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Transcriptomic insights into powdery mildew resistance in contrasting genotypes of *Triticum aestivum* L.

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Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a major fungal disease that significantly impacts wheat production worldwide. Understanding the molecular mechanisms underlying resistance in wheat (*Triticum aestivum* L.) genotypes is crucial for developing durable disease management strategies. In this study, we compared the transcriptomic responses of two contrasting bread wheat genotypes, Bolani (sensitive) and TN72 (resistant), to *Bgt* infection using RNA sequencing. Transcriptome analysis identified 2,827 DEGs in the sensitive genotype (1,507 upregulated and 1,320 downregulated) and 3,105 DEGs in the resistant genotype (1,484 upregulated and 1,621 downregulated). Functional annotation of stress-responsive genes using MapMan revealed key pathways including cell wall biosynthesis, fatty acid metabolism, redox processes, amino acid biosynthesis, and light reactions. Venn diagrams analysis highlighted two protein-coding genes: *LOC123103220* (asparagine synthetase [glutamine-hydrolyzing]) and *TraesCS6D02G392100* (xyloglucan endotransglycosylase/hydrolase protein 8-like). These genes were upregulated in the resistant genotype but downregulated in the sensitive genotype, playing roles in asparagine biosynthesis, and cell wall biogenesis/xyloglucan metabolism, respectively. The relative expression of six selected genes between resistant and sensitive genotypes, validated using real-time PCR, corroborated the RNA-seq findings. Our findings highlight the molecular basis of *Bgt* resistance in TN72 and provide valuable insights for breeding programs aimed at enhancing powdery mildew resistance in wheat.

Keywords: *Bgt*, biotic stress, bread wheat, gene ontology, MapMan, RNA-seq

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a globally significant cereal crop, cultivated extensively due to its vital role in food security. With an annual production exceeding 700 million tons, wheat accounts for nearly one-third of the world's total grain output (Mustafa *et al*., 2017). Despite its agricultural importance, wheat is vulnerable to numerous fungal pathogens, including powdery mildew (PM), caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*). This pathogen forms characteristic white, powdery colonies on leaves and stems, severely impacting plant health and yield (Li *et al*., 2019). The prevalence of *Bgt* is particularly high in humid regions, including parts of Iran, where it can lead to yield losses of up to 30% in susceptible wheat varieties (Briceno-Flix *et al*., 2008). While chemical fungicides are commonly employed to manage PM, their use raises concerns about human health and environmental sustainability due to potential respiratory, neurological, and carcinogenic effects (Oruc, 2010). Consequently, there is a growing emphasis on developing resistant wheat cultivars as a sustainable alternative to chemical interventions (Mustafa *et al*., 2017).

Conventional breeding strategies primarily focus on race-specific resistance genes (R genes). However, the rapid evolution of *Bgt* strains has rendered many R genes ineffective over time, as pathogens adapt to overcome host resistance (Li *et al*., 2017). This phenomenon, observed globally, underscores the need for continuous identification and integration of novel resistance genes into breeding programs (Leath and Murphy, 1985; Niewoehner and Leath, 1998; Clarkson, 2000; Duan *et al*., 2002; Hasaneian Khoshro *et al*., 2024). Native wheat genotypes, with their diverse genetic backgrounds, offer a valuable reservoir of resistance traits. These genotypes often exhibit enhanced adaptability to local environmental conditions and harbor multiple disease-resistance genes (Wang *et al*., 2012). Despite their potential, limited research has explored the transcriptomic responses of wheat to *Bgt* infection, particularly in resistant genotypes (Xin *et al*., 2012).

This study aims to bridge this gap by investigating the transcriptomic profiles of contrasting wheat genotypes, resistant (TN72) and sensitive (Bolani), in response to *Bgt* infection. By identifying differentially expressed genes (DEGs) and elucidating the molecular mechanisms underlying resistance, we seek to provide insights that can inform the development of durable PM-resistant wheat cultivars.

MATERIALS AND METHODS

Plant materials and inoculation

Two hexaploid wheat genotypes, Bolani (sensitive) and TN72 (resistant), were selected among thirty-one Iranian wheat genotypes in a screening experiment for this study. *Blumeria graminis* f. sp. *tritici* (*Bgt*)

samples were collected from Gorgan Province, Iran, and propagated in a greenhouse on Bolani seedlings to generate sufficient inoculum. Inoculation was performed using the rub-inoculation method, and the pots were covered with transparent lids to maintain humidity.

Growth conditions and sampling

The inoculated plants were maintained in a greenhouse at 25 ± 1 °C with a 12-hour light/dark cycle. Leaf samples were collected 24 hours post-inoculation from both genotypes, with three biological replicates per treatment.

RNA extraction and sequencing

Total RNA was extracted from control and treated leaves using the Qiagen RNeasy RNA Isolation Mini Kit (Qiagen, USA). RNA quality was evaluated using a NanoDrop ND-1000® spectrophotometer and agarose gel electrophoresis. Sequencing libraries were prepared and sequenced on an Illumina NovaSeq 6000 platform, generating 150-bp paired-end reads.

RNA-seq data analysis

Raw sequencing reads were quality-checked using FastQC. High-quality reads were aligned to the *Triticum aestivum* reference genome (IWGSC CS RefSeq v2.1) using TopHat (Trapnell *et al*., 2012). Transcript assembly and quantification were performed using Cufflinks, and differentially expressed genes (DEGs) were identified with Cuffdiff (|log2 fold change| ≥ 2, Q-value ≤ 0.01). Venn diagrams (Heberle *et al*., 2015) were used to compare DEGs between genotypes.

Functional annotation and pathway analysis

Gene ontology (GO) enrichment analysis was conducted using AgriGO v2.0 (Tian *et al*., 2017), and metabolic pathways of the DEGs were visualized using MapMan (p-value ≤ 0.05) (Thimm *et al*., 2004).

RNA Extraction, cDNA Synthesis, and quantitative real-time PCR (qPCR)

Total RNA was extracted from frozen wheat leaves using a CTAB-based method (Jaakola et al., 2001). Briefly, leaf tissues were ground to a fine powder in liquid nitrogen, and RNA was isolated using the CTAB extraction buffer. The RNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The absorbance ratios at 260/280 nm and 260/230 nm were used to assess RNA purity and to detect potential contamination by proteins or organic compounds. RNA integrity was further evaluated by electrophoresis on a 1% agarose gel stained with EcoSafe, and residual DNA was removed by treatment with DNase I enzyme (Thermo Fisher Scientific). For cDNA synthesis, 5 µg of total RNA was reverse-transcribed using the M-MuLV RT enzyme from the Maxima Reverse Transcriptase kit (Thermo Fisher Scientific). Oligo (dT)20 primers were used to initiate first-strand cDNA synthesis, following the manufacturer's protocol. The resulting cDNA was diluted and stored at −20 °C for subsequent qPCR analysis. Six differentially expressed genes (DEGs), *LOC100049055*, *LOC123111745*, *LOC123103220*, *LOC123170467*, *LOC123043752*, and *LOC123049482*, were selected for validation by qPCR. Gene-specific primers were designed (Supplementary Table S1), and their specificity was confirmed by conventional PCR using GoTaq DNA polymerase (Promega, Madison, WI, USA). The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide and visualized under UV light to ensure amplification of the expected fragment sizes. Quantitative real-time PCR (qPCR) was performed using SYBR Green I technology on a C1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Each 10 µL reaction contained 1 µL of cDNA template, 4 µL of Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Cat. No: K0221), 0.5 µL each of forward and reverse primers (100 µM), and 4 µL of PCR-grade water. The thermal cycling conditions included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A melting curve analysis was performed by gradually increasing the temperature from 65 °C to 95 °C to verify the specificity of the amplified products. PCR efficiency was determined using a standard curve generated from serial dilutions of cDNA, and the cycle quantification (Cq) values were used for relative quantification. The expression levels of the target genes were normalized to the actin housekeeping gene and calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

RESULTS

Sequencing and mapping statistics

RNA sequencing on the Illumina NovaSeq 6000 platform yielded approximately 41.59 to 46.71 million raw reads per sample, totaling ~353.38 million reads. After quality filtering, ~306.43 million high-quality reads were retained, with over 86% successfully mapped to the *Triticum aestivum* reference genome (IWGSC CS RefSeq v2.1) (Table 1).

Table 1 Summary statistics of the sequencing and mapping results.

Reads mapping	Sensitive cultivar (Bolani)						Resistant cultivar (TN72)					
	Control			Powdery mildew			Control			Powdery mildew		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
Total reads	46,714,878	44,535,462	44,859,270	46,616,314	44,834,238	45,263,539	41,595,372	43,365,190	44,593,730	43,283,994	42,440,376	41,943,420
Total mapped reads	40,161,119 [86.0%]	38,783,317 [87.1%]	38,937,846 [86.8%]	40,100,131 [86.0%]	38,603,693 [86.1%]	39,062,434 [86.3%]	36,453,727 [87.6%]	38,011,904 [87.6%]	38,930,326 [87.3%]	37,505,272 [86.6%]	36,808,856 [86.7%]	36,281,058 [86.5%]
Unique-position match	3,6210,862 [76.2%]	34,661,117 [76.5%]	34,048,185 [75.9%]	35,781,081 [75.2%]	34,121,872 [74.5%]	34,083,445 [75.3%]	32,686,994 [77.3%]	34,210,712 [77.6%]	34,426,359 [77.2%]	33,337,377 [75.5%]	33,108,887 [76.7%]	31,918,942 [76.1%]
Multi-position match	3,950,257 [9.8%]	4,122,200 [10.6%]	4,889,661 [10.9%]	4,319,050 [10.8%]	4,481,821 [11.6%]	4,978,989 [11.0%]	3,766,733 [10.3%]	3,801,192 [10.0%]	4,503,967 [10.1%]	4,167,895 [11.1%]	3,699,969 [10.0%]	4,362,116 [10.4%]

Identification of DEGs involved in powdery mildew resistance

A total of 5,932 differentially expressed genes (DEGs) were identified under *Blumeria graminis* f. sp. *tritici* (*Bgt*) stress across the two genotypes. Of these, 2,827 and 3,105 DEGs were unique to the sensitive (Bolani) and resistant (TN72) genotypes, respectively (Supplementary Table S2). Venn diagram analysis revealed 1,507 upregulated and 1,320 downregulated DEGs in Bolani, while TN72 exhibited 1,484 upregulated and 1,621 downregulated DEGs (Figure 1a). Among these, 1,622 DEGs were common to both genotypes (Figure 1b). Additionally, 734 and 749 DEGs were uniquely up- and down-regulated in TN72, respectively (Figure 1c). Numerous transcription factors (TFs) were identified among the DEGs, including well-known stress-responsive TFs such as WRKY, NAC, ERF, ARF, DREB, bZIP, and MYB. Other identified TFs included HD-ZIP I/II, GARP, NDX, zf-HD, BBX-DBB, BBX-CO, GATA, and DOF (Supplementary File S2). Based on the Venn diagram results, two notable DEGs, *LOC123103220* and *LOC123146280*, were upregulated in TN72 but downregulated in Bolani (Figure 1c). *LOC123103220* (XM_044524732.1), encoding an asparagine synthetase [glutamine-hydrolyzing] protein (*TaASN1*), is located on chromosome 5A and involved in asparagine biosynthesis. *LOC123146280* (*TraesCS6D02G392100*), encoding a xyloglucan endotransglycosylase/hydrolase protein 8-like, is located on chromosome 6D and associated with cell wall biogenesis and xyloglucan metabolism.

Gene ontology classification of DEGs

Gene ontology (GO) analysis was conducted to categorize the differentially expressed genes (DEGs) into three primary domains: biological processes (BP), cellular components (CC), and molecular functions (MF). In the sensitive genotype (Bolani), 28 GO terms were significantly enriched, while the resistant genotype (TN72) exhibited 21 enriched terms. The most prominently enriched biological processes included the 'cellular process' (GO:0009987) and the 'metabolic process' (GO:0016020). These terms reflect the active involvement of cellular and metabolic activities in the response to powdery mildew (PM) infection. Key molecular functions identified among the DEGs included 'transporter activity' (GO:0005215), 'nucleic acid binding transcription factor activity' (GO:0001071), and 'electron carrier activity' (GO:0009055). These functions highlight the roles of transport mechanisms, transcriptional regulation, and redox reactions in the defense response. Enriched cellular component terms included 'cell part' (GO:0005575), 'membrane' (GO:0016020), 'membrane part' (GO:0044425), and 'extracellular region' (GO:0005576). These findings suggest that cellular structures, particularly membranes and extracellular regions, play a critical role in mediating the plant's response to PM stress. The GO classification results, summarized in Figure 2, provide a comprehensive overview of the functional roles of DEGs in both genotypes. The enrichment of these terms underscores the complexity of the molecular mechanisms underlying PM resistance and sensitivity.

Functional annotation of PM-stress responsive genes using MapMan

To gain deeper insights into the molecular mechanisms underlying powdery mildew (PM) resistance, functional annotation of differentially expressed genes (DEGs) was performed using MapMan. This analysis revealed several key metabolic pathways that were significantly modulated during PM infection in the resistant genotype (TN72). Among these, pathways related to cell wall biosynthesis, fatty acid metabolism, redox-active metabolite biosynthesis, amino acid metabolism, and light reactions (photosynthesis) were prominently regulated (Figure 3, Supplementary Table S2).

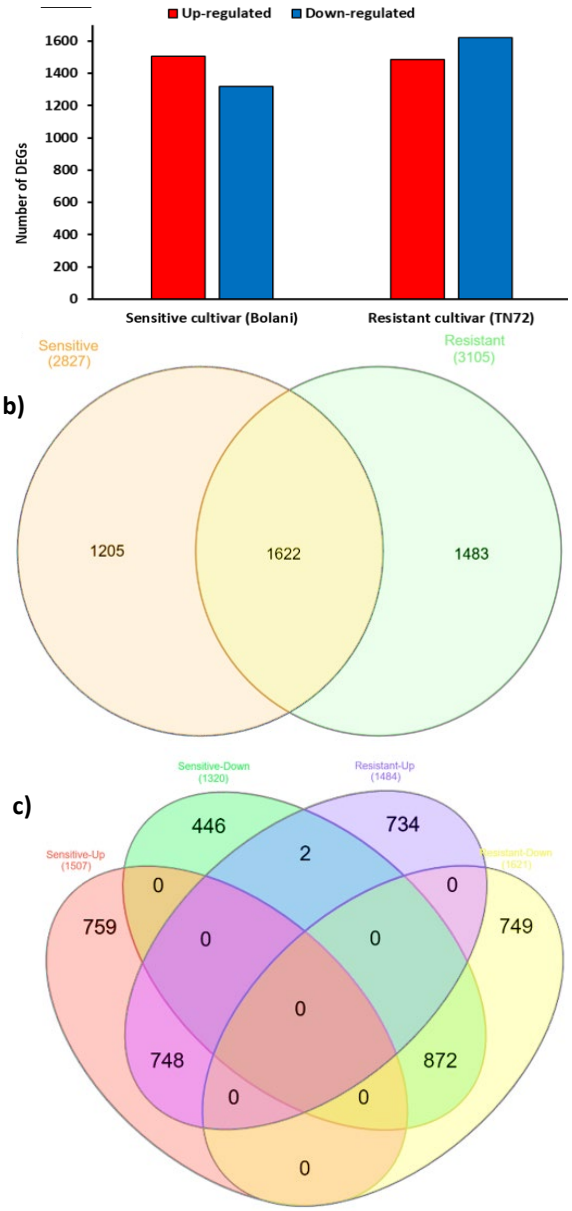

Figure 1. Differential expression genes under PM resistance in wheat genotypes. (a) The DEGs are upregulated or downregulated during PM stress. (b) Venn diagram illustrating common DEGs in both genotypes. (c) Unique and common DEGs in resistance and sensitive genotypes.

Validation of RNA-seq results by qPCR analysis

To validate RNA-seq findings, quantitative real-time PCR (qRT-PCR) was conducted on six randomly selected DEGs: *LOC100049055* (WRKY transcription factor 26), *LOC123111745* (LRR receptor-like serine/threonine-protein kinase), *LOC123103220* (Asparagine synthetase [glutamine-hydrolyzing]), *LOC123170467* (NAC domain-containing protein 22), *LOC123043752* (Stress-related protein), *LOC123049482* (Disease resistance protein RGA5) (Supplementary Table S1). The relative expression levels of these six DEGs under PM infection were validated using qRT-PCR, and the results were compared to RNA-seq data (Figure 4). The WRKY transcription factor *LOC100049055* (Figure 4a) showed increased expression in the resistant genotype (TN72) compared to the sensitive genotype (Bolani), with both RNA-seq and qRT-PCR analyses confirming its upregulation. *LOC123111745* (Figure 4b), encoding an LRR receptor-like serine/threonine-protein kinase, exhibited higher expression in the resistant genotype, and the qRT-PCR results were consistent with the RNA-seq data. The expression of *LOC123103220* (Figure 4c), which encodes asparagine synthetase, demonstrated significant downregulation in the sensitive genotype and upregulation in the resistant genotype, reflecting strong agreement between the two methods. For *LOC123170467* (Figure 4d), a NAC domain-containing protein, the expression levels were similar in both RNA-seq and qRT-PCR analyses, with slightly higher expression observed in the sensitive genotype compared to the resistant genotype. The stress-related protein *LOC123043752* (Figure 4e) was significantly downregulated in the sensitive genotype but showed increased expression in the resistant genotype, corroborating the RNA-seq results. Finally, *LOC123049482* (Figure 4f), encoding the disease resistance protein RGA5, exhibited a marked increase in expression in the resistant genotype, with consistent findings between RNA-seq and qRT-PCR results. The qRT-PCR analysis confirmed the RNA-seq data, showing minimal variation in log2 fold change values between the two methods (Figure 4). This high degree of consistency underscores the reliability and accuracy of RNA-seq analysis in detecting differential gene expression under PM infection.

DISCUSSION

In a preliminary screening, thirty-one Iranian wheat genotypes, including Bolani, were evaluated against ten *Blumeria graminis* f. sp. *tritici* (*Bgt*) pathotypes. Bolani and TN72 were identified as the most sensitive and resistant genotypes, respectively, and selected for transcriptome analysis. Among the surveyed pathotypes, Gorgan_02 was identified as the most virulent. Consequently, the effects of this pathotype on the selected genotypes were assessed in a focused experiment to identify genes and mechanisms involved in powdery mildew resistance in bread wheat.

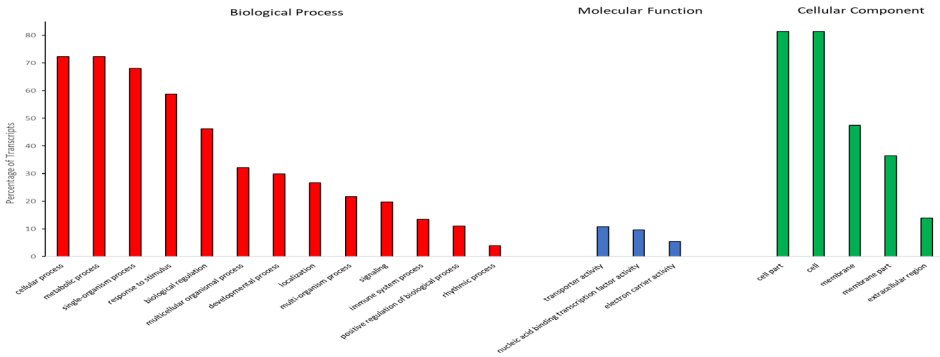

Figure 2. Summary of significantly (*p-value* < 0.01) enriched GO terms in the resistant genotype (TN72).

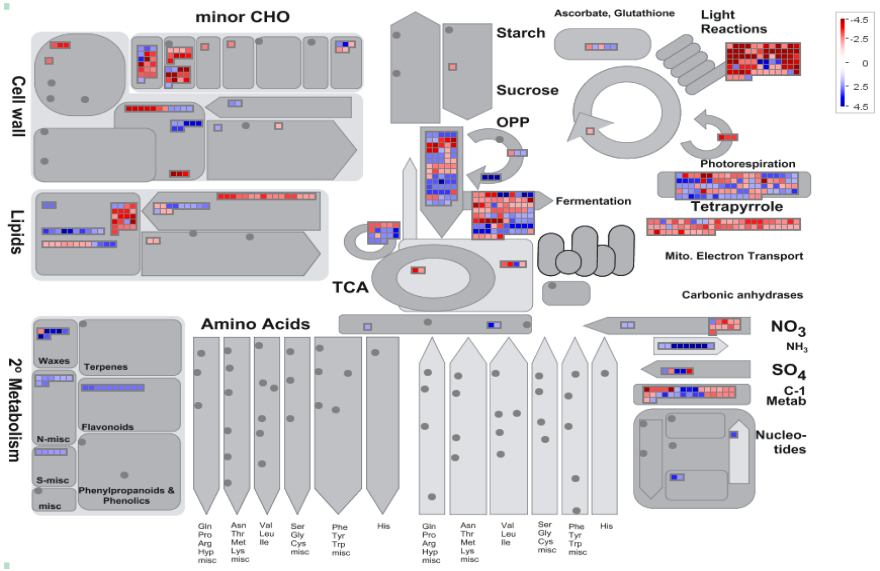

Figure 3. MapMan metabolic pathway overview showing upregulated (blue) and downregulated (red) DEGs in the resistant genotype during PM infection.

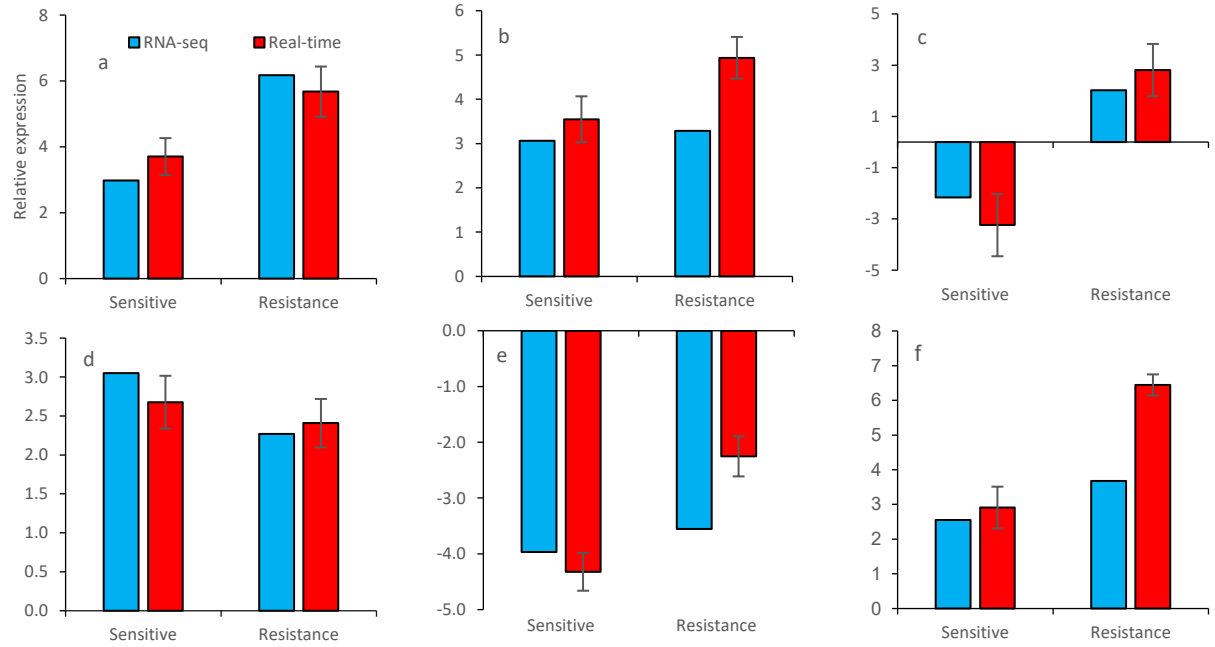

Figure 4. Relative expression of selected DEGs under PM infection as determined by RNA-seq and qRT-PCR analysis: (a) *LOC100049055*, (b) *LOC123111745*, (c) *LOC123103220*, (d) *LOC123170467*, (e) *LOC123043752*, (f) *LOC123049482*.

Comparative transcriptomic analysis revealed key stress-responsive DEGs and pathways associated with Bgt resistance (Wang *et al*., 2018). Previous studies have demonstrated that wheat infected with *Bgt* triggers defense responses in both resistant and sensitive genotypes (Xing *et al*., 2017). In this study, key pathways associated with *Bgt* resistance, including sucrose metabolism, fatty acid biosynthesis, and redox-active metabolite production, were identified in both genotypes (Fig. 3; Supplementary Table S2 and Fig. S1).

Sucrose, a primary product of photosynthesis, is directly linked to plant resistance against environmental stresses (Cheng *et al*., 2023). Pathogenic fungi, including *Bgt*, exploit sucrose absorption as a covert attack on host plants (Wahl *et al*., 2010; Chang *et al*., 2017; Wang *et al*., 2021). In this study, five cell wall invertase (CWIN) genes (*traescs6a02g060700.2*, *traescs6d02g064400.1*, *traescs5b02g557100.1*, *traescs4a02g321600.2*, *traescs4a02g321500.1*) and eleven vacuolar invertases (VIN) genes, which are crucial for sucrose hydrolysis into fructose and glucose, were identified (Fig. 3, Supplementary Table S2). Despite challenges in silencing sucrose metabolism-related genes, further studies are essential to elucidate the precise mechanisms regulating sucrose metabolism in wheat (Wang *et al*., 2021). Invertase genes have been shown to confer stress tolerance in plants (Ruan *et al*., 2010; Abbas *et al*., 2022; Cheng *et al*., 2023). LRR receptor-like serine/threonine-protein kinases such as *LOC123111745* play a critical role in pathogen recognition and activation of defense responses through the receptor-mediated cascade. The upregulation of this gene in the resistant genotype highlights its contribution to early pathogen recognition.

Additionally, redox homeostasis-related genes were prominently regulated in the TN72 genotype during *Bgt* infection. Antioxidant systems play a vital role in regulating reactive oxygen species (ROS) during pathogen attack (Gao *et al*., 2022). This study identified several redox-responsive genes, including seven genes for enzymatic ROS scavengers, three for hydrogen peroxide removal, three for chloroplast redox homeostasis (F-type thioredoxin), and eleven for low-molecular-weight scavengers (Fig. 3; Supplementary Table S2). These findings underscore the importance of redox regulation in disease resistance.

Transcription factors (TFs) are pivotal in gene regulation during stress responses. A wide array of well-known TF families, including WRKY and NAC, were identified among the DEGs (Supplementary Table S2). Notably, 44 WRKY TFs were upregulated, while 18 NAC TFs exhibited varied responses, 13 upregulated and five downregulated. WRKY transcription factors, particularly *LOC100049055* (WRKY 26), were significantly upregulated in the resistant genotype. WRKYs are well known for their involvement in activating defense-related pathways through pathogen signaling (Wang et al., 2021). Similarly, the NAC domain-containing protein *LOC123170467* showed distinct expression patterns, reflecting its role in regulating plant stress responses. These results suggest that *Bgt* resistance in wheat is controlled by a diverse set of genes, although engineering a subset of key genes may significantly enhance resistance.

CONCLUSION

Comparative transcriptome analysis between sensitive and resistant wheat genotypes provided critical insights into stress-responsive DEGs and mechanisms underlying resistance to *Bgt*. The study identified 5,932 DEGs, with 28 significantly enriched Gene Ontology (GO) terms in the Bolani genotype and 21 in the TN72 genotype. Key molecular functions, such as transporter activity, transcription factor activity, and electron carrier activity, were enriched in the resistant genotype. Key biosynthesis pathways, such as sucrose metabolism, fatty acid biosynthesis, and redox-active metabolite production, were actively expressed during *Bgt* infection. Additionally, several transcription factor families, including WRKY, NAC, and MYB, played vital roles in the resistance response. The upregulation of genes such as *LOC123103220* and *TraceCS6D02G392100* in TN72 underscores their potential contribution to resistance mechanisms. These findings not only enhance our understanding of the molecular basis of *Bgt* resistance but also provide a foundation for future genetic improvement of wheat. By targeting key genes and pathways identified in this study, breeders can develop wheat cultivars with enhanced and durable resistance to powdery mildew, contributing to sustainable wheat production.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this study. All authors prepared the manuscript.

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Not applicable

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article.

DECLARATIONS

Ethics approval and consent to participate this article does not contain any studies with human participants or animals performed.

COMPETING INTERESTS

The authors declare that they have no competing interests

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