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ORIGINAL PAPER

Isolation and Identification of Differentially Expressed Genes from Wheat in Response to *Blumeria graminis* f. sp. *tritici* (*Bgt*)

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Abstract Wheat powdery mildew, caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a serious wheat disease both in China and worldwide. Finding and cloning the key resistance genes to *Bgt* are important for wheat breeding. In this study, 23 differentially expressed genes were isolated by GeneFishing from the hybrid wheat alien disomic addition line germplasm SN6306, which is highly resistant to powdery mildew. SN6306 originated from the intergeneric hybridization between wheat Yannong 15 and *Elytrigia intermedium* (Host) Nevski. Among the 23 genes, a gene encoding for auxin-repressed protein (*TaARP1*) was selected for further study, and its full-length complementary DNA (cDNA) and DNA sequences were obtained. The function of *TaARP1* was investigated by real-time quantitative reverse transcription PCR and Barley Stripe Mosaic Virus-mediated gene silencing. Results showed that, after inoculation with *Bgt*, the expression of *TaARP1* was upregulated more than ten times compared with non-inoculated controls. Ten days after inoculation with powdery mildew, BSMV:*TaARP1* leaves clearly showed higher susceptibility to mildew infections than BSMV:00 leaves. Powdery mildew colonies and mycelia development were analyzed by microscopic observation. These data suggested that *TaARP1* may be involved in the response to *Bgt* and may be a potential resistance gene to wheat powdery mildew.

Keywords Wheat · GeneFishing · Differentially expressed genes (DEGs) · BSMV-VIGS · Auxin-repressed protein · Powdery mildew

Abbreviations

ACP	Annealing control primer
ARP	Auxin-repressed protein
BSMV	Barley Stripe Mosaic Virus
CTAB	Cetyltrimethylammonium bromide
DEGs	Differentially expressed genes
PCR	Polymerase chain reaction
VIGS	Virus-induced gene silencing

Introduction

Wheat is the most widely grown food crop in the world. Wheat powdery mildew, caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a serious wheat disease both in China and worldwide. The disease usually results in a 13 to 34 % loss in yield and can be as severe as 50 % when the flag leaves are severely infected during the heading and filling stages (Griffey et al. 1993). Therefore, an in-depth pathological study of disease-resistant varieties of wheat has important implications in food production.

GeneFishing (http://www.seegene.com/), established by Hwang et al. (2003), is a technique used to identify differences in gene expression based on polymerase chain reaction (PCR). The annealing control primer (ACP) is composed of a tripartite structure with a polydeoxyinosine [poly(dI)] linker between the 3′ end target core sequence and the 5′ end non-target universal sequence, which was designed to improve the specificity of PCR amplification (Hwang et al. 2003). The technology has been widely used to screen differently

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expressed genes in plants and animals (Ding et al. 2012; Effendy et al. 2013; Takemura et al. 2013).

Auxin, a common growth-stimulating phytohormone, regulates a wide variety of growth and developmental processes in plants (Lee et al. 2013). An increasing number of experiments reveal that auxin is involved in responses to biotic and abiotic stresses (Llorente et al. 2008; Domingo et al. 2009; Lee et al. 2009; González-Lamothe et al. 2012; Qi et al. 2012; Müller et al. 2014). Auxin-repressed protein (*ARP*) genes, including dormancy-related proteins (Stafstrom et al. 1998) and glycine-rich protein 1 (Shimizu et al. 2006), downregulate auxin. Several *ARP* genes have been isolated from plant species, such as strawberry (Reddy and Poovaiah 1990), apple (Lee et al. 1993), black locust (Park and Han 2003), tobacco (Steiner et al. 2003), cucumber (Shimizu et al. 2006), *Elaeagnus umbellate* Thunb (Kim et al. 2007), tree peony (Huang et al. 2008), peanut (Guimarães et al. 2010), tea plant, Chenopodiaceae, *Brassica rapa* (Lee et al. 2013), and pear (Shi et al. 2013). Abiotic stresses induced expression of these *ARP* genes in hot pepper (Hwang et al. 2005), *Arabidopsis* (Mlynárová et al. 2007), and *B. rapa* (Lee et al. 2013). Biotic stresses induced expression of these *ARP* genes in peanuts (Guimarães et al. 2010; Morgante et al. 2013). However, little is known about the expression of *ARP* genes under biotic stresses in wheat.

This study aimed to identify wheat powdery mildew-resistance genes from the hybrid wheat germplasm SN6306, which is highly resistant to powdery mildew and originated from the intergeneric hybridization between Yannong 15 and *Elytrigia intermedium* (Host) Nevski. In this work, wheat *TaARP1* was isolated and its expression was characterized after infection with wheat powdery mildew. Furthermore, Barley Stripe Mosaic Virus-based virus-induced gene silencing (BSMV-VIGS) was used to downregulate the expression of wheat (*Triticum aestivum* L.) endogenous *TaARP1* gene to elucidate its function in the response to *Bgt*. *TaARP1* was upregulated by powdery mildew, and BSMV-VIGS showed that it is a potential resistance gene. Results facilitate the understanding of the function of *TaARP1* in the response of wheat to biotic stress.

Materials and Methods

Plant Materials and Growth Conditions

Wheat alien disomic addition line germplasm SN6306 ($2n=44$), which originated from the intergeneric hybridization between wheat cultivar Yannong 15 ($2n=42$) and *E. intermedium* (Host) Nevski ($2n=42$) and is highly resistant to powdery mildew, was stored in our laboratory. Seeds were transferred to a mixture of substrate and vermiculite (1:1) after being soaked in water for 24 h at 24 °C. Plants were grown in a phytotron set under long-day conditions (16 h light and 8 h dark) at 24 °C during the day and 18 °C at night with 75 % relative humidity and approximately 12,000 lx (white fluorescent lamps). Chinese *Bgt* isolate E09 was used for the pathogen inoculation test by the method described previously (Zhang et al. 2012).

DNA and RNA Isolation

Genomic DNA was extracted from SN6306 leaves using cetyltrimethylammonium bromide (CTAB) (Saghai-Maroof et al. 1984). Total RNA was isolated from the control and the powdery mildew-infected plants at 12 h using EasyPure™ Plant RNA Kit (TransGen, Beijing, China) and dissolved in RNase-free water according to the protocol of the manufacturer. Concentration and purity (A260/A280) of extracted DNA and RNA were subsequently measured by NanoPhotometer P360 (Implen GmbH, München, Germany). Integrity of the genomic DNA and total RNA was evaluated by electrophoresis on 0.8 % agarose gel at 180 V and stained with ethidium bromide. First-strand complementary cDNA (cDNA) was synthesized from 3 μg total RNA with dT-ACP1 based on the protocol described in the DEG™ GeneFishing Kit (Seegene, Seoul, Korea).

GeneFishing and Cloning

Eighty different ACP primers were used to identify the differentially expressed genes in the final volume of 20 μL containing 7 μL of diluted first-strand cDNA, 1 μL of dT-ACP2 (10 μM), 2 μL of 5 μM arbitrary ACP, and 10 μL of 2× MasterMix® (Seegene). The PCR program was divided into three stages. The first stage was one cycle of a low specific reaction at 94 °C for 5 min, followed by 50 °C for 3 min and 72 °C for 1 min. The second stage was 40 cycles of a more specific reaction at 94 °C for 40 s, followed by 65 °C for 40 s and 72 °C for 40 s. The final stage was an extension at 72 °C for 5 min. Amplified PCR products were separated using 2 % agarose gel stained with ethidium bromide. The gel was screened by Tanon 3500 Gel image system (Tanon, Shanghai, China). Differentially expressed bands were extracted from the agarose gel using a gel extraction kit (Biomiga). pMD18-T TA cloning vector (TaKaRa, Biotech, Dalian, China) was used for subcloning of extracted cDNAs using T_4DNA ligase, and transformed in DH5a *Escherichia coli* competent cells. The sequencing of plasmids, identified to be positively inserted, was accomplished by Biosun (Shanghai, China). Homology of all inserted sequences was analyzed by running the BLAST programs in the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Triticeae Full-Length CDS Database (TriFLDB) (http://trifldb.psc.riken.jp/ver.2.0/blast.pl).

Isolation and Analysis of Full-Length cDNA and DNA of *TaARP1*

Results of the basic local alignment show that the primer (ARPF: GGATCTTATCGGTGGGTAG, ARPR: GCTTGC TCTGAGGCTGTTA) was designed to obtain the full-length cDNA and DNA of *TaARP1*. Analysis of the sequence was performed as described previously (Shi et al. 2013; Zhang et al. 2012).

Quantitative RT-PCR Analysis of *TaARP1*

Quantitative PCR was performed using SYBR Green Real Master Mix (Tiangen Biotech) according to protocol by the manufacturer. CFX96™ Real-Time System (Bio-Rad, Hercules, CA) was used under the following conditions: 95 °C for 90 s, 40 cycles of 94 °C for 10 s, 63 °C for 15 s, and 72 °C for 15 s. Melting curves were obtained at 65 to 95 °C with the fluorescence continuously monitored. The primers of *TaARP1* used in quantitative reverse transcription PCR (qRT-PCR) were TaARP1F: AGCAACCACCGCTA AATAAGAG and TaARP1R: AAGCTCAAGACAAACC ACATGC. Taβ-actin expression was used as the internal standard (F: CCGGCATTGTCCACATGAA, R: CCAAAA GGAAAAGCTGAACCG). Then, qRT-PCR were performed in triplicate and repeated. Fold changes were calculated by $2^{-\Delta\Delta C}{}_{T}$ (Livak and Schmittgen 2001). RNA from leaves of the control and the powdery mildew-infected plants at 12, 24, 48, and 72 h were used for qRT-PCR. RNA isolation and synthesis of the first strand were processed as described above.

BSMV-Mediated Gene Silencing of *TaARP1* in Wheat

A partial sequence (262 bp) of *TaARP1* was cloned and ligated to the vector of BSMV pCa-γbLIC with the primer (VF: AACCACCACCACCGTTTGCTGCTCTTT, VR: AAGG AAGTTTAAGCTCTGAGGCTGTTATTGTCTTAC; the adaptors are underlined) according to Yuan et al. (2011), with tobacco (*Nicotiana benthamiana*) as an intermediate host. This method is divided into two phases. First, the *Agrobacterium*-containing vectors were introduced into tobacco leaves to produce the virus, and the *NbPDS* gene of tobacco was used as a reference gene in the experiments for a photobleaching phenotype (pCaBS-α, pCaBS-β, and pCaBS-γb:*NbPDS*). Second, the leaves were harvested, ground, and soaked in 20 mM Na-phosphate buffer (pH 7.2) containing 1 % celite. The extract was mechanically inoculated into the leaves of wheat SN6306 during the two-leaf stage. Meanwhile, *TaPDS* gene of wheat was used as the reference gene for a photobleaching phenotype (pCaBS-α, pCaBS-β, and pCaBS-γb:*TaPDS*). Then, the expression level of the endogenous *TaARP1* gene in leaves was tested by semi-quantitative RT-PCR using gene-specific primers (ARPF: GGATCTTATCGGTGGGTAG, ARPR: GCTTGCTCTG AGGCTGTTA) that anneal outside the region targeted for silencing. Wheat *Taβ-Actin* gene was used as the internal standard. Microscopic analysis of *Bgt*-inoculated leaves that exhibited symptoms of powdery mildew after BSMV-induced gene silencing of *TaARP1* was conducted according to Yuan et al. (2011). Staining with Coomassie brilliant blue (CBB) according to the reported protocol (Lee et al. 2009) was employed before observation with light microscopes.

Results

Isolation and Analysis of Candidate Partial cDNAs from GeneFishing

GeneFishing was performed on the control and powdery mildew-infected plants using 80 different primers at 12 h. Partial amplification results are shown in Fig. 1. Differentially expressed bands between the control and

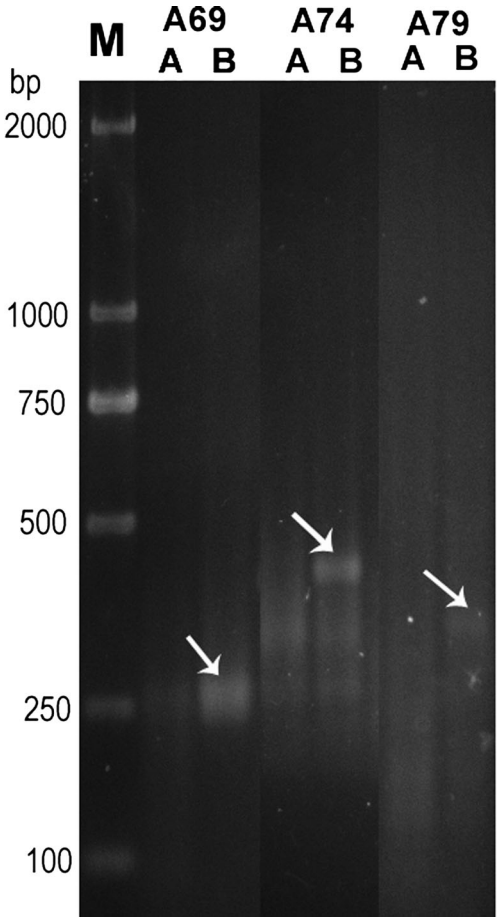

Fig. 1 Partial GeneFishing results of SN6306 cDNA amplified with the ACP primer pairs. *M* Takara DL2000 marker, *A* control of SN6306, *B* powdery mildew-infected SN6306 at 12 h, *A69*, *A74*, and *A79* different ACP arbitrary primers. *Arrows* indicate differential amplified cDNA bands

the powdery mildew-infected plants were extracted and cloned using a TA cloning vector for sequencing. Table 1 shows 23 partial cDNAs, approximately 100 to 350 nt, obtained from SN6306 inoculated with powdery mildew. Of the 23 partial cDNA sequences, 7 (30 %) were potentially associated with disease defense (clones 69B1, 74B7, 36116, 33B2, 23B7, 30B1, and 23B5), 1 (4 %) was predicted as uncharacterized protein (clone 1B1), and the rest were related to basic metabolism (11, 48 %), transcription (2, 9 %), and signal transduction (2, 9 %).

Isolation and Characterization of *TaARP1* Gene

A 168-nt cDNA sequence of the seven partial cDNAs presumed to encode for disease defense-related proteins was obtained using GeneFishing with clone number 69B1 (Table 1). The full-length sequence and the powdery mildew resistance of clone 69B1 were a prior consideration. Running BLASTN in TriFLDB revealed that a gene called *tplb0007b16* is 95 % identical with the partial cDNA of interest. Specific primer pairs were designed to obtain the full-length sequences of the cDNA and DNA. This powdery mildew-induced gene was named *TaARP1*, which is 93 % identical with *tplb0007b16* in TriFLDB. Full-length cDNA 751 bp and DNA 1072 bp of *TaARP1* were obtained with a putative open reading frame (ORF) of 348 bp encoding for 115 amino acids and containing two introns (Fig. 2a). *TaARP1* does not have a signal peptide (SignalP 4.1 Server) and thus may have a cytoplasmic function. The auxin-repressed conserved domain was detected in the protein (Fig. 2b) using BLASTX in NCBI.

Multiple Alignment and Phylogenetic Analysis of ARP Proteins

Multiple alignments and phylogenetic analyses of the amino acid sequence of TaARP1 with 14 ARPs from other species derived from NCBI were conducted. The multiple alignments showed that TaARP1 sequence is highly identical with other ARP proteins, particularly in the area of the two domains (Fig. 3a). The resulting phylogenetic tree shows that TaARP1 in wheat is similar to ARP proteins in other monocotyledons, such as *Hordeum vulgare* subsp. *vulgare*, *Aegilops tauschii*, and *Brachypodium distachyon* (Fig. 3b).

Table 1 Sequence similarities and characterization of differentially expressed transcripts

Clone	Length (bp)	Putative identity	BLAST	Identities	*E* value
6113	196	RFL_Contig927 TGA4 (TGACG MOTIF-BINDING FACTOR) factor	N	168/176 (95 %)	8e-75
69B1	168	PREDICTED: auxin-repressed 12.5 kDa protein-like isoform 2	X	48/51 (94 %)	2e-26
35B25	117	RFL_Contig857 defective chloroplasts and leaves protein (DCL)-like	N	115/115 (100 %)	5e-59
30B2	113	AK250118 thioredoxin h-like protein	N	101/115 (87 %)	3e-20
27B5	108	tplb0016a08 RNA binding protein, putative, expressed	N	102/102 (100 %)	3e-51
24B16	211	RFL_Contig3351 Endomembrane protein 70 containing protein, expressed	N	179/189 (94 %)	5e-79
33B1	209	tplb0007d10 glycosyl transferase	N	209/209 (100 %)	e-115
33B4	254	tplb0016p02 ethylene-responsive element binding protein 2	N	251/254 (98 %)	e-134
30B6	328	*Triticum aestivum* tonoplast intrinsic protein (AQP6) mRNA, complete cds	N	766/782 (98 %)	0.0
1B8	301	*Triticum aestivum* ATP synthase subunit mRNA, complete cds	N	298/298 (100 %)	1e-153
74B7	328	PREDICTED: Bowman–Birk type trypsin inhibitor-like	X	55/116 (47 %)	1e-21
79B5	228	PREDICTED: protein SRG1-like	X	128/198 (65 %)	1e-78
27B4	106	RFL_Contig4964 plasma membrane H+—ATPase	N	103/107 (96 %)	2e-42
36116	272	RFL_Contig148 metallothioneine type 2	N	226/258 (87 %)	2e-67
33B2	271	*Triticum aestivum* beta-1,3-glucanase precursor (Glb3) mRNA, complete cds	N	170/195 (87 %)	3e-54
25B28	214	Wheat FBP gene for chloroplast fructose-1,6-bisphosphatase	N	205/205 (100 %)	4e-102
1B1	236	PREDICTED: uncharacterized proteins ll1770-like	X	86/93 (92 %)	4e-48
23B7	203	*Triticum aestivum* partial mRNA for putative glyoxalase I	N	195/196 (99 %)	5e-96
30B1	139	*Hordeum vulgare* mRNA for bas1 protein	N	120/122 (98 %)	7e-53
23B5	208	RFL_Contig2497 putative salt tolerance protein 5	N	206/208 (99 %)	e-109
25B24	212	RFL_Contig3606 Cyclin, N-terminal domain, putative	N	212/212 (100 %)	e-116
38B6	231	RFL_Contig6066 Endoplasmin homolog precursor (GRP94 homolog)	N	230/230 (100 %)	e-127
22B12	328	RFL_Contig5790 RNA binding protein	N	308/322 (95 %)	e-151

A

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ggatcttatcggtgggtagtagtacgagcgagtgacacaagttcttgcaccgatcagatccaacttctgcgcaag   -1
ATGCTGGACAAGCTGTGGGACGACGTGGTGGCCGGGCCTCGCCCGGAGACGGGCCTCGACAAGCTCCGCAGGGCG   75
 M  L  D  K  L  W  D  D  V  V  A  G  P  R  P  E  T  G  L  D  K  L  R  R  A    25
GCCGCCACCCAACCTCTCGCCATCAACACAGgtacagtttacatacgttagcacagaggtaccgacatggcctga   150
 A  A  T  Q  P  L  A  I  N  T                                                  35
Aactggaagaacgtaagaactagcggtcagtctagcaccagtggttttcagaagaagatttgatgtttaagcgga   225
ggatctattgggttcattgtgtgaactgatggtggtttacatgggtgttggtggaggtgcagCTGCAGGGGAGGC   300
                                                              A  A  G  E  A    40
GATTAAGCAGTCGCCGTCGATGCCGACGACCCCGACCACGCCGGTGACGCCGTCGTCGTCGACGCCCCCGCGCGG   375
  I  K  Q  S  P  S  M  P  T  T  P  T  T  P  V  T  P  S  S  S  T  P  P  R  G    65
CGGCAGCGTGTGGCGGAGCGTTTTCCACCCCGGGAGCAACCTGGCCACCAAAAGCCTCGGCGCCAACCTCTTCGA   450
  G  S  V  W  R  S  V  F  H  P  G  S  N  L  A  T  K  S  L  G  A  N  L  F  D    90
CCGCCCGCAGCCCAACTCCCCCACCGTCTACGACTGgtgcgctggctcatgatctatctcacaatataatatcag   525
  R  P  Q  P  N  S  P  T  V  Y  D  W                                          102
tgttaataacacgatctactgggaacttgaaacgggtgaacatggcaaatagttaaaacatggggaaaaaaataa   600
cgctgccacgtacgttccatgtccagGCTTTACAGCGACGAGACCAGGACCAGGAGCAACCACCGCTAAataaga   675
                          L  Y  S  D  E  T  R  T  R  S  N  H  R  *              115
gccgtcggccgaagaagaagagagggtgctcaacaacttctgcgacgccgccatgtttgctgctctttggttat   750
ctaatcctgcatagcgtacgttgccgtccgaggctgcatgtggtttgtcttgagcttcgtactactgtctactag   825
tgcttgtaactttctttttcttatcccggtctgctcactgcatgctagcgagtgatgggtgtgtatgtgtgtactt   900
tgtgtacgctccatgtgtaaattctcgtgtgtgtggtgtggtgttctcaatgtttaaccacctactatggtaaga   975
caataacagcctcagagcaagc                                                        997
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B

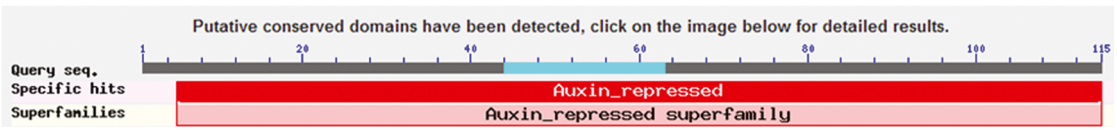


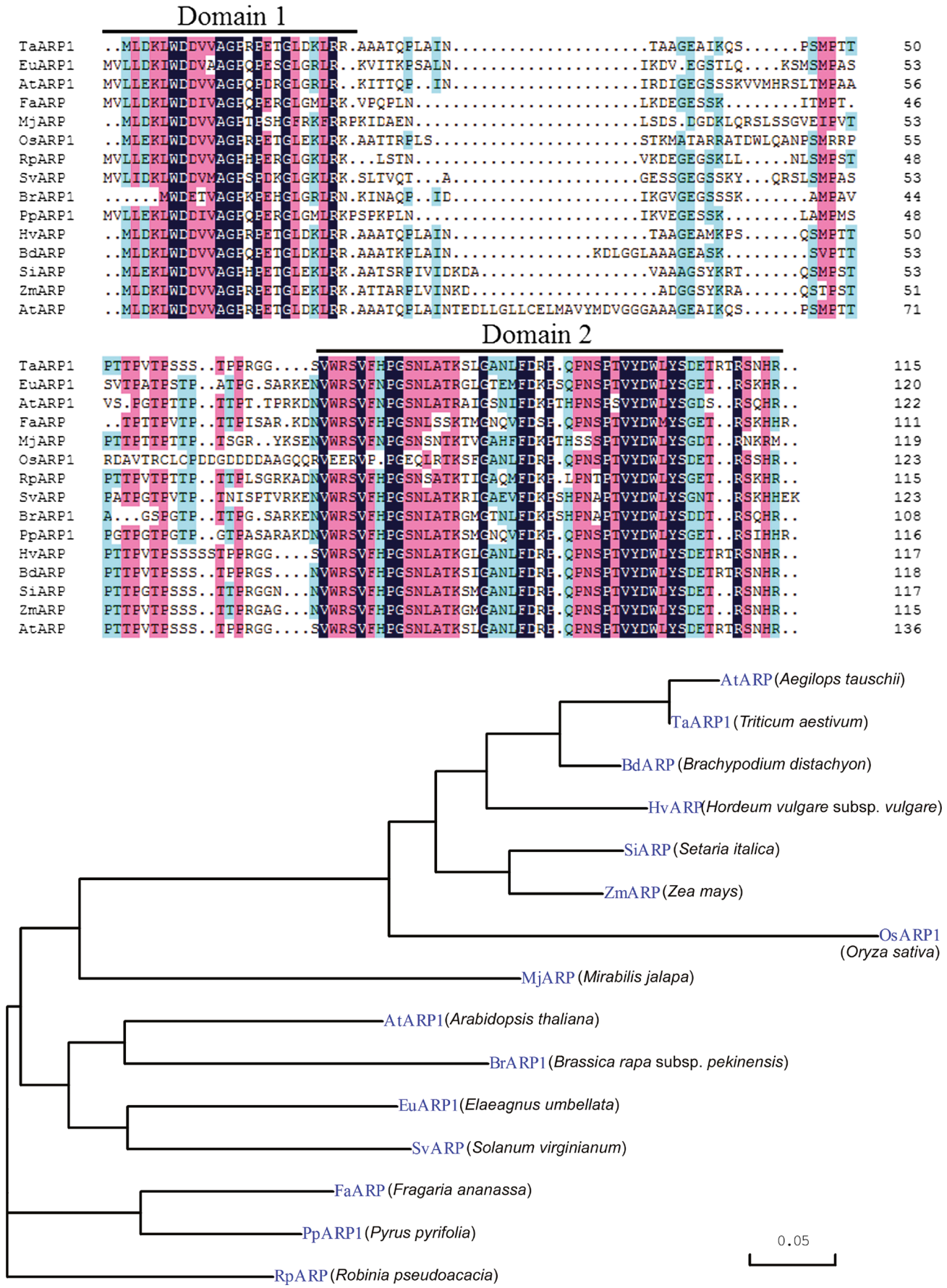
Fig. 2 The sequence and structure information of *TaARP1* from wheat. **a** Nucleotide sequence and deduced amino acid sequence of *TaARP1*. **b** Putative conserved domain of the TaARP1 protein

Expression Patterns of *TaARP1* Genes in Response to Powdery Mildew

To evaluate the mechanism of *TaARP1* in the response to powdery mildew, its expression patterns were analyzed. qRT-PCR showed that the expression level of *TaARP1* was upregulated by more than ten times compared to non-inoculated controls, especially at 12 h after inoculation (Fig. 4), and the expression pattern was the same as that with GeneFishing.

TaARP1 Gene Silencing Allowed Powdery Mildew Growth

To further investigate the mechanism of *TaARP1* in the resistance to powdery mildew, BSMV-based gene silencing was performed. The *NbPDS* gene of tobacco was used as a reference gene in the experiments, according to Yuan et al. (2011). Large areas of photobleaching occurred in leaves infected with BSMV:*NbPDS* of *N. benthamiana* (Fig. 5a). This demonstrated that


Domain 1
Domain 2
TaARP1
EuARP1
AtARP1
FaARP
MjARP
OsARP1
RpARP
SvARP
BrARP1
PpARP1
HvARP
BdARP
SiARP
ZmARP
AtARP
AtARP (Aegilops tauschii)
TaARP1 (Triticum aestivum)
BdARP (Brachypodium distachyon)
HvARP (Hordeum vulgare subsp. vulgare)
SiARP (Setaria italica)
ZmARP (Zea mays)
OsARP1 (Oryza sativa)
MjARP (Mirabilis jalapa)
AtARP1 (Arabidopsis thaliana)
BrARP1 (Brassica rapa subsp. pekinensis)
EuARP1 (Elaeagnus umbellata)
SvARP (Solanum virginianum)
FaARP (Fragaria ananassa)
PpARP1 (Pyrus pyrifolia)
RpARP (Robinia pseudoacacia)
0.05

◀ **Fig. 3** Multiple alignments (*top*) and phylogenetic analyses (*bottom*) of the amino acid sequence of TaARP1 with 14 ARPs from other species. Identical amino acid sequences are highlighted in *black* and sequence similarities in *grey*. *Domains 1* and *2* represent the regions on N- and C-terminal ends. The minimum evolution tree was constructed in DNAMAN Version 4.0. The accession numbers of *ARPs* in GenBank are listed as follows: TaARP1 (KJ513671), AtARP (EMT13445), HvARP (BAJ89735), BdARP (XP_003562172), SiARP (XP_004982572), ZmARP (NP_001152232), OsARP1 (AAL78369), EuARP1 (AAC62104), SvARP (AAS75891), AtARP1 (NP_564305), BrARP1 (AAO32054), MjARP (AAN16890), RpARP (AAG33924), FaARP (AAA73872), and PpARP1 (AGG19165)

NbPDS was silenced; thus, BSMV was successfully produced in *N. benthamiana*.

Wheat *TaPDS* was another reference gene for a photobleaching phenotype in the study. Figure 6a shows photobleaching of the leaf of wheat germplasm line SN6306 infected with BSMV:*TaPDS* (Fig. 5b), indicating that BSMV has been successfully produced.

Powdery mildew was inoculated into the leaves with silenced *TaARP1*. Ten days after conidia dusting, a microscopic analysis of *Bgt*-inoculated wheat leaves, with BSMV-silenced *TaARP1*, was conducted. The leaves with BSMV:*TaARP1* were notably susceptible to powdery mildew infections than those infected with BSMV:00 (Fig. 6a). Semi-quantitative RT-PCR was performed to confirm silencing of *TaARP1* in BSMV:*TaARP1* leaves (Fig. 6b). Ten days after conidial dusting, development of outstretched mycelia and colonies on leaf segments of SN6306 infected with BSMV:*TaARP1* was confirmed by staining with Coomassie brilliant blue (CBB) compared with BSMV:00 (Fig. 6c).

Discussion

Several methods are used to screen differentially expressed genes. Methods include suppression subtractive hybridization (SSH) (Diatchenko et al. 1996), GeneFishing (Hwang et al. 2003), microarray (DeRisi et al. 1996), two-dimensional gel electrophoresis (2-DE) (Wilkins et al. 1996), and RNA-Seq (Trapnell et al. 2010). In this study, GeneFishing was applied to find differentially expressed genes because of favorable features such as easy execution and its wide range of PCR products (Hwang et al. 2003). Twenty-three partial cDNAs were obtained in the response to the infection of powdery mildew using 80 ACP primers from wheat SN6306, as these sequences showed significant similarity to *H. vulgare* or *T. aestivum*. Several acquired cDNAs showed similarity to genes associated with plant defense, such as TGACG MOTIF-BINDING FACTOR (clone 36113) (Foley and Singh 2004), ethylene-responsive element binding protein 2 (clone 27B5) (Park et al. 2001), Bowman–Birk type trypsin inhibitor like (clone 74B7) (Chilosi et al. 2000), and auxin-repressed 12.5 kDa protein-like isoform 2 (clone 69B1) (Guimarães et al. 2010).

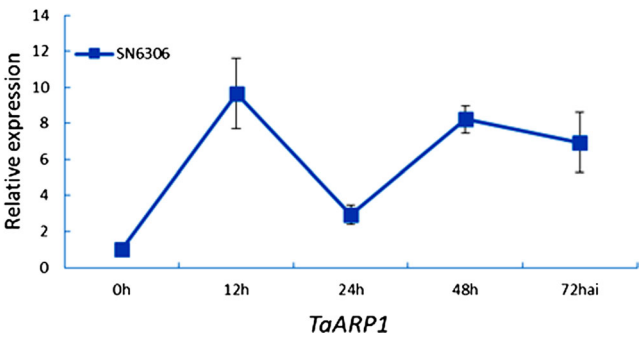


Fig. 4 Relative expression level of *TaARP1* induced by powdery mildew at different stages (0, 12, 24, 48, and 72 h after infection). Non-inoculated wheat (0 h) was used as the control. *hai* hours after inoculation

The best studied auxin, indole-3-acetic acid (IAA), is a key plant growth regulator that also impacts plant–pathogen interactions. Treatment with higher IAA can enhance disease caused by *Botrytis cinerea* or *Pseudomonas syringae* in wild-type *Arabidopsis* (González-Lamothe et al. 2012). Overexpression of the *YUCCA1* auxin biosynthesis gene, which elevated endogenous auxin levels, led to enhanced susceptibility to the bacterial plant pathogen *P. syringae* strain DC3000 in *Arabidopsis* through a mechanism that is independent of the suppression of SA-mediated defense (Mutka et al. 2013).

Auxin-repressed protein (ARP) gene was characterized as a repressor of plant growth and an activator of disease resistance based on genetic complementation, gene silencing, and overexpression analyses (Zhao et al. 2014). The dual role of ARP1 in plant growth and disease resistance is related to transcriptional regulation of inhibiting the expression of AUXIN RESPONSE FACTOR gene ARF8, and recruiting the NPR1 gene, which is essential for the salicylic-acid-mediated defense, respectively (Zhao et al. 2014). The *ARP1/GERI1* recessive mutant tobacco-repressed expression of defense response genes is consistent with impaired resistance to diseases caused by viral, bacterial, and oomycete pathogens (Zhao et al. 2014). *AsARPs* in *Arachis stenosperma* significantly responded to the infection of *Meloidogyne arenaria* (Morgante et al. 2013).

In the present study, a differential expressed gene *TaARP1* fragment was isolated by GeneFishing method, and the full-length gene was cloned. Real-time quantitative reverse transcription PCR and Barley Stripe Mosaic Virus-mediated gene silencing analyses showed that it markedly responded to the infection of the fungal pathogen *B. graminis* f. sp. *tritici* (*Bgt*) in the hybrid wheat alien disomic addition line germplasm SN6306. qRT-PCR revealed that powdery mildew upregulated *TaARP1* by three to ten times in the early stage, implying that *TaARP1* participates in the plant defense reaction. Interestingly, this gene was slightly expressed and not induced

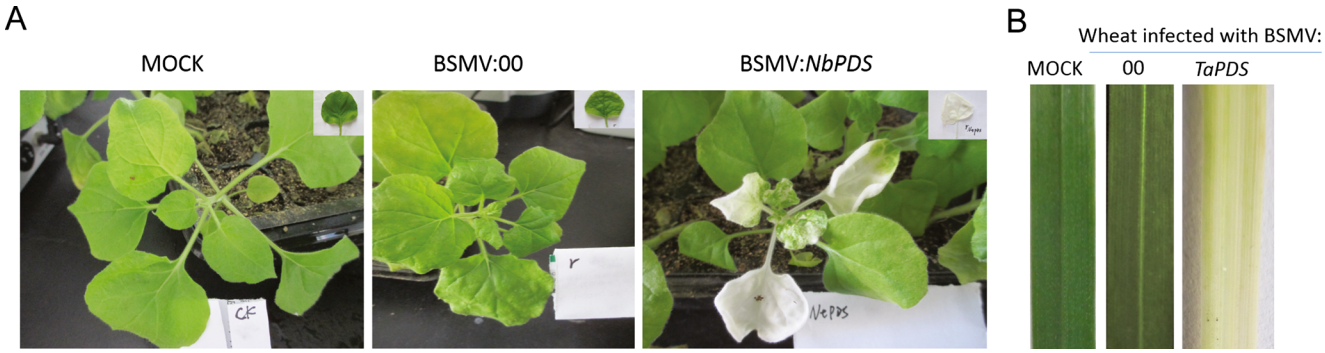


Fig. 5 Phenotype of the silencing of endogenous phytoene desaturase gene (*PDS*) by *Agrobacterium*-mediated BSMV-VIGS. **a** The silencing of endogenous *NbPDS* gene in leaves of *N. benthamiana* infected with BSMV:*NbPDS* (**a**) and *TaPDS* gene in leaves of SN6306 infected with *BSMV:TaPDS* (**b**) showed the phenomenon of photobleaching compared with untreated leaves (MOCK) and the empty vector (BSMV:00). The leaves of each treatment were shown in the insert panel of **a**

by the fungal pathogen *B. graminis* (data not shown). It is of great interest to illustrate the molecular mechanism of *TaARP1* gene in the regulation of the resistance to the fungal pathogen. And the underlying reasons of the different expression patterns of *TaARP1* gene in the powdery mildew-resistant hybrid wheat alien disomic addition line germplasm SN6306 and its susceptible parent wheat cultivar Yannong 15 needed thorough investigation.

BSMV, a tripartite genome consisting of RNAs α, β, and γ, is a positive-sense RNA virus with a broad experimental host range (Jackson et al. 2009). BSMV-mediated gene silencing is a useful and time-saving tool in the identification of gene function in the grass family (Yuan et al. 2011). Leaves whose *TaARP1* was silenced were notably susceptible to powdery mildew infections than leaves in the control. This result further proved *TaARP1* function in the plant response to powdery mildew.

In summary, a total of 23 DEGs upon inoculation of leaves with *Bgt* were isolated from wheat alien disomic addition line germplasm SN6306 with high resistance to powdery mildew by using GeneFishing™ technology. The gene sequence and expression pattern of *TaARP1* were further studied. The mechanism of *TaARP1* in the response to *Bgt* was primarily characterized by BSMV-induced gene silencing. Results of the present study may present candidate genes that are useful for breeding powdery mildew-resistant wheat cultivars. To determine the mechanism of *TaARP*, an overexpression vector of *TaARP* was constructed and used to transform wheat. Further

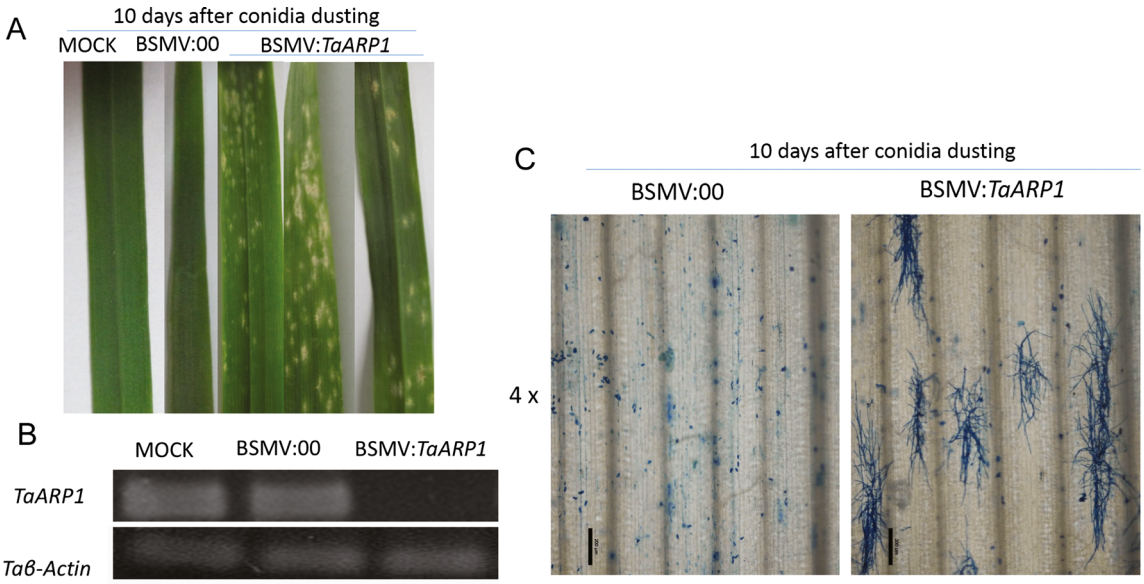


Fig. 6 The phenotype of auxin-repressed protein gene (*TaARP1*) silencing in wheat. **a** Gene silencing phenotypes on wheat leaves 10 days after powdery mildew conidia dusting infected with BSMV:00 or BSMV:*TaARP1*. **b** Effects of downregulation of *TaARP1* on powdery mildew pathogenesis and confirmation by semi-quantitative RT-PCR of the relative transcript levels of endogenesis *TaARP1* in leaves infected with the BSMV:00 or BSMV:*TaARP1* with the gene-specific oligonucleotide primers. Amplified *Taβ-Actin* gene served as an internal control. **c** Microscope observations of powdery mildew colonies and mycelia development at 10 days after conidial dusting appearing on leaf segments infected with BSMV:00 or BSMV:*TaARP1*. The *bar* is 200 μm

work will clarify the molecular mechanisms of protein interactions associated with resistance to powdery mildew infection.

The sequence described in this article was submitted to the GenBank database under accession number KJ513671.

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Conflict of Interest The authors declare that they have no competing interests.

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