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Research article

Transcriptome and proteome analyses of resistant preharvest peanut seed coat in response to *Aspergillus flavus* infection

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ABSTRACT

Background: The infection of peanut (*Arachis hypogaea* L.) seed coat by the pathogenic fungus *Aspergillus flavus* has highly negative economic and health impacts. However, the molecular mechanism underlying such defense response remains poorly understood. This study aims to address this issue by profiling the transcriptomic and proteomic changes that occur during the infection of the resistant peanut cultivar J11 by *A. flavus*.
Results: Transcriptomic study led to the detection of 13,539 genes, among which 663 exhibited differential expression. Further functional analysis found the differentially expressed genes to encode a wide range of pathogenesis- and/or defense-related proteins such as transcription factors, pathogenesis-related proteins, and chitinases. Changes in the expression patterns of these genes might contribute to peanut resistance to *A. flavus*. On the other hand, the proteomic profiling showed that 314 of the 1382 detected protein candidates were aberrantly expressed as a result of *A. flavus* invasion. However, the correlation between the transcriptomic and proteomic data was poor. We further demonstrated by in vitro fungistasis tests that *hevamine-A*, which was enriched at both transcript and protein levels, could directly inhibit the growth of *A. flavus*.
Conclusions: The results demonstrate the power of complementary transcriptomic and proteomic analyses in the study of pathogen defense and resistance in plants and the chitinase could play an important role in the defense response of peanut to *A. flavus*. The current study also constitutes the first step toward building an integrated omics data platform for the development of *Aspergillus*-resistant peanut cultivars.
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1. Introduction

Peanut (*Arachis hypogaea* L.) is a legume crop with a high nutritional value. Peanut seeds rank third among the major sources of vegetable protein worldwide and are extensively used for producing edible oil [1]. Peanuts also contain abundant unsaturated fatty acids, which can lower the risk of cardiovascular diseases [2]. Despite these benefits, peanuts are frequently infected by *Aspergillus flavus*, a soilborne fungal pathogen during both pre- and postharvest stages. The contamination subsequently leads to seed damage and accumulation of aflatoxin, a strong carcinogen. It has been shown that preharvest *A. flavus* invasion of peanut seeds and the consequent buildup of aflatoxin could increase under stressful conditions such as in the presence of insects and during drought [3]. To date, various chemically or biologically based strategies to battle contamination have been developed with limited success [3]. On the other hand, engineering the pathogen resistance of peanut is generally considered as the most promising approach, but it has traditionally been limited to conventional breeding and cultivar selection because of the lack of understanding of the underlying defense mechanism [2]. Therefore, there is an urgent necessity for agricultural researchers to further investigate the genes and pathways that contribute to the molecular changes that occur in peanut as a result of *A. flavus* invasion.

RNA sequencing is a rapid and high-throughput technology widely used in transcriptomic analysis. Its application in agriculture has greatly facilitated the elucidation of various stress-response mechanisms in plants, particularly cash crops, with excellent specificity, sensitivity, and reproducibility [4]. For example, the comparative transcriptomic profiling by RNA sequencing of different tomato cultivars in response to the infection of tomato yellow leaf curl virus, which suggested the possible involvement of pathways related to cell wall remodeling, ubiquitination, and metabolite synthesis [5]. In another study, transcriptome analysis revealed that genes related to DNA binding and ATP binding were activated in the leaves of *Brassica napus* during the

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infection of *Sclerotinia sclerotiorum* [6]. Nayak et al. [7] reported that some of the most abundantly expressed genes in the *A. flavus*-resistant peanut cultivar J11 (used also in our current study) included pathogenesis-related (PR) proteins, peroxidases, chitinases, etc. These examples lent credence to the tremendous utility of sequencing-based transcriptomic analysis in elucidating the complex regulatory networks of plants and their interactions with various environmental stimuli.

In this study, we performed transcriptomic analysis using RNA sequencing to investigate the changes in the global gene expression profile during the infection of preharvest peanut seed coat by *A. flavus*. Furthermore, we also profiled the proteomes of the same peanut specimens to aid in the interpretation of the transcriptomic data and to provide a deeper insight into the defense mechanism in peanut against *A. flavus*. Our differential expression and functional analyses led to the identification of a wide array of pathogenesis- and/or defense-related genes and pathways. Furthermore, our data suggested that oxidative stress and cell wall remodeling are two key mechanisms contributing to the defense response of peanuts against *A. flavus*. However, we noticed that there was very little overlap between the differentially expressed genes (DEGs) and proteins (DEPs), suggesting that post-transcriptional regulatory processes could also play a key role in governing pathogen defense in peanuts. This study is our first step toward building an integrated omics data platform for the development of *Aspergillus*-resistant peanut cultivars.

2. Materials and methods

2.1. Plant materials and pathogen treatment

The sample peanut cultivar J11 was obtained from the Groundnut Pathology Unit of the International Crops Research Institute for the Semi-Arid Tropics. Peanut seeds were grown in the test field of Shandong Peanut Research Institute, China. J11 was previously shown to be resistant to *A. flavus* invasion [7,8]. A wild-type *A. flavus* strain, NRRL3357, provided by the USDA-ARS Culture Collection was cultured on Czapek–Dox medium at 28 ± 1°C for 7 d. Conidia were then collected and suspended in sterile water containing 0.05% Tween-80. After 120 d of germination, peanuts were infected with *A. flavus* according to a previously described artificial inoculation method [8,9]. The seed coat samples were collected at 3 DAI (days after inoculation), 5 DAI, 7 DAI, and 10 DAI from the infected samples of J11. Seed coats of uninfected plants were used as controls and sampled at the same time points as mentioned above. The samples were stored at −80°C until use.

2.2. RNA isolation and construction of cDNA libraries

To prepare the libraries, seed coat samples collected at 3 DAI, 5 DAI, 7 DAI, and 10 DAI were pooled for RNA isolation and employed as a mixed-stress library according to the strategy for preparing a pooled cDNA library, whereas seed coat samples from the uninfected plants served as the control group. Each group comprised three biological replicates.

Total RNA was extracted from each group using RNeasy Mini Kit (QIAGEN, USA) with DNase treatment according to the manufacturer's instructions. RNA concentration was determined using NanoDrop 2000 (Thermo Fisher Scientific, USA) and Qubit 2.0 RNA Broad-Range Assay Kit (Invitrogen, USA). RNA quality was examined on a 2100 Bioanalyzer (Agilent Technologies, USA). Isolation of mRNA and the subsequent cDNA synthesis were conducted with TruSeq RNA Sample Preparation Kit (Illumina, USA) and SuperScript II Reverse Transcriptase (Invitrogen, USA) according to the manufacturers' instructions. The synthesized cDNA was quantified with Qubit 2.0 DNA Broad-Range Assay Kit (Invitrogen, USA). The resultant RNA libraries were sequenced on an Illumina HiSeq4000 Platform (Illumina, USA) to generate 2 × 150 bp paired-end reads. Sequence quality was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). Adaptors and low-quality bases were trimmed by Trimmomatic [10]. Reads with a Phred quality (Q20 ≥ 95.61% and Q30 ≥ 92.51%) were used for further alignment and assembly. The GEO submission number of the transcriptome sequencing data is GSE102782.

2.3. Alignment to the reference genome

The clean data were aligned to peanut reference genomes (NCBI Aradu1.0 and Araip1.0 assembly) using HISAT (version 2.0.5) [11]. Aligned reads from each sample were assembled into transcripts using Stringtie (v 1.3.1) [12] and compared to sequences from the above-mentioned reference genomes. Separate GTF files were generated for each of the six samples. The assembled transcripts were quantified with Fragments Per Kilobase of transcript per Million fragments mapped (FPKM) using the R package Ballgown [13]. The reads that were not aligned to the peanut reference genomes were used for alignment with the *Aspergillus* genome available at NCBI [14].

2.4. Identification and functional analysis of DEGs

Differential expression analysis was performed with Ballgown. The false discovery rate was used to determine the threshold p-values for multiple tests. A gene was considered differentially expressed if its fold change score was above one and its adjusted p-value exceeded 0.05.

Gene Ontology (GO) enrichment analysis was performed by querying the identified DEGs against the Gene Ontology database (http://www.geneontology.org/). The number of genes in each GO term was calculated, followed by the determination of significantly enriched GO functions by a hypergeometric test. GO terms with an adjusted p-value <0.05 and $\log_2$ fold change >1 were considered significantly enriched. Similarly, the gene queries were also searched against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.kegg.jp/). The KEGG terms with an adjusted p (q) value <0.05 and $\log_2$ fold change >1 were considered significantly enriched.

2.5. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

qRT-PCR primers were designed using Beacon Designer 7.0 (PREMIER Biosoft, USA), and their specificities were evaluated by performing BLAST searches against the NCBI database. Total RNA was reverse-transcribed into single-stranded cDNA using PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Japan). The *ACTIN11* gene served as an internal control for normalizing the transcript levels of all analyzed target genes [15]. PCR reactions were performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, USA) using the SYBR Premix Ex Taq reagents (Takara, Japan) following the manufacturer's recommended protocol. The experiments were conducted in triplicate, and the relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. All qRT-PCR primer sequences are listed in Table S1.

2.6. Proteomic analysis

Proteomic analysis was performed on the same six peanut samples described above using the tandem mass tag technology [16]. Total protein was extracted using Plant Protein Extraction Kit (Solarbio, China) and then quantified by the Bradford assay. Sequencing and data interpretation, such as peptide mapping and differential expression analysis, were conducted as described [16]. A protein was considered differentially expressed if its p-value was below 0.05 and fold change above 1.2. Pearson correlation analysis was conducted with SPSS (version 12.0, SPSS Inc., USA) to examine the degree of concordance between the level of a transcript and that of its protein product.

2.7. Fungistasis test of hevamine-A

Heterologous expression of the *hevamine-A* (GenBank accession no. LOC107467678; GenBank accession no. XP_016182616.1) was assessed by the prokaryotic expression method, according to a procedure detailed by Saijo et al. [17]. After purification using the Ni-Agarose His Kit (CWBIO, China), the fungistatic effect of *hevamine-A* on *A. flavus* was evaluated. Briefly, conidia of *A. flavus* NRRL 3357 were suspended in sterile water, and their concentration was determined using a hemocytometer. Based on the concentration, 0.5 mL of spore suspension (3.5×10^7 CFU/mL) was pipetted into a sterile petri dish containing Czapek–Dox medium, followed by the addition of purified *hevamine-A* (0.1 mL, 1 mg/mL). The petri dish was incubated at 28°C for five days, and the growth of *A. flavus* was monitored by direct observation.

3. Results

3.1. Transcriptome and proteome profiles of A. flavus-infected peanut seeds

Our study consisted of three group samples invaded by *A. flavus* and three controls with no infection, all of the same resistant J11 cultivar. RNA sequencing of all six transcriptome libraries generated a total of 353,279,484 raw reads as shown in Table S2. After quality control, 29,624,684–36,121,302 clean reads, equivalent to 4.4–5.4 Gb of clean bases, were obtained for each library. The Q20 (% of bases with a Phred value >20) and Q30 (% of bases with a Phred value >30) values of clean data were 95.61–97.01% and 92.51–94.31%, respectively. Over 92.61% of all uniquely mapped reads were found to be of exonic origin, whereas those derived from introns and intragenic regions comprised 4.89–5.07% and 1.2–2.39% of the total, respectively. Overall, 13,539 genes were confidently mapped to the reference genome (Table S3).

We then determined gene expression levels by calculating the number of reads mapped to each identified unique transcript (Fig. S1a). A gene was considered differentially expressed depending on the criteria of adjusted $p > 0.05$ and $\log_2$ fold change >1. In total, 663 genes exhibited differential expression levels in the *A. flavus*-infected peanut seeds compared to those of the control, where 417 of those were upregulated and 246 downregulated (Fig. S1b). Details of the 20 most upregulated and 20 most downregulated genes are summarized in Table S4. We then selectively analyzed the DEGs with possible roles in plant defense against invading pathogens, such as those encoding various transcription factors (TF), pathogenesis-related (PR) proteins, chitinases, gibberellin, plantacyanin, arabinogalactan proteins (AGPs), xyloglucan endotransglucosylase/hydrolase (XTH), protease inhibitor, and acanthoscurrin. The details of these DEGs are listed in Table S5.

We also performed proteomic analysis on the same samples to enable further understanding of the molecular changes that occurred in peanut seeds in response to *A. flavus* infection. The proteomic profiling generated a total of 15,339 spectra and led to the confident identification of 1382 proteins. Overall, 314 candidates showed differential expression in the infected peanut seeds based on the screening criteria of adjusted p-value <0.05 and fold change >1.2, among which 185 were upregulated and 129 downregulated. Again, we discovered a number of protein candidates with possible roles in plant defense, including, but not limited to, hevamine-A-like, β-1,3-glucanase, and peroxidase 15-like protein.

3.2. qRT-PCR validation of selected DEGs

To validate the gene expression data obtained from RNA sequencing, we selected 14 DEGs implicated in plant pathogen defense and quantified their levels by qRT-PCR. As illustrated in Fig. S2, both RNA sequencing and qRT-PCR analyses showed the same expression trend for each of the analyzed candidates, suggesting that the transcriptomic data were accurate and could be used for further functional analysis and mechanistic interpretation.

3.3. Functional analysis of DEGs and DEPs

Functional characterization of the identified DEGs and DEPs was performed by GO enrichment and KEGG pathway analyses. In GO analysis, their functions could be divided into three categories, namely, biological process, cellular component, and molecular function. As illustrated in Fig. 1 and Table S6, the most enriched GO categories that could be meaningfully interpreted based on the transcriptomic data were those related to the organization of cell wall or the metabolism of its components, including, but not limited to, "cell wall organization," "external encapsulating structure organization," "cell wall organization or biogenesis," "pectin metabolic process," and "galacturonan metabolic process." Several GO functions with possible implication in plant defense were also unearthed, such as "diterpenoid biosynthetic process," "gibberellin metabolic process," and "diterpenoid metabolic process." Further, the most enriched GO functions as determined by analyzing the proteomic results were "cell cycle," "negative regulation of signal transduction," "2-alkenal reductase activity," and "signal transducer activity" (Fig. 2). We also identified several GO functions with possible roles in plant defense, such as "regulation of response to stress," "detection of external stimulus," and "flavonoid biosynthetic process."

On the other hand, KEGG analysis showed that several defense-related pathways were among the most enriched as determined from the transcriptomic data, such as "phenylpropanoid biosynthesis" and "antigen processing and presentation" (Fig. 3 and Table S6), whereas those identified from the analysis of DEP candidates included "plant–pathogen interaction" and "lysosome" and "fatty acid metabolism" (Fig. 4). Taken together, these results confirmed that *A. flavus* infection could greatly alter the metabolism and induce host defense mechanisms in the peanut seeds, echoing a number of previous studies on the broad impact that the invasion of fungal pathogens could exert on global gene expression in plant [18].

3.4. Integrative analysis of proteome and transcriptome

We then compared the genes identified from our transcriptomic analysis to the protein candidates revealed by the proteomic profiling in an attempt to determine the extent of overlap between the results of the two methods. Interestingly, concordance tests revealed a poor correlation (rPearson = 0.0617) between gene and protein ratios (Fig. 5). Further analysis suggested little overlap between the panel of DEGs and that of DEPs. Overall, only 12 cases of concordant expression were identified, where protein accumulation was significantly correlated with transcript abundance (Table S7). It was worth noting, however, that the majority of the gene/protein pairs that exhibited concordant expression patterns were associated with plant defense against pathogen invasion. Among them, *hevamine-A*-like, *lectin*-like, *plant UBX domain-containing protein 4*-like, and *cell wall/vacuolar inhibitor of fructosidase 1*-like proteins were upregulated in infected peanut samples compared to those in the controls, whereas *peroxisomal multifunctional enzyme type 2* and *cysteine proteinase 15A*-like proteins were downregulated.

3.5. Analysis of hevamine-A involvement in A. flavus resistance

We next sought to experimentally verify whether *hevamine-A*, one of the candidates, shows concordant expression trends in both the transcriptome and proteome (Table S7). As shown in Fig. 6, fungistasis test revealed that the production of *A. flavus* was strongly inhibited by *hevamine-A*. Therefore, our results suggested that *hevamine-A* had a direct molecular role in the defense response of peanuts to *A. flavus* infection.

3.6. DEGs in A. flavus during infection

Expression analysis of transcripts on the *Aspergillus* genome indicated differential expression of 258 genes, of which 165 were upregulated and 93 downregulated. Notably, a large number of the upregulated genes were found to be associated with Go terms like cell-wall related, fatty acid biosynthesis, transporters and growth. Furthermore, genes encoding aflatoxin biosynthesis, such as aflD nor-1 reductase, and peroxidase activity (such as glutathione peroxidase and glutaredoxin) also exhibited enhanced expression levels.

4. Discussion

The current study aims to provide a better understanding of the mechanism underlying the development of defense response in peanuts against the infection of *A. flavus*. To this end, we compared the transcriptomic profiles between infected and uninfected peanut specimens. Although a number of excellent studies have been published on *A. flavus* infection of peanuts [2,3,7,9,19], the recent availability of diploid *Arachis* spp. reference genome [20] has offered renewed opportunities to enable more precise analysis of transcriptome sequencing data and, resultantly, better interpretation of the molecular changes stimulated by pathogen invasion. On the other hand, we also analyzed the proteomic differences between the same two groups in an attempt to provide corroborating, or at least supplementary, results to the transcriptomic analysis. Subsequent GO and KEGG analyses suggested the potential involvement of a diverse range of genes and multiple pathways such as “cell wall organization,” “external encapsulating structure organization,” “cell wall organization or biogenesis,” and “pectin metabolic process” in the activation of pathogen defense machinery in peanuts. Combined, our results constituted a significant step toward the goal of unraveling the complex regulatory events that drive the defense response to pathogens in peanuts, which could provide useful guidance in the future design and engineering of cultivars with increased resistance to infectious fungi.

We noticed a poor correlation between the panel of DEGs and DEPs. Although slightly disappointing, this was not entirely unexpected because various post-transcriptional regulatory processes also play a critical role in governing protein expression levels [21]. Nevertheless, changes that occurred on a transcriptional level still provided useful perspectives on the molecular consequences of *A. flavus* infection in peanuts. The 12 genes that showed a good correlation between the transcript and protein levels should be thoroughly investigated to elucidate their mechanistic roles.

Analysis of the genes with the greatest fold change values revealed a number of interesting candidates with potential association with plant defense and immunity. Two genes annotated as *gibberellin 20 oxidase 4*-like and *gibberellin 20 oxidase 2*-like were found to be upregulated 12- and 3-fold, respectively, in *A. flavus*-infected peanuts. Gibberellins are a class of plant hormones that contribute to the regulation of plant innate immunity [22]. One of the aspects through which gibberellins modulate plant pathogen defense is the induction of oxidative stress [23]. We identified two putative peroxidases whose mRNA levels remarkably increased following pathogen infection, which was in good agreement with the results reported in an array of studies on the significance of peroxidase-dependent plant defense machinery [24].

Our results suggested that cell well modification, such as lignification, could be another major defense mechanism that peanut employs against *A. flavus* infection. For example, the

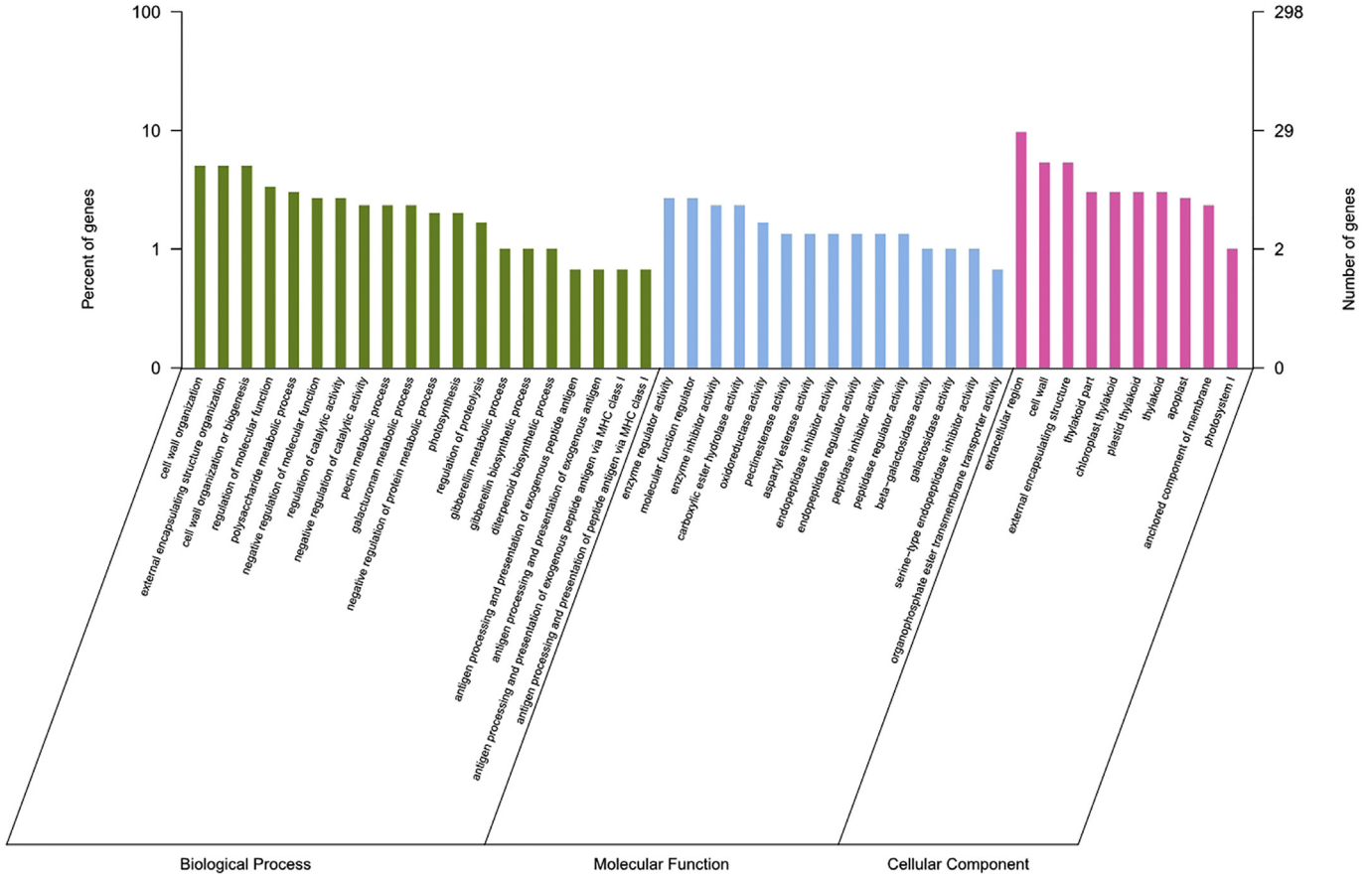

Fig. 1. GO classifications of genes differentially expressed in *A. flavus*-resistant peanuts. Frequency (absolute and percentage of total) of GO classes in DEGs. Results are divided as three main categories: biological process, molecular function, and cellular component.

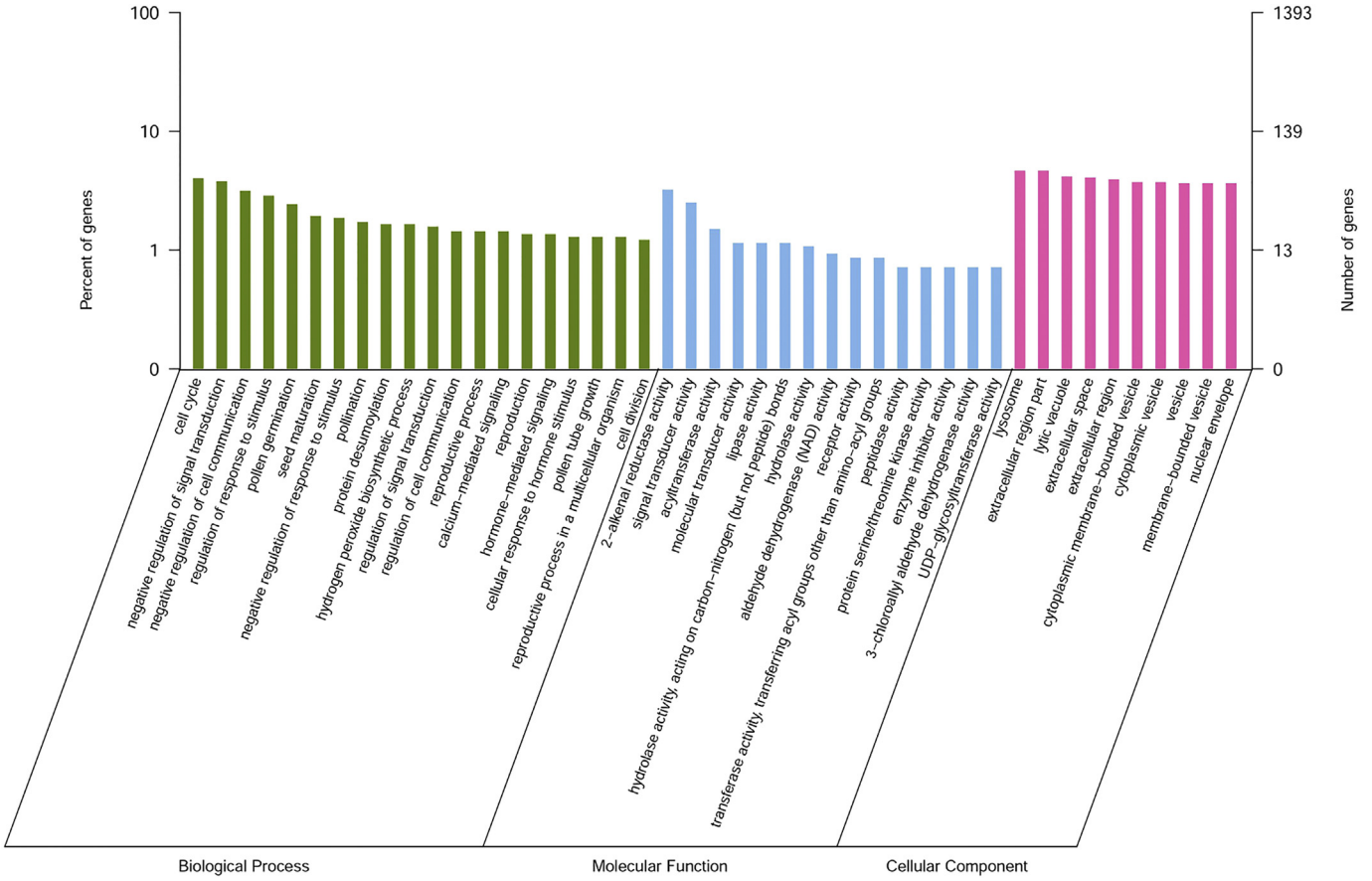

Fig. 2. GO classifications of proteins differentially expressed in *A. flavus*-resistant peanuts. Frequency (absolute and percentage of total) of GO classes in DEPs. Results are divided as three main categories: biological process, molecular function, and cellular component.

above-mentioned gibberellin has also been proposed to be able to induce metabolic changes that ultimately lead to altered cell wall composition [22]. Two genes encoding homologs to basic blue protein, also known as plantacyanin, were among the most upregulated candidates based on the transcriptomic analysis. Plantacyanin belongs to a large family of blue copper proteins previously associated with lignin deposition in plant cell wall [25,26]. Our finding of expression changes of plantacyanin mirrored previous microarray results indicating its role as a stress-induced regulatory factor [27]. Several other genes with considerably elevated expression levels, including those of *early nodulin-like protein 2* and *fasciclin-like arabinogalactan protein 9*, fall into the category of arabinogalactan proteins (AGP) [28]. Many AGPs are known to be induced under various abiotic stress conditions [29]. Moreover, Cannesan et al. [30] found that arabinogalactans were involved in containing early infection of *Aphanomyces euteiches* in pea root, which highlighted the potential importance of these proteoglycans in root–microbe interaction. Because of their apparent roles in cell wall remodeling, it would not be unimaginable that regulation of AGPs could play a significant role in plant defense. The identification of a *xyloglucan endotransglucosylase/hydrolase (XTH) 2* gene from the most upregulated transcripts is also consistent with its established role in modifying the xyloglucan–cellulose network that underpins the fundamental cell wall structure [31]. Echoing our finding, activation of XTH expression has been linked to the defense response of celery to aphids [32] and attributed to the phenotypic difference between a jute species showing susceptibility to *Macrophomina phaseolina* infection and another with strong resistance [33]. Other gene with apparent connection to cell wall remodeling, such as *cell wall/vacuolar inhibitor of fructosidase 1*-like, was also found to be greatly upregulated in infected peanuts.

We also systematically examined the transcriptional patterns of other defense- and/or pathogenesis-related genes. For example, various members of the bZIP superfamily, such as *TGA1B*, *bZIP17*, *bZIP27*, and *bZIP53*, were upregulated in infected peanut, which was in good agreement with the results of previous observations [19]. bZIP transcriptional factors have previously shown to participate in plant defense by mediating different types of stress response [34,35]. Further, the plant-specific ERF TF family includes several genes involved in the modulation of disease resistance pathways. Interestingly, more than half of ERFs identified in our current study (10 of 16) were downregulated as a result of *A. flavus* infection, which diverged from the findings of a previous study with postharvest samples [19]; 64% (43 of 67) DEGs encoding ERFs were induced in that study. Moreover, some ERFs performed differently in the two studies, such as *ERF 113*. In our study, it was downregulated. The opposite result was observed in Wang et al.'s study [19]. This disparity suggested that some ERFs might play different roles in response to *A. flavus* invasion during pre- and postharvest stages. Further work is needed to confirm these reasons.

DEGs that belong to various PR families, such as pathogenesis-related-1 (PR1), PR2, and PR5, were of particular interest in this study. Similar to that of bZIPs, upregulation of PR proteins is an important component of systemic acquired resistance in part because of its involvement in the activation of SA response [36]. Xin and coworkers identified five PR1-encoding transcripts that were upregulated in resistant and normal wheat cultivars as a result of *Blumeria graminis* invasion, two of which were shown to be activated exclusively in the former, suggesting a link between these proteins and plant defense [37]. However, it is worth noting that the authors also cautioned that the enhanced production of the PR1 proteins could be related to the

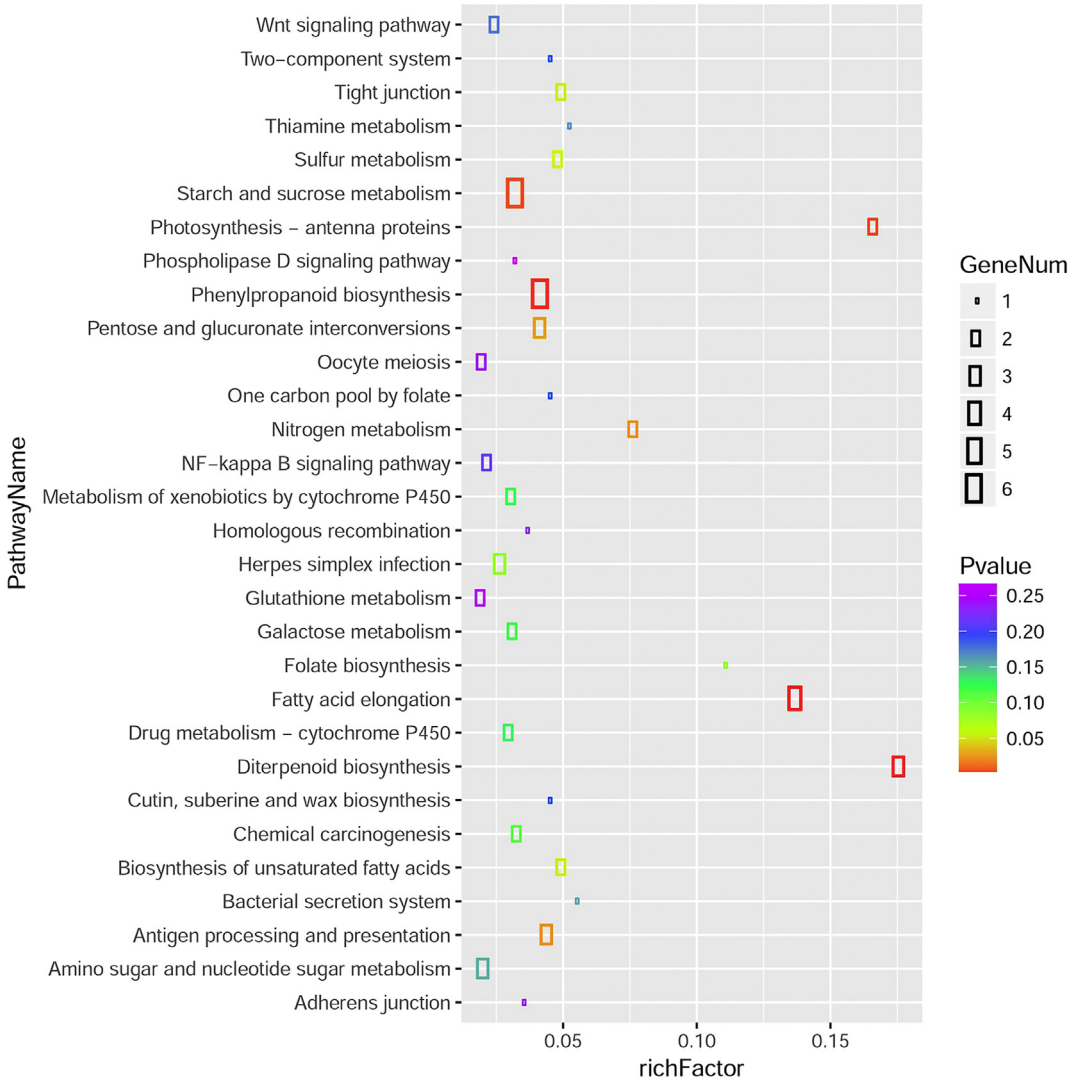

Fig. 3. KEGG analysis genes differentially expressed in *A. flavus*-resistant peanuts. KEGG enrichment of annotated DEGs in treated peanut seeds. The Y-axis shows the KEGG pathway, and the X-axis indicates the Rich factor. A high p value is represented by blue, and a low p value is represented by red.

severity of the infection rather than the resistance genotype. As mentioned above, analysis of our transcriptomic sequencing data unearthed a number of differentially expressed PR proteins. For example, a PR2-related *β-1,3-glucanase*, which can hydrolyze fungal cell wall and generate response elicitors, was found to be upregulated at both the transcript and protein levels (Table S7). Surprisingly, the PR5s identified in this study all exhibited decreased transcriptional levels in infected peanut samples despite their apparent role in disrupting the fungal plasma membrane [38]. This suggested that the effect of PR proteins on plant defense might vary significantly according to their subfamilies and the pathogen species.

Plant resistance to *A. flavus* infection is accomplished by the inhibitory actions of various antifungal proteins including chitinases [39]. Chitinases are known to inhibit fungal growth by degrading chitin in cell wall and suppressing the elongation of hyphae. In addition, they could also release pathogen-borne elicitors such as chitin oligosaccharides that activate defense mechanisms in plants [40]. It has been shown that cultivars with resistant genotypes often exhibit increased expression and/or activity of antifungal proteins when compared to susceptible lines [41]. This was echoed by the results of our current study, where a number of chitinases were shown to be upregulated at the transcriptional and/or translational level. Moreover, in vitro fungistasis tests provided direct experimental evidence that a peanut-derived *hevamine-A*-like chitinase could significantly inhibit the growth of *A. flavus*. Previously, Moore et al. [39] reported that enhanced chitinase activity in rachis was a major contributing factor to the antifungal phenotype of several resistant corn cultivars. The authors demonstrated that the growth of *A. flavus* was inhibited by 50% on exposure to 20 μg/mL of Tex6 (a chitinase protein), and no fungus survived when the protein concentration was increased to 2.0 mg/mL. In another study, in vitro seed inoculation bioassays indicated an inverse correlation between the number of transgenic events and the extent of *A. flavus* infection in a series of engineered peanut cultivars expressing a rice-derived chitinase [40]. Taken together, the results suggested that resistance to *A. flavus* in peanut could potentially be improved by introducing resistance-conferring genes from diverse sources.

The analysis of transcriptomes of *A. flavus* provided us insights into the host–pathogen interactions. In the study of sorghum leaf spot fungus, a similar method was applied successfully [42]. In our study, the pathogen transcriptome analysis indicated a large number of DEGs were related to transporters (such as ABC transporters). Transporters imply important roles in fungal nutrient uptake as well as in signal transduction. Many transporter proteins detected in the present study were an integral part of the membrane proteins. The results suggested that these proteins might play a very important role in inter-kingdom cross-talks [7].

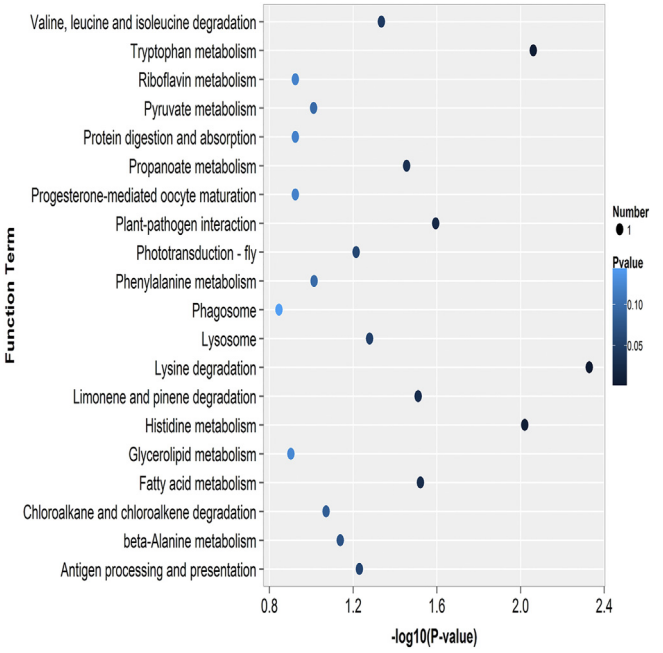

Fig. 4. KEGG analysis proteins differentially expressed in *A. flavus*-resistant peanuts. KEGG enrichment of annotated DEPs in treated peanut seeds. The Y-axis shows the KEGG pathway, and the X-axis indicates the Rich factor. A low p value is represented by a darker shade of blue.

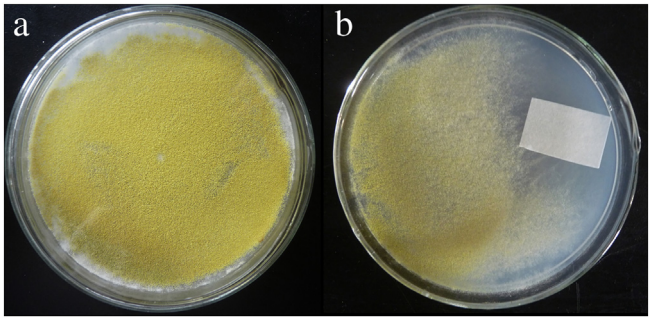

Fig. 6. Fungistasis test in vitro experiment with the *hevamine-A* protein. Control (a) and *hevamine-A* (b)-treated *A. flavus* colonies.

5. Conclusions

Comparative transcriptomic and proteomic profiling was applied to the elucidation of defense mechanisms against *A. flavus* invasion in peanuts. Based on the results, 663 DEGs and 314 DEPs were identified between the infected peanut specimens and the normal controls. Although the transcriptomic and proteomic data showed little overlap, the examination of the differential candidates revealed the possible involvement of different defense mechanisms, including, but not limited to, the induction of oxidative stress, cell wall remodeling, inhibition of proteolysis, and direct secretion of antimicrobial agents.

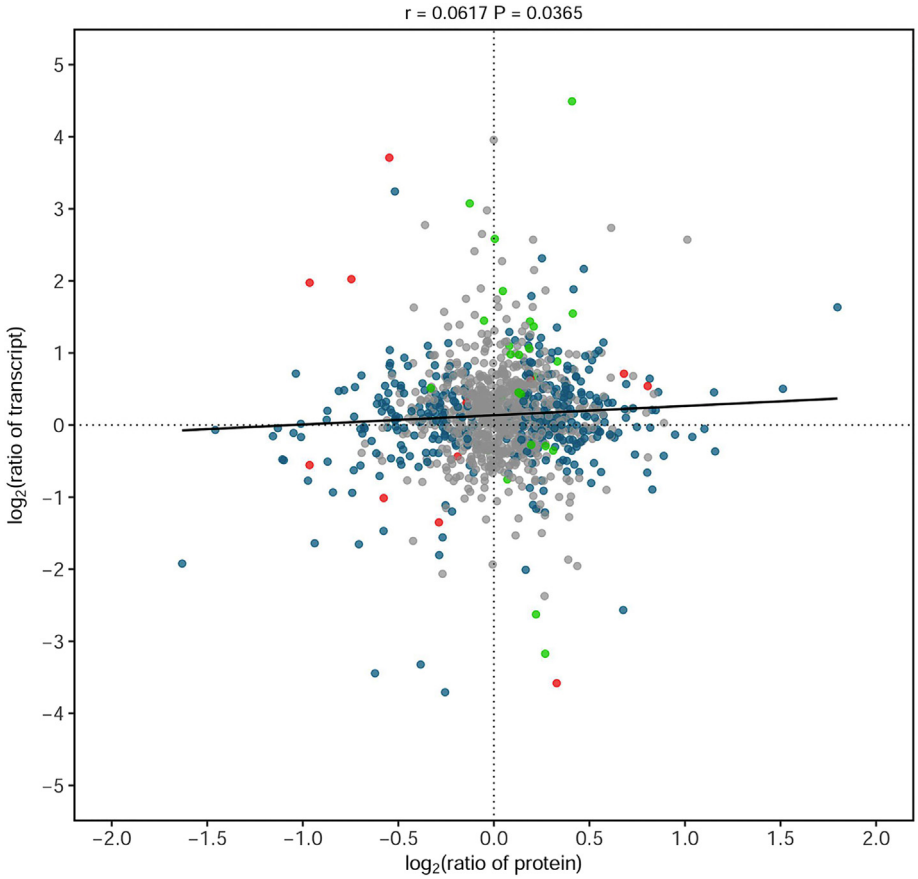

Fig. 5. Correlation between proteomic and transcriptomic data. Comparison of transcript (y-axis) and protein (x-axis) expression ratios from *A. flavus*-resistant peanuts. Log_2 expression ratios were calculated from the control and treated groups. Significant expression changes are color coded: blue, change only in protein levels; green, change only in transcript levels; red points, both protein and transcript levels changed. The fitted straight line represents the trend obtained from the comparison.

Analyses of some responsive genes were also performed in this study, such as TFs, and PRs. This confirmed that peanuts could mobilize a diverse range of defense strategies upon infection. Future functional analysis of the responsive genes will provide a better understanding of the molecular mechanism of defense against *A. flavus* invasion in peanut and will facilitate identifying major candidate genes for improving resistance to *A. flavus* invasion.

Declarations of interest

None.

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Supplementary material

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