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Long non-coding RNAs in wheat are related to its susceptibility to powdery mildew

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Abstract

To identify genes involved in the susceptibility to powdery mildew in wheat, genechip hybridization experiments were performed applying interactions between resistant and susceptible wheat cultivars and powdery mildew. Ten genes were found to express exclusively in the susceptible host after inoculation. The single cell transient expression experiments revealed that the RNA interference (RNAi) of two expressed sequence tag (EST) sequences (accession No. BQ168479 and CA648596) could enhance host resistance by 22 and 15 %, respectively. Full-length cDNA cloning revealed that they represented long non-coding RNAs (lncRNAs) and markedly accumulated during the powdery mildew primary infection.

Additional key words: expressed sequence tags, RNA interference, *Triticum aestivum*.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in the world, and wheat powdery mildew caused by the fungus *Blumeria graminis* f. sp. *tritici* is the most damaging disease causing great yield loss (Braun *et al*. 2002). Up to date, more than 40 powdery mildew resistance genes (*Pm* genes) have been identified. The interaction between wheat and powdery mildew fits the "gene-for-gene" theory and a *Pm* gene just functions against a responding pathogen's physiological race. Race-specific resistance genes have the great disadvantage that they are quickly overcome by the pathogen within a few years after a new cultivar has been introduced to the field (Hovmøller 2001, Pavan *et al.* 2010). Integrating many resistance genes into one cultivar can improve host durable resistance. However, only one of these resistance genes in wheat, *Pm3*, has been cloned up to date (Yahiaoui *et al*. 2004). This is largely due to the hexaploid nature of the exceedingly large genome of about 1.6×10^{10} bp (Bennet and Leitch 1995). Thus, the attempt to clone and integrate these resistance genes in the same cultivar seems difficult. Powdery mildew fungi belong to obligate biotrophs that are entirely dependent on nutrient supply from living host cells for their growth and reproduction. The genomic programs in some obligate biotrophic fungal pathogens have been completed in the past years. They revealed that many genes in important biosynthetic pathways are lost during the evolution although some genome sizes are relatively large (Baxter *et al*. 2010, Spanu *et al*. 2010, Duplessis *et al*. 2011, Kemen *et al*. 2011). On the other hand, some host genes have been found to be required for the pathogen growth and development, and thus they have a role as 'susceptibility factors' or susceptibility genes (Schulze-Lefert and Vogel 2000, Eckardt 2002, Pavan *et al*. 2010).

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Abbreviations: CYC - Chiyacao; EST - expressed sequence tags; HR - hypersensitive response; HY - Hongyou; hpi - hour post inoculation; *Pm* genes - powdery mildew resistance genes; qPCR - quantitative polymerase chain reaction; RT-PCR - reverse transcription PCR; lncRNAs - long non-coding RNAs; RACE - rapid amplification of cDNA ends; RNAi - RNA interference; TaS - *Triticum aestivum* susceptibility.
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The loss-of-function mutations of susceptibility genes can lead to enhanced resistance to pathogens (Pavan *et al*. 2010). Up to date, more than thirty susceptibility genes have been identified mainly in barley and *Arabidopsis.* They are involved in various functions, including transport, transcription, cell death inhibition, signal transmission, as well as defense reactions (Vogel and Somerville 2000, Vogel *et al*. 2002, 2004, Panstruga 2003, Hückelhoven 2005, Pavan *et al*. 2010). One of the most prominent susceptibility genes is *Mlo* in barley, whose loss-of-function mutations lead to broad spectum pathogen resistance and has been used in practice for more than 40 years in Europe (Jørgensen 1992, Hückelhoven 2005). Like barley *mlo* mutants, *Arabidopsis* powdery mildew resistance mutants have been found to carry recessively inherited loss-of-function mutations (Vogel and Somerville 2000). On the other hand, the presence of *PMR5*, *PMR6*, and a number of other genes promote powdery mildew pathogenesis in *Arabidopsis* (Vogel *et al*. 2002, 2004, Hückelhoven and Panstruga 2011). Apart from MLO, many other proteins, such as the WRKY transcription factors, the RHO-like GTPase RACB, the small peptide BLUFENSIN, and calmodulin are also required for full powdery mildew susceptibility in barley (Kim *et al*. 2002, Schultheiss *et al*. 2002, Eckey *et al*. 2004, Meng *et al*. 2009). Some susceptibility gene mutants, however, also bring adverse agronomic features, such as plant dwarfism (Vogel *et al*. 2002). The amplified fragment length polymorphism (AFLP) approach has been used to identify genes differentially expressed during compatible interactions of leaf rust (*Puccinia triticina*) and powdery mildew with wheat (Zhang *et al*. 2003, Zheng *et al*. 2009). However, these genes exclusively expressed in compatible hosts have not been characterized with regard to their function. In addition, no research on susceptibility genes in allohexaploid wheat to powdery mildew has hitherto been reported.

The generation and assessment of transgenic wheat is a time-consuming procedure and limits the number of transgenic plants with potential resistance traits that can be tested (Altpeter *et al*. 1996, Panwar *et al*. 2013). The pathosystem of wheat with powdery mildew is especially amenable to a functional transient assay system, since the fungus invades only the epidermal cell layer that is the main target for transformation by bombardment with particles (Schweizer *et al*. 1999a). In this study, transcriptome changes in susceptible and resistant wheat cultivars induced by powdery mildew inoculation were analyzed using *Affymetrix GeneChip* hybridization experiments. PCR fragments corresponding to candidate genes were used to construct RNAi vectors and expressed using the biolistic transient assay system (Schweizer *et al*. 1999b). The object of our study was to identify susceptibility genes which, when suppressed by RNAi, would inhibit powdery mildew infection in wheat. Here, we report two long non-coding RNAs (lncRNAs) in the compatible host which are in favour of the pathogen infection, named *TaS1* and *TaS2* (*Triticum aestivum* susceptibility).

Material and methods

Plants and treatments: Susceptible wheat (*Triticum aestivum* L.) cv. Yumai 13 and two resistant wheat cultivars Hongyou (HY) and Chiyacao (CYC) were sown in pots in a greenhouse under day/night temperatures of 20/16 °C, a relative humidity of 70 %, a 16-h photo-period, and an irradiance of 360 μmol m^{-2} s^{-1}. Seven-day-old wheat seedlings were inoculated by powdery mildew (*Blumeria graminis* f.sp. *tritici*, Swiss isolate 96229 maintained in wheat cv. Kanzler by weekly transfer) at a density of approximately 200 conidia per mm^2 (Winzeler *et al*. 1991). Leaf segments (2 cm long) were harvested at different time points after inoculation and immediately fixed in 95 % (v/v) ethanol/glacial acetic acid (3:1, v/v) for 3 - 6 h. Leaves were then boiled for 2 min in alcoholic lactophenol (20 cm^3 of ethanol, 10 cm^3 of phenol, 10 cm^3 of water, and 10 cm^3 of lactic acid; Frye and Innes 1998) and then stained with Coomassie brilliant blue R 250 at room temperature for 5 min.

H_2O_2 was detected using the diaminobenzidine (DAB) method (Thordal-Christensen *et al*. 1997). The inoculated primary leaves were sampled and then exposed to a DAB solution [1 mg cm^{-3} 3,3-DAB adjusted with 3 M HCl, pH 3.8] for 8 h. After discoloration by boiling in 95 % ethanol for 10 min, the samples were clarified overnight in saturated chloral hydrate. All the stained specimens were stored in 50 % (v/v) glycerol before microscopic examination. At least 300 interaction sites on five leaf segments were examined for each sample. Images were captured with a digital camera (*Caikon* CK-300, Shanghai, China).

Microarray hybridization analysis: Seven-day-old wheat seedlings of Yumai 13, HY, and CYC were inoculated with powdery mildew, and infected leaves harvested at 0, 24, and 48 h post inoculation (hpi) and immediately frozen in liquid nitrogen. Microarray hybridization was carried as described by Zhou *et al*. (2011). The data are available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE43930.

To validate the microarray results, 20 genes were selected because of their high expression exclusively in the susceptible cultivar, but not in the resistant cultivars upon the powdery mildew infection. RNA from relevant leaf samples was purified using a *SV* total RNA isolation kit (*Promega*, Madison, WI, USA) including a DNase I treatment. The RNA quantity and purity were determined

using a *NanoDrop-2000* spectrophotometer (*Thermo Fisher Scientific*, Waltham, MA, USA). The RNA integrity was checked by an electrophoretic separation on a 1 % (m/v) agarose gel in a 1× TAE running buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.0). First-strand cDNA was synthesized with a recombinant RNase *H Minus Moloney Murine Leukemia Virus* (M-MLV) reverse transcriptase point mutant (*Promega*).

Real time qPCR was carried out in a *Bio-Rad CFX96TM* real-time system (*Bio-Rad*, Hercules, CA, USA) using a *iQTM SYBR Green* kit (*Bio-Rad*) under the following conditions: 95 ºC for 3 min, 40 cycles of 95 ºC for 5 s, 60 ºC for 15 s, followed by melting curve generation (65 to 95 ºC). To normalize the obtained data, wheat constitutive 18S ribosomal RNA (acc. No. AY049040) was used as endogenous control. Dissociation curves were generated for each reaction to ensure a specific amplification. The relative quantitative comparative method (ΔΔCt) was used to evaluate the quantitative variation (Livak and Schmittgen 2001). Forward and reverse primers were designed using the Primer 5.0 software (*PREMIER Biosoft International*, Palo Alto, CA, USA). Three replicates for each treatment were conducted, and transcript abundance was assessed with three independent technical qPCR replications. The selected genes and corresponding specific primers were listed in Table 1 Suppl.

Plasmid constructions: To generate RNAi constructs for gene suppression, 200 - 500 bp fragments were generated by reverse transcription (RT)-PCR using RNA extracted from Yumai 13 as described above. Primers are listed in Table 2 Suppl. Fragments were digested with *Bam*HI and *Xho*I and ligated into a *pENTR*^{TM} cloning vector (*Invitrogen*). The final RNAi vector was constructed by an LR clonase reaction between the entry clone and a pANDA-mini expression vector (*Invitrogen*) by cloning two copies of each fragment in inverse orientation into the expression vector (Miki and Shimamoto 2004).

Transient RNAi assays: To suppress the expression of the corresponding endogenous genes, a biolistic transient assay system was used (Schweizer *et al*. 2000a,b). The apical 10 cm of primary leaves of Yumai 13 were cut and placed on 0.75 % (m/v) *Phytogar* plates supplemented with 50 % (m/v) benzimidazole (Leath and Heun 1990) and biolistically transformed with plasmid-coated 1.1 µm gold particles using a *PDS1000/He* apparatus (*Bio-Rad*). Gold particles were coated with a 1:1 mixture of an RNAi vector and pUbiGUS (Schweizer *et al*. 1999a). For control experiments, the empty pANDA-mini vector was used instead of the RNAi vector. Plates were incubated for 4 h, infected with approximately 150 to 250 conidiaspores per 1 mm^2 of leaf area, and incubated at 20 °C, a 16-h photoperiod, an irradiance of 360 µmol m^{-2} s^{-1}, and an 80 % relative humidity for 44 h. Afterwards, the leaves were stained in a GUS solution for 8 h (including vacuum infiltration). To visualize fungal structures, the leaves were subsequently stained with Coomassie blue before they were examined under the microscope.

The interactions of GUS-expressing cells attacked by a germinated spore with a fully matured appressorium were classified into two types: the incompatible (without a haustorium), and the compatible (containing a haustorium and forming secondary hyphae) (Schweizer *et al*. 1999a,b). Penetration efficiency was calculated by dividing the number of penetrated cells by the number of counted interaction sites. The change in penetration efficiency was taken as measure of plant resistance at the single cell level (Hoefle *et al*. 2009). For each experiment, three independent replications were analyzed.

Rapid amplification of cDNA ends: The full length of cDNAs were cloned using a *SMART™ RACE* cDNA amplification kit (*Clontech*, Palo Alto, CA, USA) as described by the manufacturer. The gene-specific primers used for RACE were designed by the *Primer 5.0* software based on the expressed sequence tag (EST) sequences (Table 3 Suppl.). PCR products were separated on 1.5 % (m/v) agarose gels in 1× TAE and purified with a *QIAEX1 II* gel extraction kit (*Qiagen*). The DNA fragments were cloned into a *pGEM-T Easy* vector (*Promega*) for sequencing. The cDNA sequence data were analyzed with the *BLAST* (http://www.ncbi.nlm.nih.gov/blast/), the *CAP3* sequence assembly program (http://pbil.univ-lyon1.fr/cap3.php/), the nucleotide (DNA/RNA) sequence translated to the protein sequence (http://web.expasy. org/translate/), and ORFs were analysed with the *ORF Finder* program (http://ncbi.nlm.nih.gov/gorf/gorf.html). Dotplots were generated using the dotter (Sonnhammer and Durbin 1995).

Expression analysis of *TaS1* and *TaS2* by real time qPCR: Leaf segments of Yumai 13 inoculated with powdery mildew were sampled at 0, 2, 6, 12, 24, 48, 72, 96, 120, 144, and 192 hpi. In addition, different wheat organs (roots, stalks, leaves) were collected from 7-d-old Yumai 13 seedlings without inoculation. The wheat *β-actin* gene (acc. No. AB181991) was used as endogenous control. Three replicates were performed for each sample using the primers listed in Table 4 Suppl.

Results

In the field, seedlings and adult plants of wheat cvs. HY and CYC display resistance to powdery mildew (Wang *et al*. 1996), whereas Yumai 13 is an extremely susceptible cultivar (Wang and Zhang 1998). After

inoculation in the greenhouse, there was no visible pathogen colony on leaves of HY and CYC at 96 hpi, whereas in Yumai 13, dense white colonies were visible with naked eyes on whole inoculated leaves.

Light microscopic observation found that most spores germinated and formed primary germ tubes in all the cultivars at 6 hpi, followed by secondary germ tube and appressoria formation at about 8 hpi. The pathogen appressoria penetrated into the host cell and formed different amounts of haustoria in the three cultivars (Figs. 1 and 2). In HY and CYC, the haustoria formation rate reached 22 and 25 % at 48 hpi, respectively. Most of the haustoria in HY and CYC remained in the undeveloped state and the haustorial central bodies could not produce the haustorial lobes and secondary hyphae, some of them started to degenerate from 48 hpi (Fig. 2*A*). However, the mature haustoria with well developed haustorial lobes were detected in most of the infected sites in Yumai 13, and the secondary haustoria produced by the secondary hyphae could be observed at 72 hpi (Fig. 1*B*).

In HY and CYC, a number of small haustoria with elliptic collars became apparent from 48 to 96 hpi, but they could not develop further (Fig. 1*C*). The formation frequency of these structures was much higher in the two resistant cultivars than in Yumai 13 at 48 hpi. There were just less than 1 % of small haustoria detected in Yumai 13 (Fig. 2*B*).

When the matured appressoria penetrated the host cell wall, some globular papillae beneath the appressorial lobes were visible (Fig. 1*D*). The number and size of the papillae increased gradually over time, and reached the maximum at 48 hpi in HY and CYC, but the papilla formation rate was much lower in Yumai 13 (Fig. 2*C*). In the susceptible host, pathogen penetration pegs could pass through most of the papillae so that those papillae were ineffective. These suggest that papilla-associated resistance in HY and CYC is an important component in the resistance mechanism (Fig. 2*A*).

Most of the infected sites both by primary germ tubes and appressoria were stained into deep brown by DAB at 12 and 24 hpi, indicating H_2O_2 accumulation (Fig. 1*E-F*). At 48 hpi, many invaded cells of HY and CYC became brown after DAB staining. It implied strong H_2O_2 accumulation in the whole cells and hypersensitive response (HR) (Fig. 1*G*). In contrast, the H_2O_2 accumulation was determined just locally in the penetration sites at 96 hpi (Fig. 1*H*), and a whole-cell DAB reaction was only observed in a few penetration sites in Yumai 13 (Fig. 2*D*).

The *Affymetrix GeneChip*® wheat genome arrays contain 61 180 probes with cDNA corresponding to RNAs extracted from the three wheat cultivars at 0, 24, and 48 hpi. From the hybridization database, 20 genes

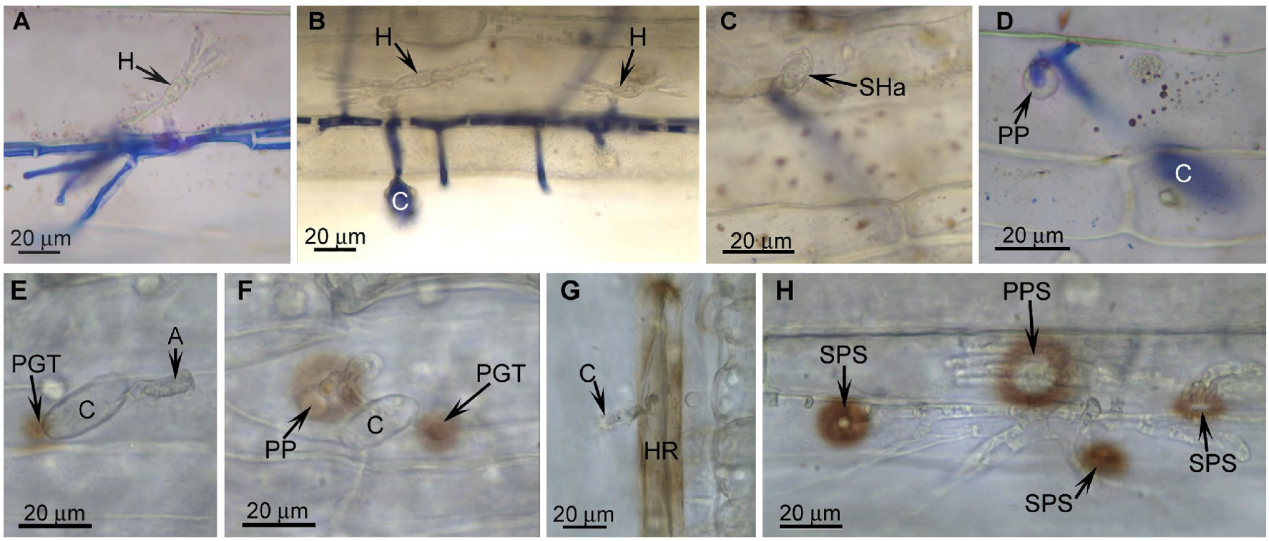

Fig. 1. *A* - A mature haustorial body (H) with fingers in Yumai 13 at 48 hpi. *B* - Primary and secondary haustoria in Yumai 13 at 96 hpi. *C* - A small haustorium (SHa) in HY at 72 hpi. *D* - A papilla (PP) arrested successfully pathogen penetration in CYC at 48 hpi. *E* - H_2O_2 accumulation was detected at the penetration sites of a primary germ tube (PGT) in HY at 12 hpi. *F* - H_2O_2 accumulation at the penetrated site by an appressorium in CYC at 24 hpi. *G* - H_2O_2 bursted in a whole epidermal cell undergoing HR in CYC at 48 hpi. *H* - Tracing H_2O_2 in the primary penetration site (PPS) and secondary penetration site (SPS) in Yumai 13 at 96 hpi. C - a conidium. A - an appresorium.

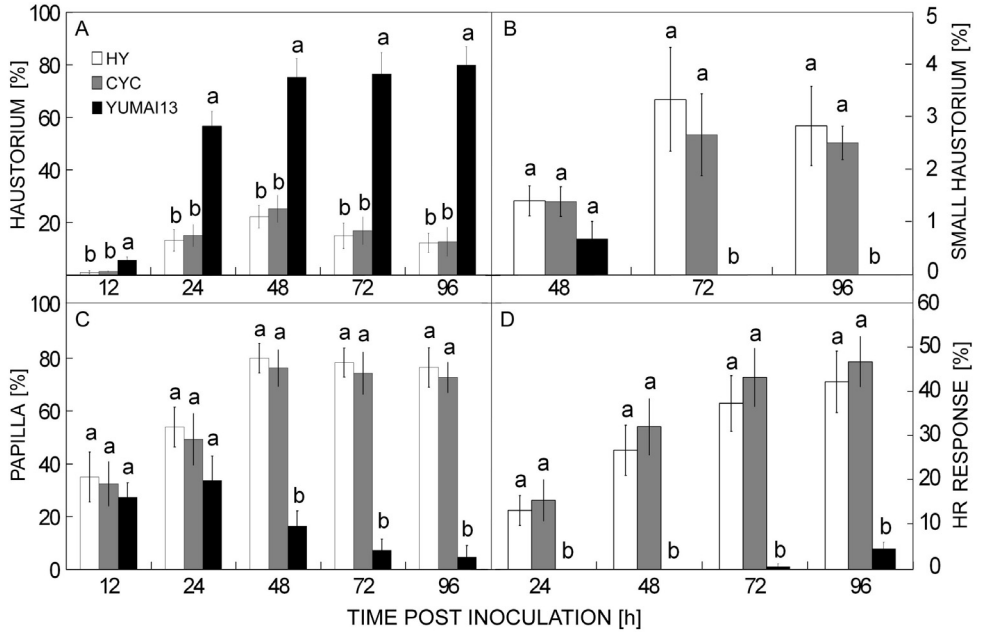

Fig. 2. Interaction events between the pathogen and various wheat cultivars. *A* - Penetration efficiency indicating the percentage of successfully attacked cells containing a haustorium among the total interaction sites. *B* - Small haustoria formation frequency. *C* - Papilla frequency was calculated on the basis of the number of the cells arrested by papillae divided by the interaction sites. *D* - The percentage of hypersensitive response. The whole-cell staining with DAB implied undergoing HR. Means ± SE of pooled data from three independent experiments with at least 300 penetration sites. Columns labelled with different letters are significantly different at $P \leq 0.05$.

Table 1. The candidate susceptibility genes and their expressions examined with gene chip hybridization. Expression signal intensities were taken from the *Gene Chip* analysis. Means of the values measured at 24 and 48 hpi, the control at 0 hpi.

Accession No.	Probe set ID	Yumai 13 infected	control	HY infected	control	CYC infected	control
CK205485	Ta.7509.3.S1_at	1296.3	857.4	7.7	8.2	8.3	8.1
CA684267	Ta.3087.2.A1_at	1215.5	1503.0	7.6	9.7	10.4	10.0
CA664935	Ta.22285.1.A1_at	1046.6	890.7	20.9	13.5	13.7	22.3
CD863680	Ta.29583.1.S1_at	971.7	803.1	20.1	15.8	20.4	26.2
CD890484	TaAffx.123103.1.S1_at	355.9	323.4	15.8	14.2	10.7	13.0
BQ168479	Ta.11068.1.A1_at	333.7	439.1	11.6	11.2	11.0	15.1
CA633933	Ta.5243.2.S1_a_at	308.4	380.5	10.5	9.7	11.1	8.2
BJ272293	TaAffx.119440.1.A1_at	318.7	330.8	22.8	21.6	23.0	23.6
CA648596	Ta.19202.1.S1_at	322.9	175.1	18.6	35.9	26.7	16.2
CD374038	Ta.30728.1.S1_at	1252.6	1152.7	63.6	65.0	75.8	68.1

were found to express strongly in the susceptible cv. Yumai 13, and their signal intensities were 5-fold higher than in the two resistant cultivars. The acc. Nos and putative functions of these candidates are listed in Table 2 Suppl. Some genes were annotated by searching for homologous sequences using the *BLASTX* or *BLASTN* software (http://www.ncbi.nlm.nih.gov/BLAST/).

To confirm the microarray hybridization results, the real time qPCR analysis was carried out for the 20 selected genes in all interactions. The relative fold-change values were calculated and normalized with respect to Yumai 13 mRNA divided by HY and CYC at 0, 24, and 48 hpi, respectively (Table 2 Suppl). These experiments confirmed that the data obtained from the gene chip hybridization experiments reliably reflected the different expression of these genes which were strongly expressed in Yumai 13, but only weakly in HY and CYC. The results are coincident with that in the gene chip assay.

Among the 20 genes, 10 ESTs exhibiting the largest expression differences between the susceptible and the resistant cultivars were selected for further RNAi experiments. The expressions of these 10 genes were absent in the resistant cultivars HY and CYC, but their

expression was high in Yumai 13 (Table 1). These ESTs were amplified and cloned as inverted repeats into the RNAi vector pANDA-mini and thus brought under the control of the maize ubiquitin1 promoter. These RNAi constructs were cotransformed with a GUS reporter construct into epidermal cells of leaf segments of Yumai 13 using the transient biolistic transformation protocol (Schweizer *et al*. 1999a,b).

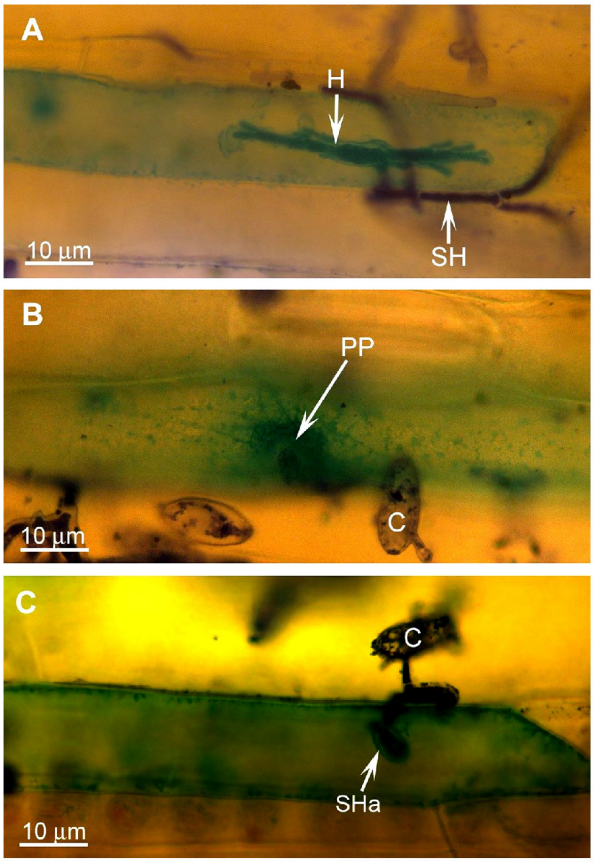

Fig. 3. *A* - A transformed cell (*blue*) successfully infected with the fungus which contained a well-developed haustorium (H) and secondary hyphae (SH). *B* - A transformed cell where fungal invasion was arrested by the formation of a papilla (PP). *C* - A transformed cell containing a small haustorium with an elliptic collar (SHa). C - a conidium.

The examples of interaction phenotypes are depicted in Fig. 3. It shows that the pathogen successfully infected a transformed epidermal cell expressing GUS and established a mature haustorium followed by the formation of secondary hyphae (Fig. 3*A*). An incompatible interaction was reflected by the fact that the fungal growth was stopped at the stage of attempted penetration and no haustorium formed (Fig. 3*B*). Sometimes, the pathogen could just produce a small haustorium with elliptic collars (Fig. 3*C*). For each candidate gene, 200 to 500 interaction sites were initially scored. The RNAi experiments with *TaS1* (corresponding to EST BQ168479) and *TaS2* (corresponding to EST CA648596) decreased the relative penetration efficiency to 78.4 and 85.2 %, and the indicating resistance enhanced by 22 and 15 % as compared to the controls, respectively. The RNAi experiments with other genes had no significant effect on the penetration efficiency.

Further cotransformation with *TaS1* and *TaS2* shows that the relative penetration efficiency was 81.4 %. There was no significant change compared to cells transformed with the single constructs. This suggests that the two genes functioned independently and there was no additive effect between them.

The expression profiles of *TaS1* and *TaS2* in Yumai 13 after inoculation were examined with the qPCR method (Fig. 4). The *TaS1* transcripts accumulated two-fold with a maximum at 12 hpi and declined thereafter, whereas *TaS2* was up-regulated three-fold, reaching a maximum at 24 hpi. These data are in accordance with the data from the gene chip hybridization experiments. *TaS1* was specifically expressed in leaves and hardly detectable in roots. *TaS2* was detected in almost all of the three organs, but preferentially expressed in roots.

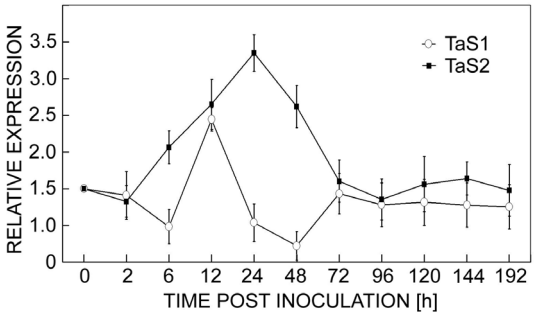

Fig. 4. The expression of *TaS1* and *TaS2* in Yumai 13 after inoculation determined by quantitative RT-PCR. Data are normalized to the wheat *β-actin* gene expression. Mean ± SE from three independent replicates.

In order to fully investigate the function of *TaS1* and *TaS2*, we obtained full-length cDNAs by 3' and 5' RACE. For *TaS1*, a 320-bp EST (acc. No. BQ168479) was identical but in reverse orientation to the 455-bp mRNA sequence whatlal6g12 (acc. No. CJ834557). The *TaS1* sequence seems to represent the 3' end of a cDNA sequence as it contains a 19-bp polyA sequence. Using 5' RACE, a 729-bp fragment was obtained which could be assembled into a putative 1 020-bp transcript sequence dubbed *TaS1_long*. Except the putative polyA tail, this cDNA sequence was 99 % identical to a sequence contained in a 1 955 bp contig (Fig. 1 Suppl.) assembled from the genome sequence of the wheat cv. Chinese Spring (http://www.wheatbp.net/WheatBP/Documents/DOC_Evolution.php) using the *CAP3* sequence assembly program. PCR was performed using genomic DNA in Yumai 13 as template. PCR product sequencing validated that the assembled contig is accurate. It suggests that

TaS1_long is derived from a transcript of the corresponding genomic region in Yumai 13.

For *TaS2* (acc. No. CA648596), a 807-bp 5' terminal fragment was obtained by RACE, resulting in a putative cDNA of 1 296-bp named *TaS2_long*. It was 98 % identical to a 1 226 bp genomic contig (Fig. 2 Suppl.) assembled from the Chinese Spring genome sequencing project, suggesting that *TaS2_long* was derived from a transcript of the corresponding Yumai 13 genomic region. The comparison of *TaS1_long* and *TaS2_long* with their respective genomic contigs revealed no introns in them. There have been no open reading frame to be detected and thus they should belong to lncRNAs. Dotplot comparisons of the sequences against themselves revealed that *TaS2_long* contained a repetitive region encompassing about 40 % of its sequence.

Discussion

Though resistance and susceptibility are the opposite sides of the same coin, most studies have focused on the resistance side, especially on resistance genes and other defense genes, in the past two decades (Cho *et al*. 2012, Zhang *et al*. 2012). Recently, the exploitation of susceptibility genes to gain broad-spectrum and durable disease resistance begins to draw the attention (Pavan *et al*. 2010). In previous studies, more than 15 susceptibility genes to powdery mildew have been identified in model plants *Arabidopsis* and barley (Hückelhoven 2005, Pavan *et al*. 2010). Here, we selected 10 genes exclusively expressed in the susceptible cultivar, and among them, only *TaS1* and *TaS2* could promote host susceptibility which contributed to the pathogen infection. It is expected that ongoing efforts will investigate wheat genes that are expressed both in resistant and susceptible hosts but up-regulated significantly in the critical stage of the pathogen infection and development.

In the past years, eukaryotic cells have been found to transcribe a vast number of noncoding RNA species. Noncoding RNAs have even been termed as junk DNA for a long time (Kung *et al*. 2013). Now they are better known for their roles as regulators of transcription and post-transcription in a variety of ways in organism development (Kung *et al*. 2013, Yoon *et al*. 2013). However, only few lncRNAs have been identified and characterized in plants (Boerner and McGinnis 2012). *TaS1* and *TaS2* were expressed only in susceptible hosts and up-regulated during the phase of the primary fungus infection and haustorial development. Judging from their high basal expression during plant development, they may have unknown functions. It would be worth to determine their subcellular localization and binding molecules in the future.

The loss-of-function mutations of susceptibility genes can lead to broad spectrum resistance to most or all physiological races of a pathogen, or to different pathogens (Vogel and Somerville 2000, Panstruga 2003, Pavan *et al*. 2010). In barley the transient gene silencing experiments seem to have a great effect on host resistance, such as silencing *RacB* and *BAX INHIBITOR-1* (Schultheiss *et al*. 2002, Eichmann *et al*. 2010). However, in our experiments, the suppression of *TaS1* and *TaS2* enhanced wheat resistance only by 15 - 22 %. This might be due to the effects of the hexaploid wheat which have more copies of alleles and they could complement each other. The mutations of some plant susceptibility genes are accompanied by pleiotropic effects, such as influencing the plant normal growth, reproduction, and grain yield (Jørgensen 1992, Nishimura *et al*. 2003, Chu *et al*. 2006). This balance between resistance and development should always be taken into account when screening for susceptibility genes.

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