines (O1, O3, and O5) with enhanced resistance at 21 d after Ggt inoculation. The RT–PCRs for TiMYB2R-1 transcription were set up with 31 amplified cycles to illustrate the higher expression of TiMYB2R-1 in the three transgenic lines, while no corresponding products were observed in WT Yangmai 12 and null-transgenic lines (Fig. 4Di). In Q-RT–PCR analysis, as TaPIMP1 transcription should be amplified at the same level in the transgenic and WT recipient plants, the relative transcript levels of
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Expression of TiMYB2R-1 were the transcript levels of TiMYB2R-1 and TaPIMP1 in transgenics relative to that of TaPIMP1 in the WT (Fig. 4Dii). As shown in Fig. 4Dii, TiMYB2R-1 in the transgenic lines was transcribed at a significantly higher level. Western blot results indicated that these lines (O1, O3, and O5) expressed high levels of TiMYB2R-1 protein, although the WT and transgenic lines contained a tiny amount of TaPIMP1 (Fig. 4E). Thus, the introduced TiMYB2R-1 gene can be highly expressed in the three transgenic lines with enhanced resistance.

Expression of TiMYB2R-1 improves resistance to take-all in transgenic wheat

Take-all reactions of transgenic wheat lines overexpressing TiMYB2R-1 along with the null-segregants and WT Yangmai 12 were evaluated 21 d after Ggt inoculation. The results showed that most plants of TiMYB2R-1-overexpressing transgenic wheat lines O1, O3, and O5 displayed significantly increased resistance relative to the WT Yangmai 12 and null-segregant plants (Table 2, Fig. 5A). The average disease severities of the three transgenic wheat lines were 9.32–25.84%, whereas those of the null-segregants and WT Yangmai 12 were 43.13–49.25% and 44.74–50.14%, respectively. The average TAI of these transgenic wheat lines were 18.75–35.47, whereas those of the null-segregant and WT Yangmai 12 were 63.33–66.75 and 61.67–68.89, respectively (Table 2). Moreover, using Ggt 18S rRNA levels as an indicator of the fungal biomass and infection, the relative Ggt abundance was significantly lower in the three TiMYB2R-1-overexpressing transgenic lines than in WT Yangmai 12 and null-segregants (Fig. 5B), further supporting that TiMYB2R-1 transgenic wheat plants were more resistant to Ggt infection. Thus, TiMYB2R-1-overexpressing transgenic wheat exhibited significantly enhanced resistance to take-all.

Expression of TiMYB2R-1 activates defence-related genes in transgenic wheat

To investigate putative molecular mechanisms of TiMYB2R-1 overexpression in enhanced disease resistance, RT–PCR and Q-RT–PCR were used to analyse the transcription levels of six defence-related genes, namely PR1a, PR17c, Chit2, Chit3, nsLTP1, and GST22, in roots of TiMYB2R-1-overexpressing transgenics and of WT Yangmai 12 and null-segregants at 21 dpi with Ggt. As shown in Fig. 6, the transcription levels of all six genes were markedly elevated in TiMYB2R-1-overexpressing lines relative to those in the null-segregant and WT plants. The results suggested that overexpression of TiMYB2R-1 activates transcription of these defence-related genes in the transgenic wheat lines.

Discussion

In plant species, some MYB proteins have been implicated in defence responses (Mengiste et al., 2003; Seo and Park, 2010; Zhang et al., 2012). Although T. intermedium possesses resistance to diverse pathogens, it is not known if and how MYB TFs in T. intermedium regulate defence responses to the pathogens. In this study, the first MYB gene of T. intermedium, namely TiMYB2R-1, was successfully isolated. Sequence analysis showed that the gene sequence of TiMYB2R-1 includes two exons and an intron, and the deduced TiMYB2R-1 protein possesses the structural characteristics of R2R3-MYB TFs. Sequence alignment and phylogenetic analysis indicated that TiMYB2R-1 protein was most closely related to a wheat R2R3 MYB TaPIMP1, followed by Arabidopsis AtMYB108. The results of subcellular localization and MBS cis-element binding analyses proved
that TiMYB2R-1 indeed is an R2R3-MYB TF in T. intermedium, consistent with its sequence traits.

Many TFs are induced under environmental stress conditions. For example, transcriptional levels of wheat TaMYB73 and Arabidopsis AtMYB44 were up-regulated under salinity stress conditions (He et al., 2011). The expression of the wheat MYB gene TaPIMP1 was increased after Bipolaris sorokiniana infection and dehydration treatment (Zhang et al., 2012). They can offer potential genes for improving biotic and abiotic stress in planta. The transcription of TiMYB2R-1 in T. intermedium was significantly induced after inoculation with Ggt or B. sorokiniana (Supplementary Fig. S3 at JXB online), suggesting that TiMYB2R-1 is probably involved in host response to infection by Ggt or B. sorokiniana.

To investigate defence roles of TiMYB2R-1 in planta, here TiMYB2R-1-overexpressing transgenic wheat lines were generated through transformation and characterized in detail. Based on PCR detection for T0–T5 transgenic wheat lines and

Table 2. Take-all responses of TiMYB2R-1 transgenic and control wheat lines.a

| Lines           | T4 generation | T5 generation |
|-----------------|---------------|---------------|
|                 | Disease severity (%) | Take-all index | Disease severity (%) | Take-all index |
| O1              | 20.495**      | 28.5**        | 23.99**          | 35.47**        |
| O3              | 22.26**       | 32.33**       | 25.84**          | 34.75**        |
| O5              | 9.32**        | 18.75**       | 19.24**          | 28.12**        |
| Null            | 43.13         | 63.33         | 49.25            | 66.75          |
| Yangmai12 (recipient) | 44.74         | 61.67         | 50.14            | 68.89          |

Significant difference between TiMYB2R-1 transgenic lines and untransformed Yangmai 12 (recipient) at **P <0.01. Null indicates the segregants lacking TiMYB2R-1.

a The values derived from the average of 70 plants of each line tested in T4 and T5 transgenics and control wheat lines.
Southern blot analyses using a transgene-specific amplicon as a probe, the results showed that the \textit{TiMYB2R-1} transgene was integrated into the genomes of three transgenic wheat lines and could be transmitted to subsequent generations. RT–PCR and western blot assays indicated that \textit{TiMYB2R-1} was highly expressed in the three transgenics. Following inoculation with the take-all pathogen \textit{Ggt}, the disease severity, TAI, and \textit{Ggt} relative biomass assays showed that the transgenic wheat lines overexpressing \textit{TiMYB2R-1} had more significantly enhanced resistance to take-all than WT Yangmai 12 and null-segregants, although \textit{TaPIMP1} exists in the WT and \textit{TiMYB2R-1} transgenic wheat lines and is induced following \textit{Ggt} infection. The enhanced degrees of resistance in the transgenic lines were correlated with \textit{TiMYB2R-1} expression levels. These suggested that overexpression of \textit{TiMYB2R-1} conferred increased resistance in transgenic wheat, whereas the expression of \textit{TaPIMP1} in WT Yangmai 12 is not enough to confer resistance after \textit{Ggt} challenge. Take-all responses of \textit{TaPIMP1}-overexpressing wheat will be tested in the future. Furthermore, overexpression of \textit{TiMYB2R-1} does not affect the development and growth of the transgenic wheat under normal growth conditions. Thus, \textit{TiMYB2R-1} can be used as an important engineering gene for improving take-all resistance of wheat. Additionally, the transgenic wheat lines expressing \textit{TiMYB2R-1} showed enhanced resistance to
common root rot caused by *B. sorokiniana* (Supplementary Table S1 at JXB online), similar to TaPIMP1-overexpressing wheat (Zhang et al., 2012). Thus, the transgenic wheat produced in this study will provide potential wheat germplasm for enhancing resistance to take-all and common root rot. This is believed to be the first report on development of take-all-resistant wheat lines.

In plants, certain activator-type TFs have been implicated in triggering disease resistance and abiotic stress tolerance through activation of defence- and stress-related genes (Ma et al., 2009; Zhang et al., 2012). Defence-related genes play vital roles in defence against pathogens in plants (Guilleroux and Osbourn, 2004; Seo and Park, 2010; Zhang et al., 2012). For example, transgenic wheat plants overexpressing a wheat *LTP* gene or a barley *chitinase* gene displayed increased resistance to fungal pathogens (Shin et al., 2008; Zhu et al., 2012). Although no natural highly resistant wheat cultivar has been identified, the transcription levels of some wheat defence-responsive genes were up-regulated during compatible interactions between wheat roots and *Ggt* using suppression subtractive hybridization and expressed sequence tag (EST) analysis (Guilleroux and Osbourn, 2004). Among them, two ESTs corresponded to *Chit3* and *GST2* genes (Guilleroux and Osbourn, 2004). TFs interact with specific DNA sequences (cis-acting elements) in target genes to modulate the transcription process. In a previous study, the microarray data showed that wheat TaPIMP1 overexpression activates the transcription of some defence-related genes in wheat, namely *PR1a*, *PR17c*, *Chit2*, *Chit3*, *nsLTP1*, and *GST22* (Zhang et al., 2012).

In this study, RT–PCR and Q-RT–PCR were used to investigate if TiMYB2R-1 overexpression activates the same defence-related genes in the transgenic wheat, using the null-segregant and WT wheat lines as controls. The results indicated that overexpression of TiMYB2R-1 indeed elevated the expression of such defence-related genes in the transgenic wheat. The promoter sequences of these defence-related genes obtained from the Wheat Draft Genome Assembly database (http://www.cerealsdb.uk.net/CerealsDB/), and prediction of MBS cis-elements in the promoters (www.dna.affrc.go.jp/PLACE/) revealed that MBS cis-acting elements exist in the promoters of wheat defence genes *PR1a*, *PR17c*, *nsLTP1*, and *GST22* (Table 1). TiMYB2R-1 possibly interacts with these promoters and thereby contributes to the increased expression of these genes. The promoters of wheat *Chit2* and *Chit3* genes could not be found due to the incomplete coverage of wheat genome sequences. TiMYB2R-1 may elevate the expression of *Chit2* and *Chit3* genes through indirect means, which has yet to be verified experimentally. The results suggested that changes in expression of a subset of wheat defence-related genes regulated by TiMYB2R-1 expression probably result in enhanced resistance to take-all in TiMYB2R-1 transgenic wheat. The results may provide a new insight into the interaction between wheat and *Ggt*.

In summary, TiMYB2R-1, the first MYB gene isolated from *T. intermedium*, was characterized. It encodes an R2R3-MYB TF and has significantly higher expression levels in host plants following *Ggt* infection. TiMYB2R-1 overexpression in transgenic wheat lines showed significantly enhanced resistance to take-all and common root rot possibly through activation of some defence-related genes. The results contribute to further understanding of the characteristics and functions of the MYB TF family in additional plant species.

### Supplementary data

Supplementary data are available at JXB online. Figure S1. Alignment of TiMYB2R-1 and TaPIMP1 sequences amplified in (Q-)RT–PCR.

Figure S2. The cDNA sequence and deduced amino acid sequence of TiMYB2R-1.

Figure S3. Transcription analysis of TiMYB2R-1 in *T. intermedium* following *B. sorokiniana* inoculation.

Table S1. *Bipolaris sorokiniana* responses of TiMYB2R-1 transgenic and control wheat.

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