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Temporal Transcriptional Changes in SAR and Sugar Transport-Related Genes During Wheat and Leaf Rust Pathogen Interactions

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Abstract

Leaf rust (*Puccinia triticina* Erikss.) is one of the most damaging pathogens of wheat (*Triticum aestivum* L.). With the rapid evolution of new races, worldwide distribution, and high genetic diversity, *P. triticina* has the ability to cause severe epidemics in wheat growing areas. In plants, salicylic acid (SA) and sugar-mediated defense pathways are expected to provide durable and broad-spectrum resistance. To understand the role of SA and sugar-mediated resistance mechanisms in wheat during early leaf rust infection, expression profiles of the key regulators of SA (*TaEDS1, TaPAD4, TaNDR1, TaRAR1, TaSGT1, TaHSP90, TaEDS5, TaPAL*, and *TaNPR1*) and sugar (*TaHTP, TaSTP13A*) pathways were analyzed in time-course experiments between two wheat near-isogenic lines (NILs) differing in the leaf rust resistance gene, *Lr24*. The quantification of candidate gene expression using reverse transcription quantitative real-time PCR at different time points post inoculation showed stage-specific transcriptional reprogramming between compatible and incompatible interactions. Interestingly, two distinct expression patterns were observed between two types of interactions. The genes acting upstream of SA in the SA pathway (*TaEDS1, TaPAD4, TaNDR1, TaRAR1, TaSGT1, TaHSP90, TaEDS5*) showed strong expressions at a later stage [48 h post inoculation (hpi)] of leaf rust infection in the compatible interaction compared to unchanged or slightly changed expressions in the incompatible interaction. Further, these genes showed similar expression patterns in either of the interactions, suggesting their cooperative or coordinated functions. On the other hand, the genes involved in SA biosynthesis (*TaPAL*), SA downstream signaling (*TaNPR1*), and sugar transportation (*TaHTP, TaSTP13A*) showed a strong expression at mid phase of infection between 6 and 24 hpi in the incompatible interaction compared to the compatible interaction. These expression patterns suggest that *TaPAL* and *TaNPR1* play a positive regulatory role in the SA-mediated resistance pathway whereas *TaHTP* (*Lr67*) plays an important role in the sugar-mediated resistance pathway activated by the leaf rust resistance gene, *Lr24*.

Keywords Pathogen · Salicylic acid · Sugar · Resistance · Wheat · Regulation

Introduction

Wheat (*Triticum aestivum* L.) is the second most important food crop after rice and has been providing nutrition to the world population over the centuries. Its annual contribution to the global economy is worth US $50 billion (Curtis and Halford 2014). With continued population growth and increasing per capita consumption, a 60% increase in wheat production is required to feed the world by 2050. However, yield levels of wheat including other major cereal crops have reached a plateau. Further, due to increasing abiotic and biotic stresses caused by changing climate, genetic homogeneity, and the need for reduced usage of input resources, the global wheat production is predicted to be lowered significantly with current crop production technologies.

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Leaf rust or brown rust (*Puccinia triticina* Erikss.) is one of the major constraints to wheat production due to its widespread distribution, rapid evolution of new virulent races, and diverse population structures (Kolmer 2005; Bolton and others 2008; McCallum and others 2016). Wheat leaf rust causes huge economic losses due to reduced yield and quality of the produce and further, additional expenditure incurred on fungicides to control the disease, thus threatening world food security and economy (Huerta-Espino and others 2011; Khan and others 2013). Breeding resistant varieties is an effective method of controlling leaf rust disease and reducing yield losses (Dubin and Brennan 2009; Singh and others 2016). As a part of resistance breeding, to date, 76 single-resistance (R) genes and several quantitative trait loci (QTLs) associated with leaf rust resistance in wheat have been identified (Cereal rust lab, USDA 2015). R gene-conferred resistance can be quickly overcome by the rapidly evolving virulent races of a pathogen. Further, pyramiding of favorable QTLs from different sources is tedious and often suffers from linkage drag. Therefore, there is a need for devising novel strategies for developing broad-spectrum and durable resistance in wheat. Developing a novel resistance breeding strategy requires a comprehensive understanding of the molecular basis of plant–pathogen interactions. However, the information on the molecular basis of defense pathways involved in wheat and leaf rust pathogen interactions is limited.

In general, pathogen defense responses involve cellular processes like recognition of a pathogen-associated pattern or effector, mitogen-activated protein kinase (MAPK) signaling, transcriptional activation of pathogenesis-related (*PR*) genes, and the hypersensitive response (HR). Systemic acquired resistance (SAR) is one of the prominent resistances induced in plants. SAR is a whole-plant resistance response, that is, enhanced disease resistance in distal tissues after a localized exposure to a pathogen. SAR provides a broad-spectrum and durable resistance against plant pathogens (Conrath 2006; Fu and Dong 2013). SAR is thought to be activated by pathogens causing cell death responses, ranging from single-cell HRs to necrotic disease lesions (Kogel and Langen 2005). Several factors are implicated as regulators of *PR* genes during the SAR-mediated defense signaling and such signaling regulators may interact synergistically and/or antagonistically to fine tune plant defenses against pathogens. The evidence for the roles of the regulators of SAR-mediated defense signaling and responses have been very well studied in model systems such as *Arabidopsis* against some of its pathogens (Dodds and Rathjen 2010; Pieterse and others 2012). Salicylic acid (SA), a phytohormone, is a key regulator of the SAR against biotrophic pathogens. In *Arabidopsis*, NDR1 (*Non-specific Disease Resistance 1, At3g20600*), EDS1 (*Enhanced Disease Susceptibility 1*), PAD4 (*Phytoalexin Deficient 4, At3g52430*), SAG101 (*Senescence Associated Gene 101, At5g 14930*), and EDS5 (also known as SID1; *At4g39030*) have been shown to regulate SA metabolism (Cao and others 1997; Ishihara and others 2008; Kawamura and others 2009; Dempsey and others 2011; Bao and others 2014). However, in rice and poplar, SA metabolism and its regulation are suggested to be more intricate than in *Arabidopsis* (Stalman and others 2003). In rice and poplar species, although SA plays an important role in disease resistance, SA synthesis is not significantly induced during biotic stresses but the basal SA levels are higher than those found during pathogen-induced SA levels in *Arabidopsis* leaves (Koch and others 2000). Studies have implicated the existence of the phenylalanine ammonia-lyase (PAL) pathway in rice (Silverman and others 1995) and poplar (Ruuhola and Julkunen-Tiitto 2003), and the isochorismate (IC) pathway in *Arabidopsis* (Dempsey and others 2011) as the predominant pathways for SA synthesis. Thus, SA metabolism and its regulation in different species could possess both shared and divergent components and regulatory mechanisms. Further, heat shock proteins (HSPs) and heat stress transcription factors (HSFs) are differentially expressed under various stresses including diseases resulting in cellular changes (Hubert and others 2003; Thao and others 2007). HSP90, a type of HSP, has been implicated to play an important role in biotic stress responses in plants. HSP90 may associate with SGT1 and RAR1, the important signaling components of R gene-mediated defense responses of various forms in *Arabidopsis*, tobacco, and rice (Hubert and others 2003; Liu and others 2004; Thao and others 2007).

Sugars serve different physiological roles in plants and have also been shown to play an important role in plant defense. In plant defense, sugars act as signal molecules and supply energy for the initiation of defense responses, for example, the synthesis of pathogenesis-related (PR) proteins (Morkunas and others 2007; Bolton 2009; Lemonnier and others 2014). Role of sugars in plant immunity is termed as "Sweet priming" and "High-sugar resistance" (Birch and others 2009; Hofmann and others 2010). Upon pathogen attack, cell wall invertase genes and sugar (hexose and sucrose) transporter genes involved in internalization of sugars (hexose) have been shown to initiate defense responses (Tauzin and Giardina 2014; Sun and others 2014). For instance, in wheat, STP4 sugar transporters are activated in response to powdery mildew (*Blumeria graminis* DC.) (Sutton and others 2007) and in maize, *SUT1* a sucrose transporter gene expression was enhanced when challenged with *Colletotrichum graminicola* (Ces.) (Vargas and others 2012). Overexpression of the cell wall invertase gene *GRAIN INCOMPLETE FILLING 1 (GIF1)* enhanced resistance to *Xanthomonas oryzae* pv. *oryzae* (Xoo) and *Magnaporthe oryzae* (B. Cough) in rice transgenic plants (Sun and others 2014). Further, the constitutive activation of defense-related genes like PR genes, *NPR1* and *WRKY45*, by several

folds and also higher accumulation of glucose, fructose, and sucrose was observed in the *GIF1*-overexpressing transgenic plants than the wild-type plants (Sun and others 2014). A durable adult-plant leaf rust resistance gene in wheat, *Lr34 (= Yr18/Pm38)*, was shown to encode an ABC transporter (Krattinger and others 2009). *Lr34/Yr18/Pm38* also confers resistance to stripe rust and powdery mildew diseases in wheat. Generally, the ABC transporters utilize the energy released from ATP hydrolysis to transport various substrates across cellular membranes (Jasinski and others 2003; Rea 2007). Expression of *Lr34* in maize and sorghum resulted in enhanced resistance against rust and other fungal diseases in transgenic plants (Sucher and others 2016; Schnippenkoetter and others 2017). Moore and others (2015) demonstrated the function of *Lr67*, a hexose transporter gene providing multiple disease resistance in wheat. Biotrophic fungi divert assimilates for their growth through the creation of a fungal sink in the infected tissues. Modeling of fungal sink competitiveness has shown that leaf rust pathogen sporulation had a competitive priority over grain filling in wheat (Bancal and others 2012). The activity of sugar transporters involved in efflux of sugars is shown to be regulated by biotrophic pathogens in plants (Chen and others 2010; Li and others 2017). A class of efflux sugar transporters called Sugars Will Eventually be Exported Transporters (SWEETs) have been speculated to facilitate pathogen nutrition (Chen and others 2010; Eom and others 2015). In rice, five SWEET (*OsSWEET11–15*) genes have been shown to support *Xanthomonas oryzae* growth (Streubel and others 2013). Interestingly, two of the SWEET genes, *OsSWEET11* and *OsSWEET13*, have been identified as recessive blight resistance QTLs, *xa13* and *xa25*, respectively (Yang and others 2006; Liu and others 2011). Recently, overexpression of *IbSWEET10* in sweet potato was found to enhance resistance to *F. oxysporum*, whereas RNA interference (RNAi) lines showed enhanced susceptibility compared to the wild-type plants (Li and others 2017). These studies suggest that sugar and related metabolite transporters play key roles in plant defense or susceptibility. Thus, understanding molecular roles of sugar transporters in plant–pathogen interactions may provide new strategies to engineer robust resistance in plants.

Moreover, the SA and sugar-mediated defense pathways are expected to provide durable and broad-spectrum resistance as different R gene signals against biotrophs converge at SA signaling (Lu and others 2016) and the sugars act as signal molecules and energy source for defense responses (Rojas and others 2014). The functions of SAR signaling and sugar transporter genes or their orthologs in response to leaf rust infection in wheat are not known. Many alien *Lr* genes have been introgressed into the hexaploid wheat cultivars from its wild relatives to enhance the durability and efficacy of leaf rust resistance. *Lr24* located on the long arm of the 3D chromosome is one such gene derived from the wild relative *Agropyron elongatum* (Gupta and others 2006). *Lr24* is one of the most potential genes that confer resistance to all the known pathotypes of leaf rust in the Indian subcontinent and many other parts of the world (Long and others 1994; Tomar and Menon 1998; Yuan and others 2007a, b; Sharma and others 2010; Mishra and others 2014). In this study, transcriptional reprogramming of key SAR regulatory and sugar transporter genes and candidate orthologs in two wheat near-isogenic lines (NILs) differing in *Lr24* was studied during leaf rust pathogen infection to understand the possible role and mechanisms of these pathways in the early resistance response of wheat plants.

Materials and Methods

Database Search for Candidate Genes and Primer Designing

The key regulators of SAR-related genes and sugar transporter genes were selected based on review of the existing literature (Dempsey and others 2011; An and Mou 2011; Fu and Dong 2013; Moore and others 2015) and a sequence database search to study the time-course expression of two pathway genes during the compatible and incompatible interactions between wheat and the leaf rust pathogen (NCBI 2017; TAIR 2017). Sequences of these genes were downloaded from the NCBI database or by homology-based search for homologs in wheat. For finding wheat homologs of selected *Arabidopsis* genes, we used the BLAST tool of the Ensembl Plant database following the protocol given in the tutorial document available at the wheat training website (http://www.wheat-training.com/wp-content/uploads/TILLING/pdfs/Finding-the-wheat-homologues-of-genes-from-model-organisms.pdf). Primers were designed using the Primer Express Software v3.0.1 primer design tool and synthesized by Sigma-Aldrich® (Table 1).

Inoculation of Wheat Seedlings and Leaf Sample Collection

Two wheat near-isogenic lines (NILs), HS240 (susceptible to leaf rust) and HW2020 (BC_7NIL of HS240, Gupta and others 2006), carrying the *Lr24* rust resistance gene (resistant to leaf rust), and the leaf rust pathotype 77–5 (121R63–1 or THTTS) were used to study the compatible and incompatible interactions between wheat and leaf rust pathogen. Leaf rust pathotype 77–5 is the most predominant and devastating leaf rust race of the Indian subcontinent (Bhardwaj and others 2016; http://rusttracker.cimmyt.org/?page_id=11). The experiment was conducted in a randomized design. Wheat NILs were grown in a growth chamber under the

Table 1 Description of genes selected for studying the SAR and sugar-mediated responses during wheat–leaf rust pathogen interactions

Gene/transcript ID	*Arabidopsis* orthologs	Gene annotation	Primer	Sequence	Amplicon size (bp)
gil959515079	AT5G26340	Hexose transport protein (Lr67)	TaHTP_F	GCCTTCCTCTCCATG CTCTG	145
			TaHTP_R	TCCACACCTTGTCGG TCATC	
TRIAE_CS42_4AS_ TGACv1_307652_ AA1022380.1	AT5G26340	Sugar transport protein 13	TaSTP13A_F	GTCTTCGTGCTCTTCTTC CTC	100
			TaSTP13A_R	GTCCATGTACCTCTTCCA GAAC	
gil672798846	AT1G64280	Non-expressor of pathogenesis-related (PR) genes 1	TaNPR1_F	TTGGATGGTGACGTT CTTCG	142
			TaNPR1_R	GATGACCAAGGGCAA ATTCC	
TRIAE_CS42_5AL_ TGACv1_374053_ AA1188920	AT3G48090	Enhanced disease susceptibility 1	TaEDS1_F	TTCAAGCTTCAGCGG GAAGT	146
			TaEDS1_R	CCCAGGTTCACCCAC TCTTC	
TRIAE_CS42_2DS_ TGACv1_177360_ AA0574510	AT3G20600	Non-specific disease resistance 1	TaNDR1_F	GCTCACGCTCGTCCT CATC	106
			TaNDR1_R	GACGTTGGGGATGCT GAAGT	
TGACv1_ scaffold_434724_5DL	AT3G52430	Phytoalexin deficient 4	TaPAD4_F	TTCAAGCTTCAGCGG GAAGT	146
			TaPAD4_R	CCCAGGTTCACCCAC TCTTC	
TRIAE_CS42_6AS_ TGACv1_485760_ AA1551380	AT4G39030	Enhanced disease susceptibility 5	TaEDS5_F	TGAAAGATGCTTGGG GTCCT	107
			TaEDS5_R	GGCACCAGCAATTCC ATATC	
gil339765023	AT2G04030	Heat shock protein	TaHSP90_F	AAGCCGATCTGGATG AGGAA	151
			TaHSP90_R	GCACAAACAGGACAG CCTTG	
EF197821	AT4G11260	SA UDP-glucosyltransferase 1	TaSGT1-B_F	TCCCATAGCAGCATT GCATC	150
			TaSGT1-B_R	CCTGGCTAGCCTCCT CTGAA	
gil723219603	AT5G51700	Required for *Mla12*-mediated resistance	TaRAR1-D_F	GTGCCACAGGGAAGC ATACA	156
			TaRAR1-D_R	CGGAGCAAAAGAAAC CCTGA	
TGACv1_ scaffold_210895_3AS	AT2G37040	Phenylalanine ammonia-lyase	TaPAL_F	CGAGCAGGTCGAGGCA	134
			TaPAL_R	CACGCTGCGCGAGGCG	

controlled conditions of temperature (22–24 °C), relative humidity (80%), and light periods (14 h day with 5000 lx light; 10 h dark periods). Single spore-derived cultures of pathotype 77–5 were used as the inoculum. Fresh urediniospores were suspended in the mineral oil Soltrol (Chevron Phillips Chemical Company, US) to form 50 mg spores/ml suspension. A spore suspension of 30 µl was sprayed per plant uniformly on the leaves of 1-week-old seedlings. Mock controls, the seedlings sprayed with Soltrol oil without urediniospores, were also maintained. The seedlings were placed in a high humidity (> 90%) growth chamber after misting for 24 h under dark. Then, the seedlings were shifted to the normal growth conditions in the glass house (22–24 °C and 80% RH). The leaf samples were harvested at 0, 1, 3, 6, 12, 24, and 48 h post inoculation (hpi) from both inoculated and mock plants for RNA isolation. Harvested leaf samples were immediately frozen in liquid nitrogen and stored at − 80 °C until RNA isolation. Three independent

biological replicates for each of the inoculated and control plants of both the NILs were maintained (Taylor and others 2010). The leaf rust infection on the wheat NILs was confirmed after 15 days post inoculation (dpi). The experiment was repeated twice under similar conditions.

RNA Isolation and cDNA Preparation

For total RNA isolation, 100 mg leaf tissue was homogenized in a FastPrep®-24 tissuelyzer (MP Biomedicals, USA). Total RNA was extracted using the QIAGEN RNeasy Mini Kit (Qiagen, Germany) and on-column DNase I digestion was done with the RNase-Free DNase I following the protocol given in the RNeasy Mini Handbook. The integrity, yield, and purity of total RNA was determined using 1.4% formaldehyde gel electrophoresis (Rio 2015) followed by measuring of absorbance ratios $A_{260/280}$ and $A_{260/230}$ in the NanoDrop 2000c® UV–Vis Spectrophotometer (Thermo Scientific, USA). cDNA was prepared from 2 µg of total RNA using a High-Capacity cDNA Reverse Transcription Kit with Oligo (dT) primer (Applied Biosystems, USA). qPCR was carried out for all the cDNA samples to ensure that cDNA yield from each RT reaction was similar (Udvardi and others 2008).

Reverse Transcription-qPCR (RT-qPCR)

Amplification efficiencies of each primer pair used in the RT-qPCR experiment were calculated using five 10-fold dilutions of template in the RT-qPCR. The selection of stably expressed reference genes across the treatment groups is crucial for relative quantification or normalization in a real-time PCR study. In our earlier experiments, expression analyses using NormFinder and Best keeper software (Andersen and others 2004; Pfaffl and others 2004) showed *Ubiquitin* (*UBI*) to be stably expressed at different time points after inoculation of leaf rust among the often used reference genes *glycerol-6-phosphate dehydrogenase* (*G6PDH*), *elongation factor-1α* (*EF-1α*), and *18S ribosomal RNA* (*18SrRNA*). Gene expression stabilities were determined and *UBI* was found to be the most stably expressed reference gene. Hence, *UBI* (Forward primer: 5′ CCTTCACTTGGTGCTCCGTCT 3′; Reverse primer: 5′ AACGACCAGGACGACAGACACA 3′) was used for relative quantification of gene expressions. For the RT-qPCR analyses, a 20-µl reaction was set up using 1 µl of 1:10 diluted cDNA template, 10 pM of each primer, 10 µl FG-Power SYBR Green PCR Master Mix (Applied Biosystems, USA), and total volume was made up with sterile distilled water. Three technical replicates were set for each of the biological replicates at different hpi. RT-qPCR reactions were performed in Applied Biosystems' 7500 HT Fast Real-time PCR System following the program: initial activation step at 95 °C for 7 min, followed by 40 cycles of 10 s at 95 °C and 30 s at 60 °C. Finally, slow heating at a rate of 0.1 °C/s from 60 to 95 °C was included to obtain the melting curve for each primer pair. Instrument operation, data acquisition, and processing were performed using Sequence Detection System v 1.2.2 software (Applied Biosystems, USA). The melting program at the end of the cycling program followed by agarose gel electrophoresis of RT-qPCR amplicons ensured the specificity of amplification by each primer pair. Relative quantification of gene expressions was carried out using the comparative Ct (ΔΔCt) method (Schmittgen and Livak 2008). The comparative relative expression of individual genes at different hpi is represented in $\log_2$ fold changes and that among different genes is represented in $\log_2$ changes.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Tukey's post hoc test at $P \leq 0.05$ was performed using the XLSTAT software (2017) to determine the statistical significance of temporal expressions of selected candidate genes.

Results

Disease Symptoms in Compatible and Incompatible Interactions

Inoculated seedlings were observed for leaf rust disease development and scored for symptom development at fifteen days post inoculation (dpi). The susceptible NIL displayed infection type 4, whereas resistant NIL exhibited ;– and ; infection types on the basis of a scale consisting of infection types in the range 0, ;, 1, 2, X, 3, 4 (Roelfs 1984) (Fig. S1) suggesting the two NILs have contrasting leaf rust resistance phenotypes. The mock inoculated plants did not show any infection. Similar results were observed in the repeated inoculation experiments.

Candidate Genes, Amplification Specificity, and Efficiency of Primer Pairs

Mining of NCBI and Ensembl Plant databases for the regulators of SAR and sugar-related genes resulted in identification of the following genes: *TaNPR1* (gi|672798846), *TaSGT1* (EF197821), *TaHSP90* (gi|339765023), *TaRAR1* (gi|723219603), *TaHTP* (gi|942473027), *TaSTP13A* (*AT5G26340*) and candidate orthologs of *Arabidopsis AtNDR1* (*AT3G20600*), *AtEDS1* (*AT3G48090*), *AtPAD4* (*AT3G52430*), *AtPAL* (*AT2G37040*), and *AtEDS5* (*AT4G39030*) (Table 1). Melt curve analysis for each gene during the RT-qPCR showed a single sharp peak (Fig. S2) and agarose gel electrophoresis showed single amplicons

of the expected size in the RT-qPCR products, indicating the absence of non-specific PCR products. The amplification efficiencies of all the primer pairs used in the RT-qPCR experiments ranged between 97 and 105%, suggesting optimal reaction conditions for exponential amplification of target gene transcripts.

Differential Gene Expression Patterns During Compatible and Incompatible Interactions

All the RNA samples displayed two prominent rRNA bands, 28 and 18 s with 2:1 intensity on a formaldehyde agarose gel suggesting intact RNA. Further, $A_{260/280}$ readings were > 1.8 and $A_{260/230}$ readings were > 2.0 indicating lack of protein and phenol contamination in the RNA, respectively. Consistency in both purity and integrity across all RNA samples minimized variability between biological replicates. Further, qPCR of cDNA samples showed that variation in the mean range of threshold cycle (Ct) values was within ± 1 for the reference gene *UBI* across all samples indicating a similar cDNA yield from each RT reaction.

The relative expression profiles of SAR-related genes (*TaEDS1, TaNDR1, TaPAD4, TaSGT1, TaHSP90, TaRAR1, TaEDS5, TaNPR1*, and *TaPAL*) and sugar transporter genes (*TaHTP* and *TaSTP13A*) at 0, 1, 3, 6, 12, 24, and 48 hpi were determined to understand the transcriptional changes in these genes during compatible and incompatible interactions between leaf rust pathogen and wheat. The transcript profiles of all the candidate genes indicated differential expression patterns during compatible and incompatible interactions at different time points after inoculation. The results of RT-qPCR based time-course expression of the candidate genes are presented in the following sections.

Expression of SAR-Related Defence Genes

The analyses of expression data of SA-related genes showed two distinct patterns of expression in the two contrasting interactions between wheat and leaf rust. Genes acting upstream of SA in the SA pathway (*TaEDS1, TaNDR1, TaPAD4, TaSGT1, TaHSP90, TaRAR1*, and *TaEDS5*) showed significantly higher regulations at a later phase of leaf rust infection (48 hpi) in the compatible interaction compared to the incompatible interaction (Fig. 1A, B). Genes involved in SA biosynthesis (*TaPAL*) and acting downstream of SA (*NPR1*) in the SA pathway showed significant upregulations between 6 and 24 hpi (Fig. 1B). *TaEDS1* expression levels varied from 2.5- to 13-fold in the compatible interaction compared to 1–7-fold in the incompatible interaction. *TaEDS1* was significantly upregulated in susceptible NIL at 0 and 48 hpi and in general, *TaEDS1* expression was greater in the susceptible NIL than in the resistant NIL at most of the time intervals after inoculation (Fig. 1A). The expression levels of *TaPAD4* in the susceptible NIL remained unchanged up to 3 hpi and after that the expression gradually increased to significantly high levels at 24 and 48 hpi. However, in resistant NIL, *TaPAD4* expression oscillated, that is, significantly high levels of expression were observed initially, which decreased to very low levels at 3 hpi and significantly increased at 6 hpi and again decreased after 12 hpi (Fig. 1A). Similarly, the expression of *TaNDR1* was induced at low levels in either of the NILs till 24 hpi and suddenly its expression was significantly upregulated by 40-fold in the susceptible NIL at 48 hpi, whereas in the resistant NIL its expression was consistently low (Fig. 1A).

The levels of *TaRAR1* transcripts were low at all the time intervals post inoculation in both the NILs except at 48 hpi in the susceptible NIL, where the transcripts accumulation was enhanced significantly by 36-fold. The expression pattern of *TaSGT1* in both the NILs was identical to that of *TaRAR1* except that *TaSGT1* was induced up to 16-fold in the susceptible NIL (Fig. 1A). The *TaHSP90* gene was induced to a significantly high level at 24 and 48 hpi in the susceptible NIL. However, *TaHSP90* inductions were low and without much variation in both the NILs up to 12 hpi and it continued to be low in the resistant NIL at later stages (Fig. 1A). Induction of *TaEDS5* expression ranged between 4- and 60-fold in the susceptible NIL, whereas in the resistant NIL expression varied between 1- and 31-fold at different time intervals. Significantly very high levels of *TaEDS5* were recorded at 48 hpi in the susceptible NIL compared to the resistant NIL (Fig. 1B).

Expression levels of *TaNPR1* were lower than tenfold and without significant differences in either of the NILs except at 24 hpi in resistant NIL, in which the expression was enhanced significantly up to 29-fold and there was a drop in the expression of *TaNPR1* at 48 hpi in the susceptible NIL (Fig. 1B). Phenylalanine ammonia-lyase (PAL) is a key phenylpropanoid pathway enzyme involved in biosynthesis of phenolic compounds including SA. *PAL* expression is potentiated by SA (Zhu and others 1996). Expression of *TaPAL* induction was more than 80-fold at 6 and 12 hpi followed by 37-fold at 48 hpi and 15-fold at 24 hpi in resistant NIL, whereas in the susceptible NIL its expression levels were significantly low at most of the time points (Fig. 1B). The comparison of relative expression of SA-related genes at different time intervals after inoculation indicated that *TaPAL* and *TaNPR1* showed the highest expression at the mid phase of early leaf rust infection in the resistant NIL, whereas the *TaEDS1, TaEDS5, TaHSP90, TaNDR1, TaPAD4, TaRAR1,* and *TaSGT1* genes showed higher upregulation at a later phase of leaf rust infection in the susceptible NIL (Fig. 1A, B). Comparison of normalized expression data of these SA-related genes in

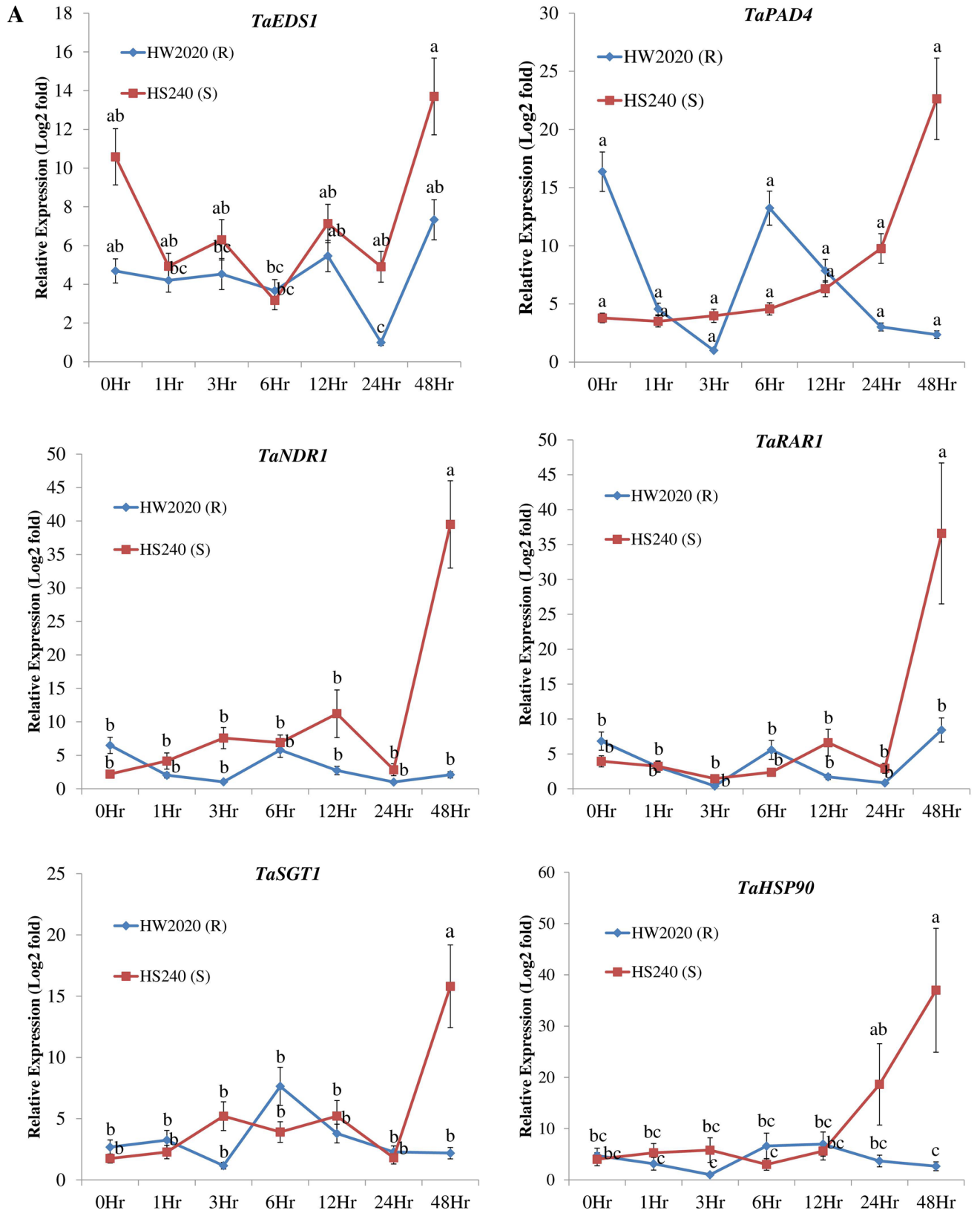

Fig. 1 Relative expression levels of **A** SA pathway-related genes (*TaEDS1, TaPAD4, TaNDR1, TaSGT1, TaRAR1, TaHSP90*, and *TaEDS5*) and **B** sugar transporter genes (*TaHTP* and *TaSTP13A*) during compatible and incompatible interactions of wheat and leaf rust pathogen

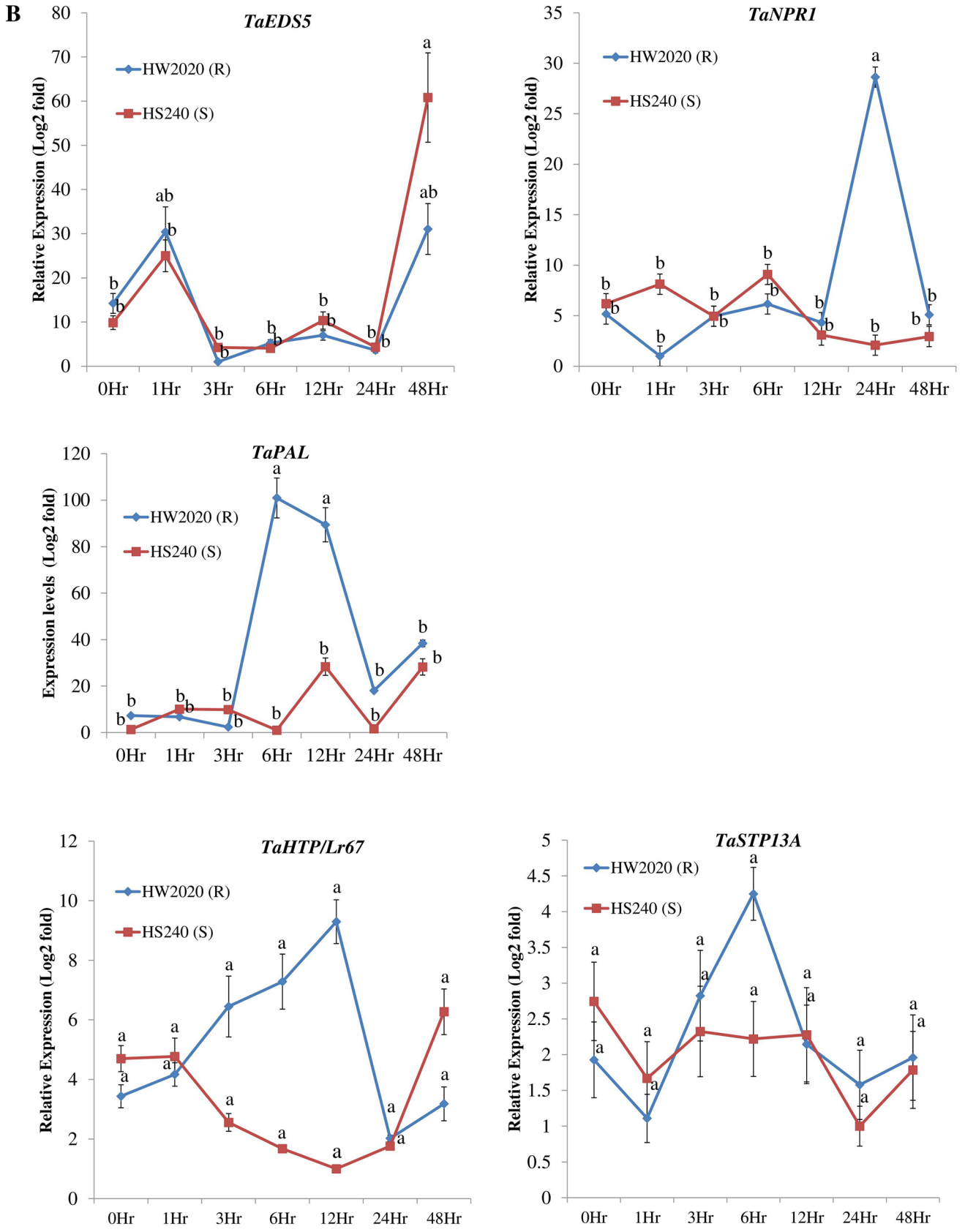

Fig. 1 (continued)

compatible and incompatible interactions suggested a very high differential expression at 48 hpi due to significantly high upregulation of genes acting upstream of SA in the compatible interaction (Fig. S3a).

Expression of Sugar Transporter Genes

In the incompatible interaction, the expression of *TaHTP (Lr67)* increased gradually from threefold at 0 hpi to ninefold at 6 hpi and then gradually decreased after 12 hpi. High expressions were observed between 3 and 12 hpi (Fig. 1B). In contrast, an inverse expression pattern was observed in the compatible interaction. Expression levels of *TaSTP13A* varied from one to fourfold in the incompatible and 1- to 2.5-fold in the compatible interactions. High expression levels were observed at 6 hpi in the incompatible interaction (Fig. 1B). However, no significant differences in expression were observed between the two NILs at all time intervals. Further, the comparison of normalized expression data of two sugar transporter genes between the two NILs showed relatively higher expressions at initial stages (0 and 1 hpi) and later the expression repressed significantly from 3 to 24 hpi in the compatible interaction compared to the incompatible interaction (Fig. S3b).

Discussion

A number of resistance pathways (SAR, ISR, and plant defensin) are induced during plant defense against pathogens. Salicylic acid (SA) is an important phytohormone implicated to play a critical role in both local and systemic defense responses. Therefore, the SA signaling mechanism provides broad-spectrum and long-lasting resistance to pathogen infections throughout the plant (Gao and others 2015). Besides defense-related pathways, primary metabolism pathways like sugar metabolism and sugars have been shown to play an important role in plant innate immunity as energy sources and signaling molecules (Kano and others 2011; Aliferis and others 2014; Cabello and others 2014; Zhao and others 2015). Further, although the components of induced resistance could be conserved among plant species, their spatial and temporal regulation vary with pathosystems. Understanding of the molecular components and their regulation in cereal crops including wheat and rice is limited. The results of the present study indicate a stage-specific transcriptional reprogramming of SA-related and sugar transportation genes during compatible and incompatible interactions between leaf rust and wheat and thus, gaining an insight of SA- and sugar-mediated resistance mechanisms in wheat during leaf rust infections.

Transcriptional Changes in SA-Related Genes During Wheat–*P. triticina* Interactions

R gene-mediated SAR is an effective strategy of resistance in plants against a broad spectrum of biotrophic pathogens mediated by the signaling molecule, SA. The major SAR signaling components include the SA molecule, genes acting upstream of SA molecules, and the SA responsive genes acting downstream of the SA. SAR has been extensively studied in the dicotyledonous species *Arabidopsis* and tobacco. However, investigations of SAR in monocot species including rice and wheat are limited. Recent availability of wheat genome and transcriptome sequence data has facilitated the search for orthologs of *Arabidopsis* SAR components and investigation into their role and regulations in plant–pathogen interactions to gain an insight into the molecular mechanisms involved in SA-mediated resistance in wheat. Further, modulation of SA-related and signaling network genes might confer broad-spectrum resistance against biotrophic fungi like wheat rusts because they act downstream of R genes, where signals perceived by different R genes converge (Tanaka and others 2015; Lu and others 2016).

SA biosynthesis occurs via two distinct pathways, the isochorismate (IC) pathway mediated by IC synthase (ICS) and the phenylalanine ammonia-lyase (PAL) pathway. In *Arabidopsis*, the ICS pathway is predominant, whereas the PAL pathway is predominant in monocots like rice (Silverman and others 1995; Ruuhola and Julkunen-Tiitto 2003). Results of the current study showed significant upregulation of *TaPAL1* in resistant NIL between 6 and 48 hpi compared to non-significant changes in susceptible NIL and as a consequence SA production, suggesting the role of the SA biosynthetic pathway in wheat defense against *P. triticina*. These results are in corroboration with other studies on plant–biotic stress interactions in rice (Duan and others 2014; Kumari and others 2016) and wheat (Sorahinobar and others 2016) suggesting *TaPAL* as an important component of SA-mediated resistance in wheat against leaf rust. In *Arabidopsis*, the genes acting upstream of SA: *EDS1, NDR1, PAD4, RAR1, SGT1*, and *HSP90* are considered key mediators of the SA pathway for resistance against biotrophs (Kawamura and others 2009; Dempsey and others 2011; Bao and others 2014). The expression of orthologs of these genes in wheat during leaf rust infection showed a significantly high upregulation in susceptible NIL at 48 hpi, the stage reported to be involved in rapid formation of haustorial mother cells (hmc) and secondary hyphal growth in susceptible NIL compared to the resistant NIL (Wang and others 2013; Serfling and others 2016). Expression of some of these genes is similar to other studies in monocot host–pathogen interactions (Qiu and others 2007; Kumari and others 2016). However, these are not consistent with the observations made on the role of these genes in *Arabidopsis*

(Clarke and others 2001; Makandar and others 2015), soybean (Youssef and others 2013; Wang and others 2014), and tobacco (Schornack and others 2004). Thus, results of the temporal differential expression of these SA upstream acting genes in the two wheat NILs used in this study suggest the role of these genes in SA-mediated resistance but regulated by a molecular mechanism different from those in dicotyledonae host–pathosystems. *EDS5* is implicated to a play role in SA accumulation possibly by controlling transport of specific molecules across the plastid membrane (Ishihara and others 2008). Evidence suggests that *EDS1, PAD4*, and *NDR1* are at least partially required for HR-induced *EDS5* expression and therefore, *EDS5* seems to function downstream of these regulators (Nawrath and others 2002). In the current study, significantly high levels of *TaEDS5* transcripts were observed at 48 hpi in the susceptible NIL compared to the resistant NIL. Further, comparison of *TaEDS5* expression in the two interactions suggest that *TaEDS5* is co-expressed or has similar temporal expression patterns with *TaEDS1, TaPAD4*, and *TaNDR1* in either of the NILs suggesting a cooperative or coordinated action during early leaf rust infection in wheat (Fig. 1A). Further, expression of *EDS5* seems to be negatively regulated by NPR1 (a SA downstream acting SAR regulator) as suggested by elevated *EDS5* transcripts in *npr1* plants (Nawrath and others 2002; Ishihara and others 2008). So our results are consistent with the observations on host–pathogen interaction studies by Ishihara and others (2008) and Nawrath and others (2002).

Expression of all the SA mediators acting upstream of SA accumulation observed in this study is in contrast to the expression of *NPR1*, a downstream regulator of SA accumulation. We found significantly high transcript levels of *TaNPR1* in the resistant NIL at 24 hpi in contrast to highly repressed expression in the susceptible NIL (Fig. 1B). Results of the *TaNPR1* expression pattern in the two contrasting wheat–*P. triticina* interactions at different time points after the inoculation suggest that a positive role in SA-mediated resistance is consistent with the studies from both the types of angiosperms during various biotic stresses (Liu and others 2002; Lin and others 2004; Yuan and others 2007a, b; Duan and others 2014; Kumari and others 2016). These suggest NPR1 as the key regulator of SA-mediated resistance in plants. Through the ankyrin motif, NPR1 mediates a wide range of protein–protein interactions and as a consequence modulates multiple pathways which may be governed by pathogen-specific signals to drive activation of the required set of defense genes (Shah 2003). In this study, the *Lr24*-mediated leaf rust recognition signals could be activating *TaNPR1* transcription to drive the expression of *PR* genes, the executers of SAR defense. Some of the pathogen effectors are known to target NPR1 to compromise SA signaling through interference with NPR1 turnover, which would impact the *PR* gene induction (Schellenberg and others 2010; Ustun and others 2013). Similarly, in this study *TaNPR1* expression was not induced in the leaf rust-infected susceptible NIL, suggesting that a pathogen effector or pathogenicity factor might be having a repressional impact on *TaNPR1* transcription, which in turn fails to activate *PR* genes and as a result the failed defense against leaf rust. Further, NPR1 is known to regulate SA accumulation in a negative feedback loop to avoid the uncontrolled accumulation of SA that may compromise other defense or growth pathways as suggested by compromised defense and dwarfism in the *npr1* mutants (Kunkel and Brooks 2002). Hence, it is important to have feedback regulation of SA synthesis and signaling for the fine-tuning of plant defense signaling against biotrophs like leaf rust fungi.

Transcriptional Changes in Sugar Transporter Genes

Expression of a cytosolic yeast-derived invertase in transgenic tobacco plants with increased levels of sugars without SAR responses suggests that hexose sensing in the secretory pathway is required for mediating defense responses in plants (Herbers and others 1996). Further, in tomato plants, the hexose content correlated with the resistance and the expression levels of hexose sugar transporter, *LeHT1*; Sade and others 2013 propose the role of *LeHT1* in regulating sugar content of tomato during host–pathogen interactions. In this study, expression levels of two orthologs of *AtSTP13: TaSTP13* and *TaHTP/Lr67* encoding two hexose transporter proteins showed upregulation in incompatible interactions. *TaSTP13* showed high expression, although not significantly, in the resistant NIL at 6 hpi, that is, mid stage of the early infection period. Further, interestingly, *TaHTP/Lr67* transcript levels were contrasting at all stages of early infection in the compatible interaction (Fig. 1B). These results are consistent with other studies on sugar transporters in several plants against different biotic stresses (Sade and others 2013; Lemonnier and others 2014). Moore and others (2015) concluded that *Lr67* encoded HTP, whose activity is regulated by amino acid sequence differences at the critical regions, and the differences in these amino acids in HTP proteins suggest the existence of resistant and susceptible alleles of *Lr67* which control broad-spectrum rust defense. In this study, results of expression analysis indicated that *TaHTP* (*Lr67)* is an effective regulator of sugar-mediated leaf rust resistance in wheat compared to *TaHTP13A*. Further, the upregulation of *TaHTP* in the incompatible interaction could be triggered directly by *Lr24* or indirectly due to the need for meeting the energy needs for expression of *PR* genes induced by *Lr24* gene.

Based on the results of stage-wise transcription reprogramming of SA-related and sugar transporter genes in the two contrasting NILs, we propose a model for their

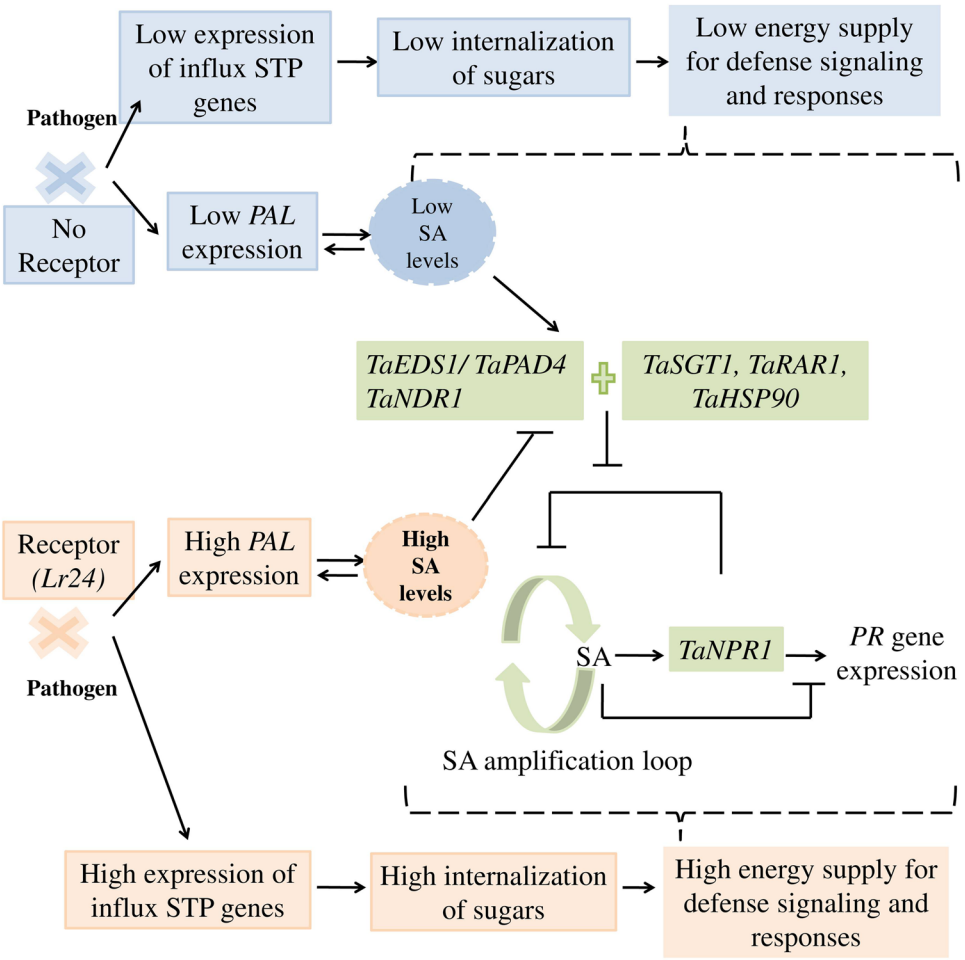

Fig. 2 Proposed model for SA and sugar signaling-mediated resistance in wheat against leaf rust (*P. triticina*)

mechanism of action in the early response of wheat plants during leaf rust infection.

Model for SA and Sugar-Mediated Resistance in Wheat Against Leaf Rust (*P. triticina*)

Salicylic acid (SA) levels play a central role in the establishment of local resistance as well as systemic resistance (Shah 2003; Tsuda and others 2008). In the susceptible NIL, low *TaPAL* (a key SA biosynthesis pathway enzyme) expression possibly due to repression activity of leaf rust factors might result in low SA accumulation. The low SA levels could have a positive feedback to activate transcription of upstream regulators of SA metabolism: *TaEDS1, TaNDR1, TaPAD4, TaSGT1, TaRAR1, TaHSP90*, and *TaEDS5*. In contrast, in the resistant NIL, a very high *TaPAL* expression induced by *Lr24* and leaf rust pathogen interactions could result in higher SA accumulation. The high levels of SA are expected to have a negative feedback on the activity of upstream regulators of SA metabolism and, therefore, repressed expression of *TaEDS1, TaNDR1, TaPAD4, TaSGT1, TaRAR1, TaHSP90*, and *TaEDS5*. Further, uncontrolled synthesis of SA may compromise other defense or growth pathways that are inhibited by SA, therefore, turnover of SA is regulated by a SA amplification loop. *TaNPR1* is a key downstream regulator of SA signaling which has a negative feedback on SA synthesis to avoid the effects of uncontrolled accumulation of SA. High expression of *TaNPR1* in the resistant NIL might result in a faster and effective PR protein-mediated defense. At the same time, *TaNPR1* might have inhibitory effect on SA biosynthesis possibly through transcription suppression of SA upstream regulators. On the other hand, low expression of *TaNPR1* in the susceptible NIL could be due to the leaf rust-mediated repression or repression by upstream regulators of SA metabolism, to inhibit the repressing activity of NPR1 on the SA amplification loop. Thus, transcriptional reprogramming of genes between the two NILs in this study suggests that expression levels of NPR1, a downstream regulator of SA signaling, might regulate SA levels which in turn could modulate the transcription and activity of upstream acting SA regulators (Fig. 2).

Further, the results of hexose transporter genes, *TaHTP* and *TaSTP13A*, expression in this study are in accordance with the results of *STP13* expression in *Arabidopsis–Botrytis*

fungus interaction (Lemonnier and others 2014) and the model of Berger and others (2007), reporting changes in sugar metabolism during pathogen infection. Accordingly, when the leaf rust pathogen attacks a wheat plant, it initiates rapid changes, resulting in the reduction of the photosynthesis rate. It is followed by an increase in invertase expression and release of hexose which may either activate a defense response or may promote pathogen multiplication, if failed to activate the plant defense. The upregulation of *HTP* genes in infected resistant plants may result in internalization of larger amounts of hexose into the cells. The larger ratio of mono to disaccharides is implicated to induce the plant defense (Sade and others 2013). The enhanced amounts of internal hexose may activate phytohormone (like SA)-mediated responses like SAR or may act as energy source for defense responses (Swarbrick and others 2006). In susceptible plants, the hexose transporter expression levels may not be sufficient and hence, internalization of hexoses into the cell may not be rapid and sufficient to incite defense signaling or to meet the energy needs of defense responses and thereby results in host susceptibility (Bolouri Moghaddam and Van den Ende 2012) (Fig. 2).

In summary, the quantification of expressions of the key genes involved in these two important defense pathways suggests a stage-specific transcriptional reprogramming during the two contrasting types of wheat–leaf rust pathogen interactions. Further, considering the potential role of these two pathways in broad-spectrum and durable resistance, manipulations of key regulators of these pathways could be a novel strategy for engineering broad-spectrum and durable leaf rust resistance in wheat. Results of this study provide a probable model and clue to key regulators of the two defense pathways expected to be involved in durable and broad-spectrum resistance, and thereby their prospective application in wheat rust resistance breeding.

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References

Aliferis KA, Faubert D, Jabaji S (2014) A metabolic profiling strategy for the dissection of plant defense against fungal pathogens. PLoS ONE 9:e111930

An C, Mou Z (2011) Salicylic acid and its function in plant immunity. J Integrat Plant Biol 53:412–428

Andersen CL, Ledet-Jensen J, Orntoft T (2004) Normalization of real-time quantitative RT-PCR data: a model based variance estimation approach to identify genes suited for normalization-applied to bladder- and colon-cancer data-sets. Cancer Res 64:5245–5250

Bancal MO, Hansart A, Sache I, Bancal P (2012) Modeling fungal sink competitiveness with grains for assimilates in wheat infected by a biotrophic pathogen. Ann Bot 110:113–123

Bao F, Huang X, Zhu C, Zhang X, Li X, Yang S (2014) *Arabidopsis* HSP90 protein modulates RPP4-mediated temperature-dependent cell death and defense responses. New Phytol 202:1320–1334

Berger S, Sinha AK, Roitsch T (2007) Plant physiology meets phytopathology: plant primary metabolism and plant–pathogen interactions. J Exp Bot 58:4019–4026

Bhardwaj SC, Prashar M, Kumar S, Gangwar OP, Gupta N, Prasad P, Khan H (2016) Patterns of physiologic diversity of *Puccinia triticina* on wheat in Indian subcontinent during 2008–2013. Indian J Agric Sci 86:55–64

Birch ANE, Shep herd T, Hancock R, Goszcz K (2009) Understanding sugar sensing in induced plant defences and stress tolerance. In: Proceedings of the 25th meeting of the international society of chemical ecology, 23–27 August 2009, Neuchatel, Switzerland, p 230

Bolouri Moghaddam MR, Van den Ende W (2012) Sugars and plant innate immunity. J Exp Bot 63:3989–3998

Bolton MD (2009) Primary metabolism and plant defense-fuel for the fire. Mol Plant Microbe Interact 22:487–497

Bolton MD, Kolmer JA, Garvin DF (2008) Wheat leaf rust caused by *Puccinia triticina*. Mol Plant Pathol 9:563–575

Cabello S, Lorenz C, Crespo S, Cabrera J, Ludwig R, Escobar C, Hofmann J (2014) Altered sucrose synthase and invertase expression affects the local and systemic sugar metabolism of nematode-infected *Arabidopsis thaliana* plants. J Exp Bot 65:201–212

Cao H, Glazebrook J, Clarke JD, Volko S, Dong X (1997) The *Arabidopsis* NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88:57–63

Chen H, Lai Z, Shi J, Xiao Y, Chen Z, Xu X (2010) Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. BMC Plant Biol 10:281

Clarke JD, Aarts N, Feys BJ, Dong X, Parker JE (2001) Constitutive disease resistance requires EDS1 in the *Arabidopsis* mutants *cpr1* and *cpr6* and is partially *EDS1*-dependent in *cpr5*. Plant J 26:409–420

Conrath U (2006) Systemic acquired resistance. Plant Signal Behav 1(4):179–184

Curtis TY, Halford NG (2014) Food security: the challenge of increasing wheat yield and the importance of not compromising food safety. Ann Appl Biol 164:354–372

Dempsey DMA, Vlot AC, Wildermuth MC, Klessig DF (2011) Salicylic acid biosynthesis and metabolism. Arabidopsis Book 9:e0156

Dodds PN, Rathjen JP (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539–548

Duan C, Yu J, Bai J, Zhu Z, Wang X (2014) Induced defense responses in rice plants against small brown plant hopper infestation. Crop J 2:55–62

Dubin H, Brennan JP (2009) Combating stem and leaf rust of wheat: historical perspective, impacts, and lessons learned. International Food Policy Research Institute. IFPRI Discussion Paper No. 00910. 2020 Vision Initiative. http://www.ifpri.org/millionsfed

Eom JS, Chen LQ, Sosso D, Julius BT, Lin IW, Qu XQ, Frommer WB (2015) SWEETs, transporters for intracellular and intercellular sugar translocation. Curr Opin Plant Biol 25:53–62

Fu ZQ, Dong X (2013) Systemic acquired resistance: turning local infection into global defense. Annu Rev Plant Biol 64:839–863

Gao QM, Zhu S, Kachroo P, Kachroo A (2015) Signal regulators of systemic acquired resistance. Front Plant Sci 6:228

Gupta SK, Charpe A, Koul S, Haque QMR, Prabhu KV (2006) Development and validation of SCAR markers co-segregating with an

Agropyron elongatum derived leaf rust resistance gene *Lr24* in wheat. Euphytica 150:233–240
Herbers K, Meuwly P, Frommer WB, Metraux JP, Sonnewald U (1996) Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. Plant Cell 8:793–803
Hofmann J, El Ashry A, Anwar S, Erban A, Kopka J, Grundler F (2010) Metabolic profiling reveals local and systemic responses of host plants to nematode parasitism. Plant J 62:1058–1071
Hubert DA, Tornero P, Belkhadir Y, Krishna P, Takahashi A, Shirasu K, Dangl JL (2003) Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. EMBO J 22:5679–5689
Huerta-Espino J, Singh R, German S, McCallum B, Park R, Chen W, Bhardwaj S, Goyeau H (2011) Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179:143–160
Ishihara T, Sekine KT, Hase S, Kanayama Y, Seo S, Ohashi Y, Kusano T, Shibata D, Shah J, Takahashi H (2008) Overexpression of the *Arabidopsis thaliana EDS5* gene enhances resistance to viruses. Plant Biol 10:451–461
Jasinski M, Duco E, Martinoia E, Boutry M (2003) The ATP-binding cassette transporters: structure, function, and gene family comparison between rice and *Arabidopsis*. Plant Physiol 131:1169–1177
Kano A, Hosotani K, Gomi K et al (2011) D-Psicose induces upregulation of defence-related genes and resistance in rice against bacterial blight. J Plant Physiol 168:1852–1857
Kawamura Y, Takenaka S, Hase S, Kubota M, Ichinose Y, Kanayama Y et al (2009) Enhanced defense responses in *Arabidopsis* induced by the cell wall protein fractions from *Pythium oligandrum* require *SGT1, RAR1, NPR1* and *JAR1*. Plant Cell Physiol 50:924–934
Khan M, Bukhari A, Dar Z, Rizvi S (2013) Status and strategies in breeding for rust resistance in wheat. Agric Sci 4:292–301
Koch JR, Creelman RA, Eshita SM, Seskar M, Mullet JE, Davis KR (2000) Ozone sensitivity in hybrid poplar correlates with insensitivity to both salicylic acid and jasmonic acid. The role of programmed cell death in lesion formation. Plant Physiol 123:487–496
Kogel KH, Langen G (2005) Induced disease resistance and gene expression in cereals. Cell Microbiol 7:1555–1564
Kolmer JA (2005) Tracking wheat rust on a continental scale. Curr Opin Plant Biol 8:441–449
Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H et al (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363
Kumari C, Dutta TK, Banakar P, Rao U (2016) Comparing the defence-related gene expression changes upon root-knot nematode attack in susceptible versus resistant cultivars of rice. Sci Rep 6:22846
Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5:325–331
Lemonnier P, Gaillard C, Veillet F, Verbeke J, Lemoine R, Coutos-Thévenot P et al (2014) Expression of *Arabidopsis* sugar transport protein STP13 differentially affects glucose transport activity and basal resistance to *Botrytis cinerea*. Plant Mol Biol 85:473–484
Li Y, Wang Y, Zhang H, Zhang Q, Zhai H, Liu Q, He S (2017) The plasma membrane-localized sucrose transporter *IbSWEET10* contributes to the resistance of sweet potato to *Fusarium oxysporum*. Front Plant Sci 8:197
Lin WC, Lu CF, Wu JW, Cheng ML, Lin YM, Yang NS, Black L, Green SK, Wang JF, Cheng CP (2004) Transgenic tomato plants expressing the *Arabidopsis* NPR1gene display enhanced resistance to a spectrum of fungal and bacterial diseases. Transgenic Res 13:567–581
Liu Y, Schiff M, Dinesh-Kumar SP (2002) Virus-induced gene silencing in tomato. Plant J 31:777–786
Liu Y, Burch-Smith T, Schiff M, Feng S, Dinesh-Kumar SP (2004) Molecular chaperone hsp90 associates with resistance protein n and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. J Biol Chem 279:2101–2108
Liu Q, Yuan M, Zhou Y, Li X, Xiao J, Wang S (2011) A paralog of the MtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. Plant Cell Environ. https://doi.org/10.1111/j.1365-3040.2011.02391.x
Long DL, Roelfs AP, Leonard KJ (1994) Virulence and diversity of *Puccinia recondite* f. sp. *tritici* in United States in 1992. Plant Dis 78:901–906
Lu H, Greenberg JT, Holuigue L (2016) Editorial: salicylic acid signaling networks. Front Plant Sci 7:238
Makandar R, Nalam VJ, Chowdhury Z, Sarowar S, Klossner G, Lee H, Burdan D, Trick HN, Gobbato E, Parker JE et al (2015) The combined action of ENHANCED DISEASE SUSCEPTIBILITY1, PHYTOALEXIN DEFICIENT4, and SENESCENCE-ASSOCIATED101 promotes salicylic acid-mediated defenses to limit *Fusarium graminearum* infection in *Arabidopsis thaliana*. Mol Plant Microbe Interact 28:943–953
McCallum BD, Hiebert CW, Cloutier S, Bakkeren G, Rosa SB, Humphreys DG, Saville BJ (2016) A review of wheat leaf rust research and the development of resistant cultivars in Canada. Can J Plant Pathol 38:1–18
Mishra AN, Prakasha TL, Kaushal K, Dubey VG (2014) Validation of Lr24 in some released bread wheat varieties and its implications in leaf rust resistance breeding and deployment in central India. Indian Phytopathol 67:102–103
Moore JW, Herrera-Foessel S, Lan C, Schnippenkoetter W, Ayliffe M, Huerta-Espino J, Kong X (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. Nat Genet 47:1494–1498
Morkunas I, Kozłowska M, Ratajczak L, Marczak M (2007) Role of sucrose in the development of *Fusarium* wilt in lupine embryo axes. Physiol Mol Plant Pathol 70:25–37
National Center for Biotechnology Information (NCBI) (2017) [Internet] Bethesda (MD): National Library of Medicine (US) National Center for Biotechnology Information, 1988. https://www.ncbi.nlm.nih.gov/. Accessed 04 Oct 2017
Nawrath C, Heck S, Parinthawong N, Métraux J (2002) EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family. Plant Cell 14:275–286
Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP (2004) Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper-Excel-based tool using pair-wise correlations. Biotechnol Lett 26:509–515
Pieterse CMJ, van der Does D, Zamioudis C, Leon-Reyes A, van Wees SCM (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521
Qiu D, Xiao J, Ding X, Xiong M, Cai M, Cao Y, Li X, Xu C, Wang S (2007) OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol Plant Microbe Interact 20:492–499
Rea PA (2007) Plant ATP-binding cassette transporters. Annu Rev Plant Biol 58:347–375
Rio DC (2015) Denaturation and electrophoresis of RNA with formaldehyde. Cold Spring Harb Protoc. https://doi.org/10.1101/pdb.prot080994
Roelfs AP (1984) Race specificity and methods of study. In: Bushnell WR, Roelfs AP (eds) The cereal rusts vol. I. Origins, specificity, structure, and physiology. Acadamic Press, Orlando, pp 132–164
Rojas CM, Senthil-Kumar M, Tzin V, Mysore K (2014) Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Front Plant Sci 5:17

Ruuhola T, Julkunen-Tiitto R (2003) Trade-off between synthesis of salicylates and growth of micropropagated *Salix pentandra*. J Chem Ecol 29:1565–1588

Sade D, Brotman Y, Eybishtz A, Cuadros-Inostroza A, Fernie AR, Willmitzer L, Czosnek H (2013) Involvement of the hexose transporter gene *LeHT1* and of sugars in resistance of tomato to *Tomato yellow leaf curl virus*. Mol Plant 6:1707–1710

Schellenberg B, Ramel C, Dudler R (2010) *Pseudomonas syringae* virulence factor syringolin A counteracts stomatal immunity by proteasome inhibition. Mol Plant Microbe Interact 23:1287–1293

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative Ct method. Nat Protoc 3:1101–1108

Schnippenkoetter W, Lo C, Liu G, Dibley K, Chan WL, White J, Godwin I (2017) The wheat *Lr34* multipathogen resistance gene confers resistance to anthracnose and rust in sorghum. Plant Biotechnol J. https://doi.org/10.1111/pbi.12723

Schornack S, Ballvora A, Gurlebeck D, Peart J, Baulcombe D, Ganal M, Baker B, Bonas U, Lahaye T (2004) The tomato resistance protein *Bs4* is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of *AvrBs4* and overexpressed *AvrBs3*. Plant J 37:46–60

Serfling A, Templer SE, Winter P, Ordon F (2016) Microscopic and molecular characterization of the prehaustorial resistance against wheat leaf rust (*Puccinia triticina*) in Einkorn (*Triticum monococcum*). Front Plant Sci 7:1668

Shah J (2003) The salicylic acid loop in plant defence. Curr Opin Plant Biol 6:365–371

Sharma AK, Singh DP, Singh AK, Saharan MS, Jat MC, Babu KS, Singh SS (2010) Progress report of all India coordinated wheat and barley improvement project 2009–10, vol. III, crop protection. Directorate of Wheat Research, Karnal, p 247

Silverman P, Seskar M, Kanter D, Schweizer P, Métraux JP, Raskin I (1995) Salicylic acid in rice: biosynthesis, conjugation and possible role. Plant Physiol 108:633–639

Singh RP, Singh PK, Rutkoski J, Hodson DP, He X, Jørgensen LN, Huerta-Espino J (2016) Disease impact on wheat yield potential and prospects of genetic control. Annu Rev Phytopathol 54:303–322

Sorahinobar M, Niknam V, Ebrahimzadeh H et al (2016) Central role of salicylic acid in resistance of wheat against *Fusarium graminearum*. J Plant Growth Regul 35:477

Stalman M, Koskamp AM, Luderer R, Vernooy JHJ, Wind JC, Wullems GJ, Croes AF (2003) Regulation of anthraquinone biosynthesis in cell cultures of *Morinda citrifolia*. J Plant Physiol 160:607–614

Streubel J, Pesce C, Hutin M, Koebnik R, Boch J, Szurek B (2013) Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. New Phytol 200:808–819

Sucher J, Boni R, Yang P, Rogowsky P, Buchner H, Kastner C, Kumlenh J, Krattinger SG, Keller B (2016) The durable wheat disease resistance gene Lr34 confers common rust and northern corn leaf blight resistance in maize. Plant Biotechnol J. https://doi.org/10.1111/pbi.12647

Sun L, Yang DL, Kong Y, Chen Y, Li XZ, Zeng LJ, He ZH (2014) Sugar homeostasis mediated by cell wall invertase *GRAIN INCOMPLETE FILLING 1 (GIF1)* plays a role in pre-existing and induced defence in rice. Mol Plant Pathol 15:161–173

Sutton PN, Gilbert MJ, Williams LE, Hall JL (2007) Powdery mildew infection of wheat leaves changes host solute transport and invertase activity. Physiol Plant 129:787–795

Swarbrick PJ, Schulze-Lefert P, Scholes JD (2006) Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. Plant Cell Environ 29:1061–1076

Tanaka S, Han X, Kahmann R (2015) Microbial effectors target multiple steps in the salicylic acid production and signaling pathway. Front Plant Sci 6:349

Tauzin AS, Giardina T (2014) Sucrose and invertases: a part of the plant defense response to the biotic stresses. Front Plant Sci 5:293

Taylor S, Wakem M, Dijkman G, Alsarraj M, Nguyen M (2010) A practical approach to RT-qPCR—publishing data that conform to the MIQE guidelines. Methods 50:S1–S5

Thao NP, Chen L, Nakashima A, Hara S, Umemura K, Takahashi A, Shirasu K, Kawasaki T, Shimamo K (2007) RAR1 and HSP90 form a complex with Rac/RopGTPase and function in innate-immune responses in rice. Plant Cell 19:4035–4045

The *Arabidopsis* Information Resource (TAIR) (2017) http://www.Arabidopsis.org/about *Arabidopsis*.html on http://www.Arabidopsis.org Oct 04

Tomar SMS, Menon MK (1998) Introgression of alien genes for leaf rust (*Puccinia recondita*) resistance in to bread wheat (*Triticum aestivum* L.) cultivars. Indian J Agric Sci 68:675–681

Tsuda K, Glazebrook J, Katagiri F (2008) The interplay between MAMP and SA signaling. Plant Signal Behav 3:359–361

Udvardi MK, Czechowski T, Scheible WR (2008) Eleven golden rules of quantitative RT-PCR. Plant Cell 20:1736–1737

Ustun S, Bartetzko V, Bornke F (2013) The *Xanthomonas campestris* type III effector XopJ targets the host cell proteasome to suppress salicylic-acid mediated plant defence. PLoS Pathog 9:e1003427

Vargas WA, Martin JM, Rech GE, Rivera LP, Benito EP, Diaz-Minguez JM et al (2012) Plant defense mechanisms are activated during biotrophic and necrotrophic development of *Colletotricum graminicola* in maize. Plant Physiol 158:1342–1358

Wang X, McCallum BD, Fetch T, Bakkeren G, Marais GF, Saville BJ (2013) Comparative microscopic and molecular analysis of Thatcher near-isogenic lines with wheat leaf rust resistance genes *Lr2a, Lr3, LrB*, or *Lr9* upon challenge with different *Puccinia triticina* races. Plant Pathol 62:698–707

Wang J, Shine MB, Gao QM, Navarre D, Jiang W, Liu C et al (2014) Enhanced disease susceptibility1 mediates pathogen resistance and virulence function of a bacterial effector in soybean. Plant Physiol 165:1269–1284

XLSTAT (2017) A user-friendly statistical software for Microsoft Excel. http://www.xlstat.com

Yang B, Sugio A, White FF (2006) *Os8N3* is a host disease susceptibility gene for bacterial blight of rice. Proc Natl Acad Sci USA 103:10503–10508

Youssef RM, Kim KH, Haroon SA, Matthews BF (2013) Post-transcriptional gene silencing of the gene encoding aldolase from soybean cyst nematode by transformed soybean roots. Exp Parasitol 134:266–274

Yuan JH, Liu TG, Chen WQ (2007a) Postulation of leaf rust resistance genes in 47 new wheat cultivars at seedling stage. Sci Agric Sin 40:1925–1935

Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Yang D, He Z (2007b) Functional analysis of rice *NPR1-like* genes reveals that *OsNPR1/NH1* is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. Plant Biotechnol J 5:313–324

Zhao HB, Xu LF, Su T, Jiang Y, Hu LY, Ma FW (2015) Melatonin regulates carbohydrate metabolism and defenses against *Pseudomonas syringae* pv. *tomato* DC3000 infection in *Arabidopsis thaliana*. J Pin Res 59:109–119

Zhu B, Chen TH, Li PH (1996) Analysis of late blight resistance and freezing tolerance in transgenic potato plants expressing sense and antisense genes for osmotin-like protein. Planta 198:70–77