Comparative Transcriptome Analysis of Genes Involved in Penicillium Chrysogenum Induced Resistance to Root-knot Nematode in Tomato

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Research article

Keywords: Tomato, Meloidogyne incognita, Penicillium chrysogenum, induced resistance, transcriptome analysis, RNA-seq

Posted Date: December 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-117883/v1

Comparative transcriptome analysis of genes involved in *Penicillium chrysogenum* induced resistance to root-knot nematode in tomato

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Abstract

Background: Tomato root-knot nematode is a soil-borne disease caused by *Meloidogyne incognita*. Enhancement of natural plant-defense mechanisms to provide resistance against pathogens may be a promising alternative environmentally friendly nematode management approach. Recently, the biocontrol effect against different pathogens in the presence of *Penicillium chrysogenum* has been reported in a wide range of plants and pathogens. For understanding the molecular mechanisms of the resistance induced by *P. chrysogenum* Snef1216 to RKN, transcriptomes of inducer control 'IRCK' (induced by Snef1216 only) and pathogen + inducer 'IRN' were compared to those of control groups, namely negative control 'CKCK' (no inoculum), pathogen control 'CKN' (inoculum of RKN only).

Results: Numerous high-quality reads were generated by Novogene, means of the RNA-seq method. After being aligned to the reference genome, four comparative transcriptomic profile maps between any pairwise comparisons were obtained to find significantly differentially expressed genes (DEGs) and three databases of induced resistance (IR)-related, nematode pathogenesis (NP)-related and basal resistance (BR)-related genes were gotten. By hierarchal clustering, the phylogenetic relationship between highly and fewer DGEs were obtained and classified the resistance and susceptible responses into two clusters after inoculation with RKN. Finally, the results were verified by RT-qPCR and analysis of important plant defense enzymes.

Conclusions: Within an integrated and more sustainable management approach, the use of biocontrol organisms, like *P. chrysogenum*, seems to be a promising alternative.

Keywords: Tomato, *Meloidogyne incognita*, *Penicillium chrysogenum*, induced resistance, transcriptome analysis, RNA-seq

Background

Meloidogyne spp. are pathogenic sedentary RKN, which have a broad host range and sometimes even display symptoms of nutrient deficiency, particularly for nitrogen[2], the damage potential cause by them can be as high as 100% in various crops including tomato[3]. Tomato (*Solanum lycopersicum*) is one of the most economically important crops throughout the world. RKN is a very important disease of tomatoes and causes severe reductions in yield and quality in many parts of the world. It forces producers to seek alternative strategies to control nematode because of the restriction on the use of nematicides around the world[5]. In terms of plant protection, to provide resistance against pathogens by enhancing the mechanisms of natural plant-defense is a potential approach[6]. In addition to a broad spectrum of direct and indirect approaches to protect crops from damage, the concept of induced resistance (IR) by natural plant activators will be a widely used method and replace

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nematicides as sustainable management methods[7, 8]. Within an integrated and more sustainable management strategy the use of biocontrol organisms, like *Penicillium chrysogenum*, seems to be a promising alternative. The biocontrol effect of *P. chrysogenum* against different pathogens has been observed in a wide range of plants and seems to involve both localized and systemic resistance in different forms and different mechanisms in three active forms mainly, like **PAF**, an antifungal protein from *P. chrysogenum*, which was a_cysteine-rich, cationic and low weight molecular[9], like **Pen**, an aqueous extract, which was from the dry mycelium of *P. chrysogenum*, a non-pathogenic ascomycete[10, 11],_and like the dry mycelium of *P. chrysogenum* (**DMP**), a waste product, which was from the pharmaceutical industry after inactivating by high temperature, which was generated by the residual product after producing penicillin[12]. To sum up, the possible action modes of *P. chrysogenum* may be distinguished into four categories just as Whipps described[13], as followed: (i) changing or improving the nutrition, morphology and development of the plant[12], (ii) competing directly or suppressing the pathogen[9], (iii) altering the biochemical related with mechanisms of defense response and IR in plant[10, 11, 14, 15], and (iv) evolution of an antagonistic microbiota in the rhizosphere[16, 17]. Additionally, its application in agriculture has been promoted to some extent in recent years[18].

Over recent years, the biocontrol effect against different pathogens in the presence of *P. chrysogenum* has been reported in a wide range of plants and pathogens, thus providing evidence for an indirect effect of *P. chrysogenum* on nematode infection through the modulation of host plant metabolism, but the data available concerning the application of *P. chrysogenum* to control nematode is little so far. Gotlieb *et al.* (2003) reported that DMP enhanced plant growth and caused a dose-dependent reduction in root galling index to protect tomato and cucumber plants against the RKN *M. javanica* in the field, besides, and the protection does not operate via IR[19]. In 2009, Siddiqui *et al.* demonstrated that *P. chrysogenum* used alone and in groups of two (*Aspergillus niger*, plant growth-promoting rhizobacteria, arbuscular mycorrhizal fungi) could promote plant growth and reduce the reproduction of *Meloidogyne incognita* in tomato in the glasshouse for the first time[20]. So, the modes of action of *P. chrysogenum* -IR against nematodes remain unknown to a higher degree.

Resistance breeding is one of the most efficient strategy for managing root knot nematode currently, especially on Solanaceous plants[21]. So far, many genes related to RKN resistance have been identified in plants, such as *Mi-1, Mi-3, Mi-7, Mi-8, Mi-9, CaRKNR, Rk, CaSn, Me1, Me3, Me4, Me7, Mech1, Mech2, rkn1, GHWTR1, MIC3, Ma* and *CaMi*[21-33]. Moreover, some of them have been applied to transgenic plants successfully. Besides, since the first RKN-resistance genes *Mi-1*, which is from *Solanum peruvianum*, which encodes NB/LRR protein with a novel amino-terminal domain, and which is located on chromosome 7ALz[22], was identified and cloned in tomato, and since the first detailed transcription analysis of giant cells induced by RKN infecting was reported[34], more and more researchers have made an effort to explore new resistant genes, virulent genes, and mechanisms related to responses to plant-RKN infection by studying differentially expressed mRNA. Through the comparison between the transcriptome profiles of compatible and incompatible interactions between tomato plants and RKN, it was discovered that a gene encoding glycosyltransferase was regulated by nematode infection significantly, and was necessary for resistance to root knot nematode in the resistant (*Mi-1*) cultivars 'Motelle' [35]. At present, RKN resistance utilized for commercial tomato cultivars is conferred only by the *Mi* gene, which has a more restricted spectrum and a reduced efficiency at high temperature. For controlling the disease, novel methods were needed to develop. Therefore, it is demanded to better understand the interaction molecular modes between plant and the pathogen in tomato. To achieve the goal, it is essential to discover the variation of gene expression in infected tomato. In turn, it will contribute to the employment of genes which are both crucial in susceptible and resistant responses to RKN. In addition, it may be an alternative mean to the development of cultivars which are with persistent resistance that to take advantage of multiple resistant genes or to knock down (out) crucial

susceptible genes in resistance breeding. Due to different genetic backgrounds of incompatible and compatible host plants, cross-comparisons among different treatments of resistant and susceptible plants cannot be achieved, like the commonly or differently expressed genes between the resistant plants with no inoculation and susceptible plant after inoculation.

Although some investigations on resistance-related genes of resistant lines or pathogenicity-related genes of susceptible lines have been performed respectively, there has not been a systematic study on the mechanism of induced resistance to RKN and the pathogenic mechanism of RKN in the same line, moreover reflecting the natural immune mechanism of plant to a certain extent at the same time. The development of natural genes means a new disease-resistant breeding pathway, which reduces the difficulty to some extent; means no instability and no potential danger of transgenes to some extent; means avoiding the embarrassing situation that the fruit taste and plant resistance are opposite to some extent. Previously, *P. chrysogenum Snef1216* was screened by seed-coating which showed a high activity of IR to RKN in tomato, and the control effect was verified by splitting the root in our earlier researches[36]. The histopathological study indicated that the giant cell in tomato roots treated by *Snef1216* fermentation broth with a slow metabolism cannot provide sufficient nutrition for the further development of nematodes. So, the treatment could reduce the second-stage juveniles (J2) number of initial infecting as well as restrain the development of nematode[37]. These findings indicate that *Snef1216* could be a promising biocontrol agent. However, no information is available regarding the molecular mechanism of *Snef1216* inducing resistance to RKN.

In the present study, next-generation high-throughput RNA-seq was used to monitor and compare DEGs in tomato inoculated with RKN or not with those in a control group, and our objectives were (i) to investigate and compare, using comparative transcriptome analysis, the global gene expression changes in *Snef1216* fermentation broth seed-coating tomato roots with those in a control group inoculated with and without RKN, (ii) to identify and validate the potential genes which involved in *Snef1216* induced RKN resistance and which involved in susceptible responses, (iii) to perform biochemical analysis of the enzymes involved in the disease response pathways during compatible and incompatible interactions, (iv) to know about possible plant natural immune mechanism. The results revealed a large number of DEGs of *Snef1216*-induced tomato roots infected by RKN with control groups, and have the potential to assist in the development of new disease control strategies and resistance breeding. All the knowledge on the underlying mechanisms of *P. chrysogenum Snef1216*-IR against plant-parasitic nematodes could lead to the improvement of the use of *P. chrysogenum* as biocontrol organisms.

Methods

Biological Materials

Plant Materials and Growth Conditions

Tomato seeds (*Solanum lycopersicum* L. cv. L-402, which is a susceptible tomato cultivar to *M. incognita* [38], and which was purchased from Liaoning Horticultural Seedling Co., Ltd.) were sterilized with ethanol (75% v/v) for 1 min, and then disinfected with sodium hypochlorite (2.5% v/v) for 1 min. To wash seeds with sterile water for 5 times was followed after each sterilization step. Subsequently, to coat dry seeds with *P. chrysogenum Snef1216* fermentation broth (control seeds were sterilized similarly, but coated with sterile water) in a volume ratio of 1:4 (*Snef1216*: seeds) and to air-dry in a dry and ventilated place. Tomato plants were grown in 1L pots which were filled with substrate mixture in a volume ratio of 1:1:1 (sand: loam: potting soil) and kept in the greenhouse conditions with 75% relative humidity and 20-27℃ day/night. After four true leaves were fully expanded, RKN were inoculated to the roots of L-402 seedlings.

Nematode Inoculum

The inoculum of RKN, *M. incognita*, originally isolated from naturally infected tomato for the experiments was obtained by pure culture raised from a single egg mass and maintained on the roots of *M. incognita* susceptible tomato cultivar L-402 under greenhouse conditions. The total root system of the infected plants was immersed in water and removed

adhering soil by gentle washing after being uprooted from soil. Second-stage juveniles (J2) of *M. incognita* were obtained from egg masses which were hand-picked from roots infected with RKN in tomato cv. L-402. For dissolving the gelatinous matrix, egg masses were placed in sodium hypochlorite (0.5% v/v) after rinsing with sterile water and agitating for 4 min, followed by washing with sterile water on a sieve which was with 26mm pores. According to the modified Baermann funnel method, freshly hatched J2 were obtained and in use for three days. After being watered to field capacity, per plant were inoculated with 2000/5ml (J2/water) in five holes which were 1cm deep and around the stem base in the potting substrate. In the control experiment, 5ml of sterile water substituted the nematode suspension with similar treatment. The experiment was made up of four treatment, and organized in a design which were randomized completely, namely negative control 'CKCK' (no inoculum), pathogen control 'CKN' (inoculum of *M. incognita* only), inducer control 'IRCK' (induced by *P. chrysogenum* only) and pathogen + inducer 'IRN' (induced by *P. chrysogenum* and inoculum of *M. incognita*). 15 days after inoculation (dpi), five plants in each group were selected randomly, followed by root collection and gentle washing for removing attached substrate. RNAs were extracted from the roots of each group after being combined, immediately frozen and stored at -80℃, and the samples were labeled.

RNA Extraction, Library Construction, and RNA-Seq

RNA extraction, library construction and RNA-seq were operated following the method of Liu *et al.* with modifications[39]. RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked by using the Nano Photometer spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured by using Qubit RNA Assay Kit in Qubit 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was assessed by using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). A total amount of 3μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA by using poly-T oligo-attached magnetic beads. Fragmentation was carried out by using divalent cations under elevated temperature in NEBNext First-Strand Synthesis Reaction Buffer (5X). First strand cDNA was synthesized by using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H). Second-strand cDNA synthesis was subsequently performed by using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/ polymerase activities. After adenylation of 3' ends of DNA fragments, NEB Next Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 150~200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37℃ for 15min followed by 5min at 95℃ before PCR. Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

Sequence Data and Differentially Expressed Gene Analysis

The data of sequence and the expression of differential genes were analyzed by using the method described by Liu *et al.* with modifications[39]. Raw data (raw reads) of fastq format were firstly processed through in-house perl scripts. In this step, clean data (clean reads) were obtained by removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data. At the same time, Q20, Q30 and GC content clean data were calculated. All the downstream analyses were based on the clean data with high quality. The *Solanum lycopersicum* reference genome (ftp://ftp.ensemblgenomes.org/pub/release-23/plants/fasta/solanum_lycopersicum/dna/) and gene model annotation files

(ftp://ftp.ensemblgenomes.org/pub/release-23/plants/gtf/solanum_lycopersicum/) were downloaded from the genome website directly. Index of the reference genome was built by using Bowtie v2.2.3 and paired-end clean reads were aligned to the reference genome by using TopHat v2.0.12, and generated a database of splice junctions based on the gene model annotation file. HTSeq v0.6.1 was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis of two groups was performed by using the DESeq R package (1.18.0). DESeq provided statistical routines for determining differential expression in digital gene expression data by using a model based on the negative binomial distribution. The resulting *P*-values were adjusted by using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted *P*-value <0.05 found by DESeq were assigned as differentially expressed.

Functional Analysis of Differentially Expressed Genes

The functional analysis of DEGs were carried out by applying the method of Liu *et al* with modifications[39]. Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the GOseq R package, in which gene length bias was corrected. GO terms with corrected *P*-value less than 0.05 were considered significantly enriched by differentially expressed genes[40], which was categorized in biological processes, cellular component, and molecular functions. Differentially expressed genes were also subjected to KOBAS analysis by using KOBAS software[41-43] to test the statistical enrichment of differential expression genes in Kyoto Encyclopedia of Genes (KEGG) pathways (http://www.genome.jp/kegg/). Protein-Protein Interaction (PPI) analysis of differentially expressed genes was based on the STRING database, which was knew and predicted protein-protein interactions. For the species existing in the database, the networks were constructed by extracting the target gene list from the database. Additionally, Blastx (v2.2.28) was used to align the target gene sequences to the selected reference protein sequences, and then the networks were built according to the known interaction of selected reference species. The Cufflinks v2.1.1 Reference Annotation Based Transcript (RABT) assembly method was used to construct and identify both known and novel transcripts from TopHat alignment results. Alternative splicing events were classified to 12 basic types by the software Asprofile v1.0. The number of AS events in each sample was separately estimated. Picard-tools v1.96 and samtools v0.1.18 were used to sort, mark duplicated reads and record the bam alignment results of each sample. GATK2 (v3.2) software was used to perform SNP calling.

Quantitative Real-Time PCR (RT-qPCR) Validation of RNA-Seq Data

According to the protocol of manufacturer, the Ultrapure RNA Kit (CWBIO) was used to extract the total RNA of the collected samples. Then the cDNA of the first-strand was synthesized by applying the PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara) in accordance with the instructions of manufacturer, followed by measuring the concentration of cDNA by the NanoVue Plus instrument (BIOCHROM LTD CAMBRIDGE, ENGLAND). Primers of tomato actin genes as the control and 20 interested genes which may play a role of plant defense for RT-qPCR as listed in Table S1, were designed by using the Primer Premier 5.0 software. TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara) was used to perform the RT-qPCR reactions on a CFX96 Real-Time PCR Detection System (BIO-RAD) platform. The amplification was made up of one cycle of 95℃ for 30s, followed by the denaturation which consisted of 40 cycles of 95℃ for 30s, annealing and extension at 60℃ for 30s. At the last step of each cycle, fluorescent products were detected. For ensuring the proper of amplification of target fragments, a melting curve was examined after all cycles. Subsequently, the $2^{-\Delta\Delta CT}$ method was used to normalize and calibrate the relative expression levels[44]. Three technical replicates and three biological replicates were performed of all reactions of interested genes.

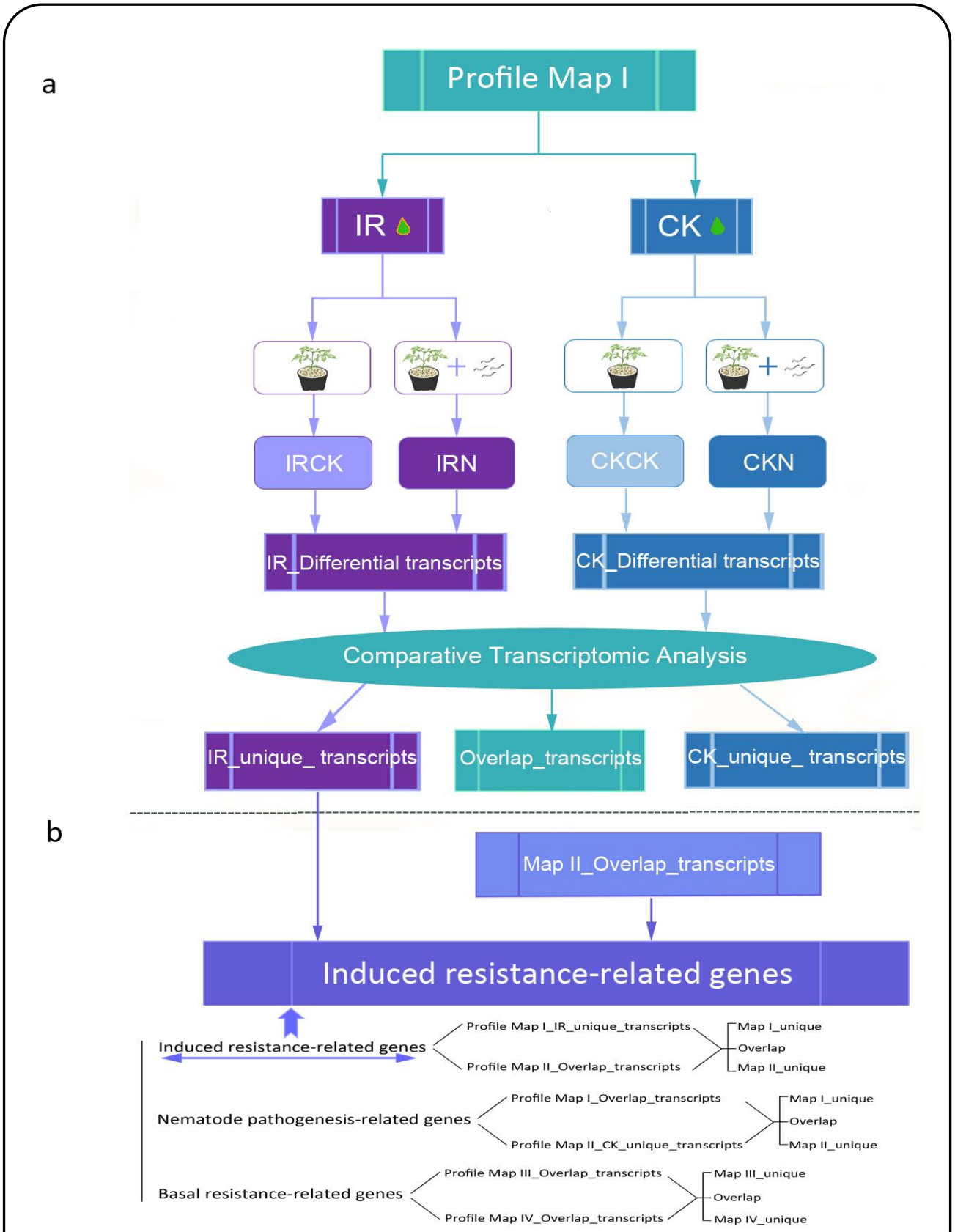

Fig. 1 a. One of the profile maps for comparative transcriptomic analysis, namely profile map I— (IRN vs IRCK) vs (CKN vs CKCK). Firstly, constructed mRNA-seq libraries of different treatments; Secondly, generated transcriptome data of each mRNA library; Thirdly, obtained differential transcripts of induced resistant (IR) group and control (CK) group, respectively; Finally, comparatively analyzed between the IR and CK groups, and obtained their own and shared transcripts. The remaining few were profile map II— (IRCK vs CKCK) vs (IRN vs CKN), profile map III— (IRCK vs CKCK) vs (CKN vs CKCK), profile map IV— (IRCK vs CKCK) vs (IRN vs IRCK), respectively. **b. Graphic presentation of the mapping design of results analysis.**

Assay of Defense Enzyme Activities

The methods of seed treatment, nematode inoculation and sampling in tomato were as described above, and the sampling times were selected at two-leaf stage, pre-transplant, 0, 5, 10, 15, 20, 25, and 30 dpi, respectively. phenylalanine ammonia lyase (PAL) activity was estimated with L-phenylalanine as the substrate by using the method of Chen *et al.* with modifications[45]. PAL specific activity was monitored at 290 nm. Peroxidase[4] activity was determined following the oxidation of guaiacol in the presence of hydrogen peroxide following the method described by Yan *et al.* with modifications[46]. POD activity was expressed as protein changes in absorbance at 470 nm. Polyphenol oxidase (PPO) activity was examined spectrophotometrically following the oxidation of catechol by applying the method of Mohammadi and Kazemi with modifications[47]. The absorbance was read at 398 nm against the blank solution, which included the same volume of reaction mixture but replacing the enzyme extract with buffer. One unit of enzyme activity was defined as the change in the OD value of 0.1, and the activity is expressed in U/g. Calculation formula:

$$\text{The enzyme activity (U/g)} = \frac{\Delta OD \bullet V_R \bullet V_G}{0.1 \bullet A \bullet T \bullet G}$$

ΔOD: change in absorbance before and after reaction

V_R: total volume of the reaction system

V_G: total volume of enzyme crude extract

A: dosage of enzyme solution used in the measurement

T: reaction time (min)

G: fresh weight of root (g)

Results

In order to observe the influence of the *P. chrysogenum Snef1216*-induced expression level of the genes involved in resistance against the *M. incognita* in L-402 tomato, four cDNA samples of the IR, namely IRCK and IRN as well as CK, namely CKCK and CKN were extracted at 15 dpi, then 4 comparative transcriptomic profile maps between different pairwise comparisons were obtained (Fig.1a). Subsequently, three databases of IR-related genes (resistance), NP-related genes (pathogenicity) and BR-related genens were gotten by analyzing the result, respectively.

Basal resistance is a defense response which is unsuccessful eventually to infection with a virulent pathogen in plant, which is believed to be triggered by recogniting the pathogen-associated molecular patterns in host, with inhibition of particular components by pathogen effectors subsequently[48]. The graphic presentation of the analysis roadmap was shown in Fig.1b.

Sequence Analyzing and Aligning to the Reference Genome

After constructing the mRNA-seq libraries of different treatments, sequencing was completed by Illumina mRNA-Seq technology. For further analysis, high-quality reads of approximately 460-560 million (M) reads with 95% Q30 bases (those with a base quality greater than 30) were selected following stringent quality assessment and data cleaning. An average 'G+C' content of above 40% was found. Of the selected reads, 87.63, 89.89, 90.24, 89.94% from the IRN, CKN, IRCK and CKCK samples were

TABLE 1 | Summary of read numbers aligned onto the tomato reference genome.

Statistical content	IRN		CKN		IRCK		CKCK	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Total reads	49703467	100%	55484460	100.00%	46017771	100.00%	47091116	100.00%
Total mapped	43693519	87.63%	49866552	89.89%	41531857	90.24%	42349546	89.94%
Multiple mapped	256227	0.51%	338631	0.60%	236570	0.52%	263266	0.56%
Uniquely mapped	43437292	87.12%	49527921	89.30%	41295287	89.73%	42086280	89.38%
Reads map to '+'	21717016	43.55%	24752173	44.63%	20625771	44.81%	21021399	44.65%
Reads map to '-'	21720276	43.56%	24775748	44.67%	20669516	44.91%	21064880	44.74%
Non-splice reads	29207866	58.59%	32167624	58.34%	25212817	54.76%	25859408	54.92%
Splice reads	14229426	28.52%	17360297	30.96%	16082470	34.97%	16226872	34.46%

aligned onto the tomato reference genome and matched either unique or multiple genomic locations (Table1).

Analysis of Differentially Expressed Genes

To study the DEGs between different pairwise comparisons, genes of twofold up-regulated and twofold down-regulated employing adjusted *P*-value <0.05 found by DESeq were identified. In the case of IR-related group, a total of 1504 DEGs were identified, of which 419 were up-regulated, whereas 1085 genes were found to be down-regulated. Correspondingly, a total of 534 genes were found to be differentially expressed in NP-related group. Of these, the up- and down-regulated respectively were 87 and 447. In total, in the case of BR-related group, 732 DEGs were identified, of which 236 were up-regulated and 496 were down-regulated. Moreover, the database of the induced resistance-related group which were obtained from two parts of Map I_IR _unique transcripts and Map II _overlap transcripts, and the nematode pathogenesis-related database which come from two parts of Map II_CK _unique transcripts and Map I _overlap transcripts were all independent and did not intersect each other, respectively. In IR-related database, only two down-regulated genes were from Map II _overlap transcripts, the rest all came from Map I_IR _unique transcripts, of which 419 were up-regulated and 1083 were down-regulated. In the case of NP-related database, 4 up-regulated and 14 down-regulated were from Map II_CK _unique transcripts. All the rest came from Map I _overlap transcripts. Of these, up- and down-regulated were 83 and 433, respectively. However, the situation was different in the last database, which was derived from the two parts of Map III _overlap transcripts and Map IV _overlap transcripts. Of it, 236 genes were uniquely up-regulated and 491 genes exclusively downregulated in Map III _overlap transcripts. Similarly, in the Map IV _overlap transcripts, unique up- and down-regulated genes were 3 and 28, respectively. In addition, 3 up-regulated and 23 down-regulated genes were shared between the two parts in all. (Fig.2, Supplementary Table S1,2,3)

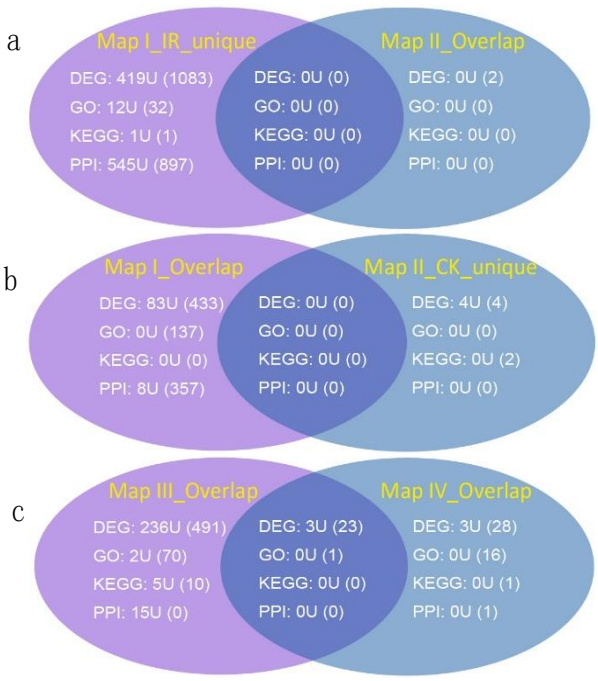

Fig. 2 Venn diagrams of differential transcripts in (a) induced resistance-related, (b) nematode pathogenesis-related and (c) basal resistance-related groups following *M. incognita* inoculation at 15day post inoculation (dpi). Numbers in parentheses indicate down-regulated

Functional Annotation and Classification of Different Expression Analysis

It was considered that differentially expressed genes (DEGs) were the significant cause of either induced defense response or pathogenic response of nematode, or basal resistance response. To better investigate the biological behavior of these responses, it is necessary to understand the functional distribution of these DEGs. Thus, GO, KEGG, and PPI analyses of the main functional groups of the DEGs were implemented and generated respective databases. In IR-related group, the whole differential expression was from Map I_IR _unique. Overall, 51, 2, 1442 different GO terms, KEGG pathways and PPI respectively were subjected, of which 12, 1, 545 were up-regulated, while 32, 1, 897 were found to be down-regulated. In the case of NP-related groups, the differential up- and down-regulated genes of the above three terms were 0, 0, 8 and 137, 2, 357, respectively. Among them, only two down-regulated KEGG pathways were from Map II_CK _unique transcripts, the rest 0, 0, 8 up-regulated and 137, 0, 357 down-regulated of the above three terms were all from Map I _overlap transcripts. In the BR-related group, 96, 18, 16 of the above three terms were found to be differential. Of these, the up- and

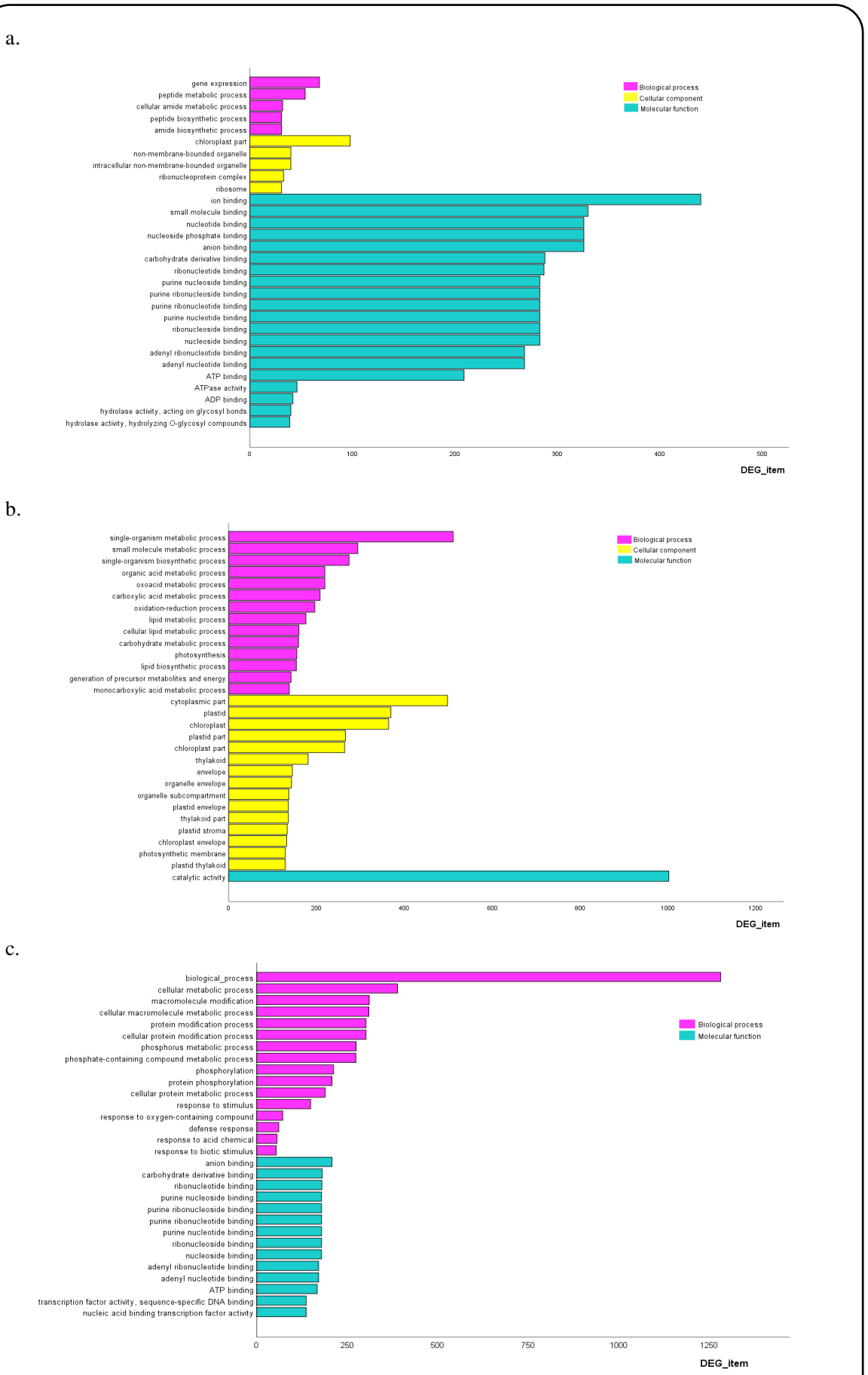

Fig. 3 GO annotations of differentially expressed genes (**a**) GO annotation related to induced resistance, (**b**) GO annotation related to nematode pathogenesis and (**c**) GO annotation related to

down-regulated respectively were 2, 5, 15 and 85, 10, 1 of every term. Additionally, different from the first two groups that had no co-expression, the last database consisted of three parts, some of which were uniquely expressed in Map III _overlap transcripts (2, 5, 15 up-regulated and 70, 10, 0 down-regulated), another part was exclusively expressed in the Map IV _overlap transcripts (16, 1, 1 down-regulated). Furthermore, one down-regulated GO term (GO:0010200: response to chitin) was shared between the two parts. (Fig. 2)

Genes Annotation-Related with Induced Resistance

It grouped genes associated with induced resistance specifically in accordance with their involved pathways. According to Gene Ontology (GO), a gene functional classification system which is standardized internationally, GO terms with corrected *P*-value less than 0.05, which were knows as significant enrichment by differentially expressed genes, were classified into three major functional ontologies. For the unique annotations of IR-related group, 'gene expression' (68) and 'peptide metabolic process' (54) were the main dominant subcategories, followed 'cellular amide metabolic process' (32), 'peptide biosynthetic process' (31), 'amide biosynthetic process' (31) and 'translation' (30) in the category of biological process (BP). For molecular function (MF), 'ion binding' (440), 'small molecule binding' (330), 'anion binding' (326), 'nucleotide binding' (326) and 'nucleoside phosphate binding' (326) were highly represented. Among cellular component (CC) terms, which were almost up-regulated, dominant subcategories were 'chloroplast part' (98), 'intracellular non-membrane-bounded organelle' (40) and 'non-membrane-bounded organelle' (40). Different from the GO terms, there was only one up-regulated (Ribosome) and one down-regulated (Photosynthesis -antenna proteins) differentially expressed KEGG pathways in this group. (Fig.2,3)

Genes Annotation-Related with Nematode Pathogenesis

The annotations exclusively belonging to NP-related group were different. In the BP category, the most represented was 'single-organism metabolic process' (511), following 'small molecule metabolic process' (294) and 'single-organism biosynthetic process' (274). For MF, 'catalytic activity' (1002) were highly represented. In the category of CC, dominant subcategories were 'cytoplasmic part' (498), 'plastid' (369) and 'chloroplast' (364). Furthermore, in this group, only two down-regulated KEGG pathways 'Linoleic acid metabolism' and 'alpha-Linolenic acid metabolism' were differentially expressed. (Fig.2,3)

Genes Annotation-Related with Basal Resistance

For the exclusive annotations of BR-related group, the most DEGs were localized to 'biological_process' (1282) in the BP category. A few DEGs were localized to 'cellular metabolic process' (389). Among MF terms, a large number of DEGs were involved in 'anion binding' (208). In addition, a few DEGs belonged to two functional subclasses, 'carbohydrate derivative binding' (181) and 'ribonucleotide binding' (180). In the CC category, there was only one GO term, which was 'photosystem II' (14). Based on the results of KEGG annotation, most DEGs were localized to 'Metabolic pathways' (1961) and 'Biosynthesis of secondary metabolites' (1036). In addition, a few DEGs belonged to two functional subclasses, 'Plant hormone signal transduction' (301), 'Carbon metabolism' (253), 'Phenylpropanoid biosynthesis' (190), 'Phenylalanine metabolism' (152) and 'Glycolysis / Gluconeogenesis' (122). (Fig.2,3)

Hierarchical clustering

The hierarchical clustering analysis classified the resistant and susceptible responses into two clusters after inoculation with *M. incognita*. By hierarchal clustering, obtained the phylogenetic relationship between highly and less differentially expressed genes. The expression changes among the gene group members in eight clusters: (i) defense-associated response, (ii) signal transduction, (iii) cell death, (iv) transcription factor, (v) oxidoreductases, (vi) defense enzymes activity, (vii) protease inhibitor, (viii) phenylpropanoid metabolism, were represented by different colors in the heat map. The color intensities indicate the expression level of each gene. (Fig.4; Supplementary Table S4)

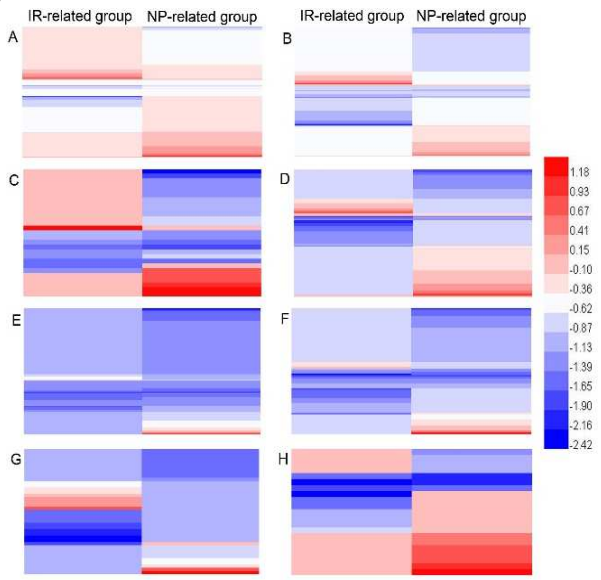

Fig. 4 Expression pattern was represented by the heat map profile of IR-related group (resistance) versus NP-related group (pathogenicity). Color changes show the level of gene expression in their respective clusters. Red, highly expressed genes; blue, highly suppressed genes. The clusters are genes for (A) defense-related response, (B) signal transduction, (C) cell death, (D) transcription factor, (E) oxidoreductases, (F) defense enzymes activity, (G) protease inhibitor, and (H) phenylpropanoid metabolism.

Validation of Differentially Expressed Genes by RT-qPCR

12 DEGs with a putative role in plant defense, which belong to the categories of defense, signal transduction, cell death, transcription factor, oxidoreductases, defense enzymes activity, protease inhibitor or phenylpropanoid metabolism, were selected and examined with specific primers by RT-qPCR to validate the reliability of the RNA-Seq data (Supplementary Table S1). Gene expressions in control plants (CKCK), Snef1216-coated plants (IRCK), nematode-infected plants[1] and Snef1216 nematode-infected plants (IRN) were compared. Then the relative expression of genes in the control group (CKN vs CKCK) and the treatment group (IRN vs IRCK) were obtained. Genes encoding myb-related protein 315-like, peroxidase P7-like, putative disease resistance protein RGA4 showed high up-regulation only in Snef1216-induced nematode-infected roots [49]. Genes encoding disease resistance protein RPP13-like, auxin-responsive protein IAA. 15.4 kDa class V heat shock protein, apoptosis-inducing factor homolog A-like, extra-large guanine nucleotide-binding protein 1-like were higher up-regulated in the treatment group than in control group (Fig.5defgh). The relative expressions of genes encoding protein BREAST CANCER SUSCEPTIBILITY 1 homolog, peroxidase 3, probable glycosyltransferase At3g07620 and transcription factor MYB48 were lower in treatment group than in control group (Fig.5ijkl). According to the expression patterns of RT-qPCR_at 15dpi, the results of the DEGs which were selected randomly were consistent with the RNA-seq data in each of three biological replicates, demonstrating that the expression patterns which were obtained by RNA-seq reflected the real gene expressed patterns in the compatible interaction and induced resistance interaction in tomato.

Biochemical assay of enzymes involved in defense response

Besides transcription level, the possible roles of induction and inoculation at the translational level were also examined. Thus, the activities of different important defense enzymes were measured in CKCK, CKN, IRCK and IRN plants at two-leaf period, pre-transplanting period (four-leaf period), 0, 5, 10, 15, 20, 25 and 30dpi individually, and compared between different treatments to evaluate the influences of induction and RKN infection on plant defense response enzymes.

Both induction and inoculation improved PAL activity, while increased enzyme activity caused by induction only (IRCK) faster than nematode inoculation (CKN or IRN). PAL activity began to drastically increase at 5dpi in IRCK. Compared with it, the enzyme activity grew slowly at 5dpi in IRN, and even more so, the activity of PAL was continuously decreasing at the same time in CKN. In the latter two, the activity started to elevate at 10dpi. Additionally, the activity rose to peak simultaneously in all three treatments at 15dpi, and declined thereafter. At 25dpi, the activity was continuously falling in CKN, or did not change drastically in IRCK, while reached a small maximum peak in IRN. In CKCK, the activity of PAL had been continuously stable. Besides, before inoculation, the changes of PAL activity had remained

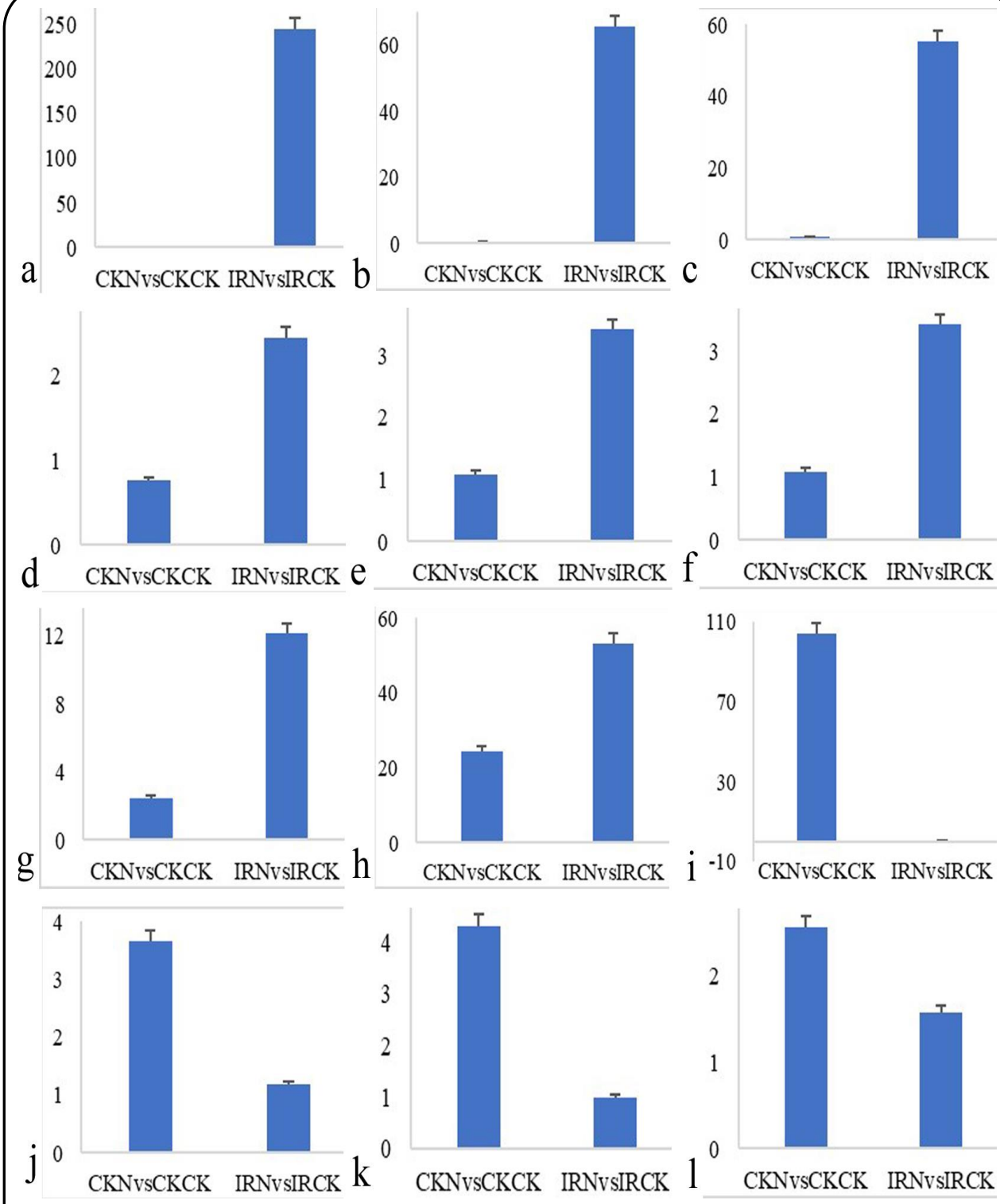

Fig. 5 Validation of RNA-seq data by RT-qPCR of selected genes from tomato subjected to RKN (*Meloidogyne incognita*) infection with or without induction. The bars indicate relative gene expression of the control values (CKN vs CKCK) in non-induced plants with or without inoculation and the treatment values (IRN vs IRCK) in induced plants with or without inoculation, respectively. (**a**) myb-related protein 315-like, (**b**) peroxidase P7-like, (**c**) putative disease resistance protein RGA4, (**d**) disease resistance protein RPP13-like, (**e**) auxin-responsive protein IAA, (**f**) 15.4 kDa class V heat shock protein, (**g**) apoptosis-inducing factor homolog A-like, (**h**) extra-large guanine nucleotide-binding protein 1-like, (**i**) protein BREAST CANCER SUSCEPTIBILITY 1 homolog, (**j**) peroxidase 3, (**k**) probable glycosyltransferase

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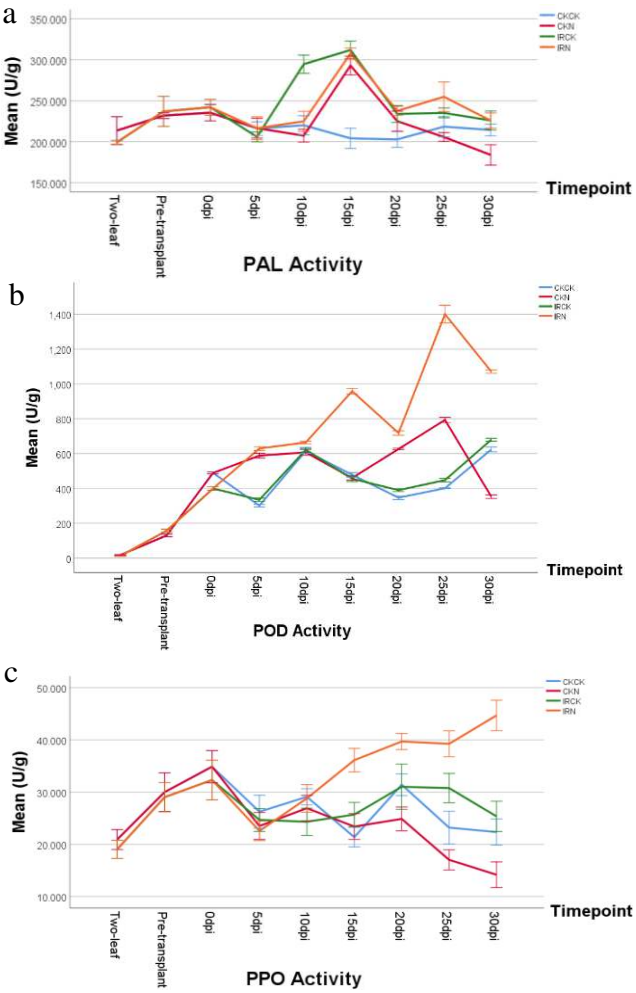

Fig.6 The activities of different enzymes, as measured in the crude extract prepared from roots harvested at various stages after *M. incognita* inoculation in *Snef1216* induced plants (IRN), were compared with those in non-induced plants [1] and their respective mock-inoculated

gentle and had no significant differences in all treatments. (Fig.6a)

The time-courses of peroxidase[4] activity were similar between induction and control, just as between IRCK and CKCK, or between IRN and CKN. Without inoculation, there were two minimum peaks (5dpi and 20dpi) and one maximum peak (10dpi) in IRCK and CKCK, and the whole values between the two treatments were almost consistent. After inoculation, the POD activity as a whole was higher than when no nematodes were inoculated, and the activity in IRN was further higher than that in CKN. The similarity of the enzyme activities between IRN and CKN was that they all indicated an early and sharp induction at 5dpi and reached the maximum at 25dpi, while the most difference between the two treatments was at the interval from 10dpi to 20dpi. The enzyme activity was a maximum peak at 15dpi in IRN, while it reached a minimum at the same time in CKN. (Fig.6b) The activity of polyphenol oxidase (PPO) in IRN plants, which was already high, began increasing after 5dpi and thereafter elevated consistently up to 30dpi. Although the activity of PPO was also higher in the IRCK, it did not perform any evident promotion after 5dpi compared with control treatments (CKN and CKCK). However, in no induction plants, the enzyme was slightly increased at 10dpi but decreased immediately until 15dpi, and was continuing to decline after 20dpi. (Fig.6c)

Discussion

At present, some studies have been conducted on the molecular interaction between tomato and nematode, and some progress has been made, while the research is not thoroughly enough[35, 50, 51]. Studies involving plant-nematode interactions provide an opportunity to elucidating plant defense signaling in root tissues, and this research field has usually received little attention as yet. Here was the first study by omics to research the mechanisms of induced resistance to RKN and pathogenicity of RKN in the same variety systematically, and to reflect plant autoimmunity to a certain extent. To develop the autoimmune genes is to develop a new disease-resistant breeding pathway, which reduced the difficulty to some extent; which has no instability just like transgenic technology, and avoided the potential danger of transgenes to some extent; to some extent, which avoided the embarrassing situation that the fruit taste and the plant resistance are opposite.

After examining the differential expression of genes, it demonstrated that the down-regulated genes were obviously more than the up-regulated, which is similar with the findings of Schaff et al, who found that after feeding sites (the position during reproduction of nematode in roots and in infected roots with mixed stage nematode) was established, there were substantially less genes up-regulated than repressed in tomato[35]. It was also consistent that the conclusion made by Jammes *et al.* after analyzing

DEGs in roots infected with RKN at weekly intervals post-infection in Arabidopsis[52].

For understanding the functional distribution of DEGs to better investigate the biological behavior of these responses, functional annotation and classification were implemented and generated. In IR-related group, the whole differential expression was from Map I_IR _unique that 12 up-regulated (mainly related to cell structure and amino acid related metabolism) and 32 down-regulated (mainly involving nucleic acid metabolism / glucose metabolism / JA metabolism related pathways) of GO terms, and one up-regulated (Ribosome) and one down-regulated (Photosynthesis-antenna proteins) of KEGG terms. In the case of NP-related group, the situation was similar in that GO enrichment was uniquely belonged to Map I. In addition, there were only 137 down-regulated, most of which were related to sugar metabolism, such as ‘glyceraldehyde-3-phosphate metabolic process’ / ‘isoprenoid metabolic process’ / ‘pentose-phosphate shunt’ / ‘glucose 6-phosphate metabolic process’ / ‘pyruvate metabolic process’ / ‘starch metabolic process’ / ‘maltose metabolic process’ / ‘glucan metabolic process’ / ‘disaccharide metabolic process’ / ‘cellular polysaccharide metabolic process’ / ‘oligosaccharide metabolic process’ / ‘cellular carbohydrate metabolic process’. Furthermore, in this group, only two down-regulated KEGG pathways (‘Linoleic acid metabolism’ and‘alpha-Linolenic acid metabolism’) were differentially expressed. In the BR-related group, among the GO terms of differential expression, it found that the only 2 up-regulated were associated with transcription factors, and the down-regulated 85 items were almost all related to plant defense. Among the differential expressed KEGG pathways, up- and down-regulated respectively were 5 (the most significant were phytohormone signaling and phenylpropanoid metabolism) and 11 terms (mainly involving energy metabolism: photosynthesis and metabolism of three major nutrients including sugar/fat/amino acid).

The tomato genes *Mi-1.2* (*Solanum lycopersicum*) for resistance to three major RKNs, *Meloidogyne arenaria*, *Meloidogyne javanica*, and *Meloidogyne incognita*[53], which triggers hypersensitive response or localized tissue necrosis, was the first RKN-resistance gene to be cloned[22]. It is a member of the NBS- LRR family and located on chromosome 6[24]. To date, it has reported 9 tomato genes of the *Mi* family[54]. In this study, *Mi-1.2* (Solyc06g008480.1) showed induction specifically during the control of *M. incognita* infection in *Snef1216*-induced tomato roots, suggesting that induction was useful against RKN. *Mi-1.2* is a resistance gene which could offer a significant fitness benefit under heavy pressure of nematode in tomato, just like Corbett *et al.* had found. Under heavy pressure of nematode (200,000 eggs per plant), the fruit production of plants carrying *Mi-1.2* was ten-fold greater than that of plants which was susceptible to nematode, and the seed production in lifetime was almost forty-fold greater in greenhouse[55]. At present, in commercially available tomato cultivars, only the *Mi* gene conferred the resistance to RKN[23], and was used exclusively for 60 years[21]. Therefore, to discover and exploit novel nematode resistance genes is critical for nematode management.

Hsp90 (heat shock protein 90) which is famous for participating early in resistance gene signaling pathways has a critical role in disease-resistant signals[56, 57]. It has indicated that *Hsp90* in solanaceous plants are necessary for specific R-gene-mediated resistance responses using virus-induced gene silencing (VIGS). For instance, *Hsp90* is required for the full function of *Rx*-mediated *Potato virus X* resistance, *N* that provides resistance to *TMV*, *Pto*-mediated resistance to *Pseudomonas syringae* expressing *AvrPto*, and *Mi-1* that confers resistance to potato aphid (*Meloidogyne euphorbiae*) and nematode (*Meloidogyne javanica*) in tomato[57-60]. Thus, the up-regulated Hsp90 of IR-related group in this study may also be required for *Mi-1.2* mediated resistance to *M. incognita* in tomato.

It was confirmed that the expression of glycosyltransferase was the requirement to the expression of the resistance phenotype to RKN by a VIGS approach[35]. In addition, it might act in an all-or-nothing manner, or it might function (or not) to effect resistance via a threshold effect, perhaps as some sort of switch. Glycosyltransferases also have a role in the synthesis of cell wall[61, 62], and may imply a role in resistance to RKN via this function. In this study,

the differentially expressed glycosyltransferase was only present in NP-related group, and was down-regulated.

Expression of genes encoding MYB and WRKY transcription, factors also seems to be related to the priming of defense-related processes in *Snef1216*-induced tomato roots with *M. incognita* inoculation, since they were upregulated in IR-related group, and were downregulated in NP-related group. The differences in the number of MYB domain repeats contained in the proteins of MYB family determine their DNA-binding ability, which in turn affects their functions[63]. They play a key role in several processes, such as regulation of secondary metabolism[64]. In Arabidopsis, AtMYB44 regulated plant defense responses and was pertinent in tolerance to acetylation in the ethylene-signaling pathway after treatment with a harpin protein which was from a bacterial pathogen[65], or caused higher antioxidant enzyme activity and a stronger ROS burst in AtMYB44-overexpressing transgenes compared to wildtype plants following infection with Botrytis[66], such functions may be associated with induced resistance to root knot nematodes.

Other defense-related genes such as those encoding PIs (proteinase inhibitors, namely PR-6) were also specifically induced in the IR-related group (Solyc08g080630.2: Up2.4661). Currently, using proteinase inhibitors is one of the most advanced strategies to defense against nematode, which could hinder nematode feeding cell development by interaction with the digestive system. These protease inhibitors are usually found in plant seeds and are proteins that interfere with digestive enzymes. Both the application of a promoter which was up-regulated and the application of PIs which were specific properly for nematode proteinases[67], could obviously impact the possible system that may regulate the activity of "constitutive" promoter, that is, the promoter was down-regulated locally where (feeding sites) its activity was required, to prevent the production of PIs' effective levels[68]. The most of these nematode resistance proteins should hardly have impact on cells in plant, and therefore there was no necessary to restrict expression to feeding cells. The shortcomings of this method might be to take advantage of virulent pathotypes overcoming the influence of nematode resistance proteins (unlikely for collagenase) or a specificity that was limited to correct nematode species under certain circumstances[69].

While the responses of incompatible interactions are triggered in resistant plants or resistance-treated plants, a series of basal defense responses of compatible interactions are initiated in susceptible plants[35]. Even if they show analogous expression profiles, the occurrence of inducing defense genes in compatible interactions was later than that in incompatible interactions. For example, in the compatible interaction between tomato plants and *Verticillium dahliae*, the basal defense responses were activated, and lead to great amount of plant pathogen interaction, phenylalanine metabolism and phenylpropanoid biosynthesis, and plant hormone signal transduction being induced in tomato[70]. Our results show a similar activation of basal defense response-related genes, suggesting that there may be overlap among the susceptible responses of tomato to fungal pathogens and nematode. In addition, the induced resistance treatment may also launch a basal defense response before inoculation with nematode in plant, just as the forming of a symbiont between mycorrhizal fungi AMF and plants in tomato is thought to predispose the mycorrhizal roots to a more efficient or rapid activation of defense mechanisms after being attacked by a pathogen subsequently, in a way similar to priming[71, 72].

Besides transcription level, the possible roles of induction and inoculation at the translational level, namely the involvement of different important defense enzymes, were also studied and compared between different treatments to evaluate the influences of induction and RKN infection on plant defense response enzymes to support our findings. Plants can secure themselves against phytopathogenic agents by generating a wide range of antimicrobial compounds among which defense-related enzymes, such as phenylalanine ammonia lyase (PAL), peroxidase [4] and polyphenol oxidase (PPO), have been involved in cellular protection and disease resistance, and have been indicated the role in the induction of systemic resistance[73-76]. PAL, POD and PPO are enzymes important in the biosynthesis of phenolic compounds

and lignin, which are not only necessary during plant development, but also are essential in establishing defense against various stresses[77].

PAL is a mediator which involving the biosynthesis of lignin, and is the first important enzyme catalyzing various phenylpropanoid products synthesis from phenylalanine, and is with high expression in resistant plants which have infested, and has been participated in resistance to insect[78]. These phenylpropanoid polymers could be part component of the cell wall structure which could resist the stylet of parasitic nematode through primary physical defense, due to the cell wall could play a role of a protectant to wound, a physical barrier against certain infections, UV damage and flavonoid pigments[79]. The first step which catalyzed by PAL is a regulatory step of the deamination response from phenylalanine to cinnamic acid in the phenylpropanoid pathway. This reaction produced many products involve the resistance to pathogen, including lignin, coumarin, condensed tannins, flavonoids and salicylic acid (SA) as a signaling molecule[80].

POD (PR-9), which is a key enzyme in the biosynthesis of lignin, functions as part of an antioxidant system and represents one of the components of the early defense system invaded by pathogen and pest in host plant, involving degradation of cytotoxic levels of hydrogen peroxide generated in plant tissues due to pathogen and pest attack[81]. Furthermore, it could also be a catalyst to oxidize phenolic compounds, whose accumulation activates a cellular oxidative burst, which is not only toxic to the host but also necessary for resistance against pathogens and pests. POD are further in charge of the cross-linking of the components of cell wall with repression to nematode infestation[76, 82]. It was that the combination of the properties of peroxidase supplied evidence that POD may involve the formation of barrier substances to limit the extent to which the nematodes can feed on an induced resistant plant. In this study, the POD activity of IRN was significantly enhanced compared with other treatments, while peaked at 15dpi and 25dpi when a large number of nematodes had invaded the roots. Thus, the high POD activity could not prevent nematode infestation completely, however, it could be used as an early physiological indicator to determine whether the host was infected with *M. incognita* in tomato.

Plants are capable of protecting themselves from phytopathogenic agents by generating a majority of antimicrobial compounds among which defense-related oxidoreductases, just like POD and polyphenol oxidase (PPOs), have been participated in protection and disease resistance in cell[81, 83]. PPO acts as a catalyst to the oxidation of phenolic compounds to highly toxic quinones and tannins, which are toxic to the growth of pathogenic mycelium. In addition, since phenolic substances are precursors of lignin formation in the cell, PPO can promote the synthesis of lignin from phenols and accelerate cell wall lignification against pathogens which play a crucial role in plant disease resistance[84, 85]. The change of PPO activity is related to disease resistance, and pathogen infection and other external stimuli could induce to elevate PPO activity. The PPO activity of most resistant varieties or sensitive varieties after induction is significantly increased after inoculation[75]. The biosynthesis of POD and PPO plays an important role in determining the degree of host resistance in primed plants with beneficial microbes[86-89].

Conclusion

In conclusion, by concentrating on differentially expressed genes in the IR-related, NP-related and BR-related groups based on RNA-seq approach, new insights were discovered into the molecular mechanisms that underlay the *Penicillium chrysogenum Snef1216*-induced resistance against *M. incognita*, nematode pathogenicity and basal resistance, respectively. Comparative transcriptome analysis can supply significant insights into the global changes in gene expression following induction and nematode infection. RT-qPCR was applied to validate the RNA-seq output, and the enzymes which were participated in defense response had also been analyzed to support our discovery. The higher expressed defense-related genes specifically in the biocontrol interaction, requires the existence of defense responses primed by the induction of *P. chrysogenum*, as has been proposed for the interaction between *P. chrysogenum* and other types of pathogens[10, 11, 16,

17]. It will be advantage to the understanding of the interactions between plant and nematode, and serve for the breeding of nematode resistance cultivars that the illustration of the molecular resistance basic. The breeding of disease resistance cultivar is the most efficient method for the control of RKN, especially in Solanaceous plants[21]. These findings could contribute to the breeding of new tomato cultivars which are resistant to nematode. Overall, within an integrated and more sustainable management approach, the application of biocontrol organisms, like *P. chrysogenum*, seems to be a prospective alternative.

Supplementary Data

Supplementary Table S1. Differentially expressed genes of induced resistance-related groups in *Snef1216*-induced plants following inoculation of *M. incognita*.

Supplementary Table S2. Differentially expressed genes of nematode pathogenesis-related groups in control plants following compatible interaction with *M. incognita*.

Supplementary Table S3. Differentially expressed genes in basal resistance-related groups.

Supplementary Table S4. Differentially regulated genes associated with resistant and susceptible responses of eight categories in *Snef1216*-induced and control plants infected with *M. incognita*.

Supplementary Table S5. Primers used in RT-qPCR validation of gene expression analysis.

Competing Financial Interests

All authors declare that they have no competing financial interests.

Abbreviation

RKN: root-knot nematode; AS: alternative splicing; DEG: differentially expressed gene; IR: induced resistance; NP: nematode pathogenesis; BR: basal resistance; NB/LRR: nucleotide-binding/leucine-rich repeat; J2: second-stage juveniles; CKCK: negative control; CKN: pathogen control; IRCK: inducer control; IRN: pathogen + inducer; dpi: days post inoculation; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes; PPI: protein-protein interactions; RABT: reference annotation-based transcript; SNP: single nucleotide polymorphism; RT-qPCR: quantitative real-time PCR; PAL: phenylalanine ammonia lyase; POD: peroxidase; PPO: polyphenol oxidase; PIs: proteinase inhibitors.

Acknowledgements

The research was funded by Special Fund for Agro-scientific Research in the Public Interest (201103018), National Natural Science Foundation of China (31171823).

Authors' Contributions

M.Z. and Y.D. conceived and designed the experiments. M.Z. performed the experiments, analyzed the data, and wrote the paper. A.S., X.Z., Y.W., X.L., H.F., Y.X., L.C. and Y.D. modified the article. All authors read and approved the final manuscript.

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Received: Accepted:
Published:

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Figures

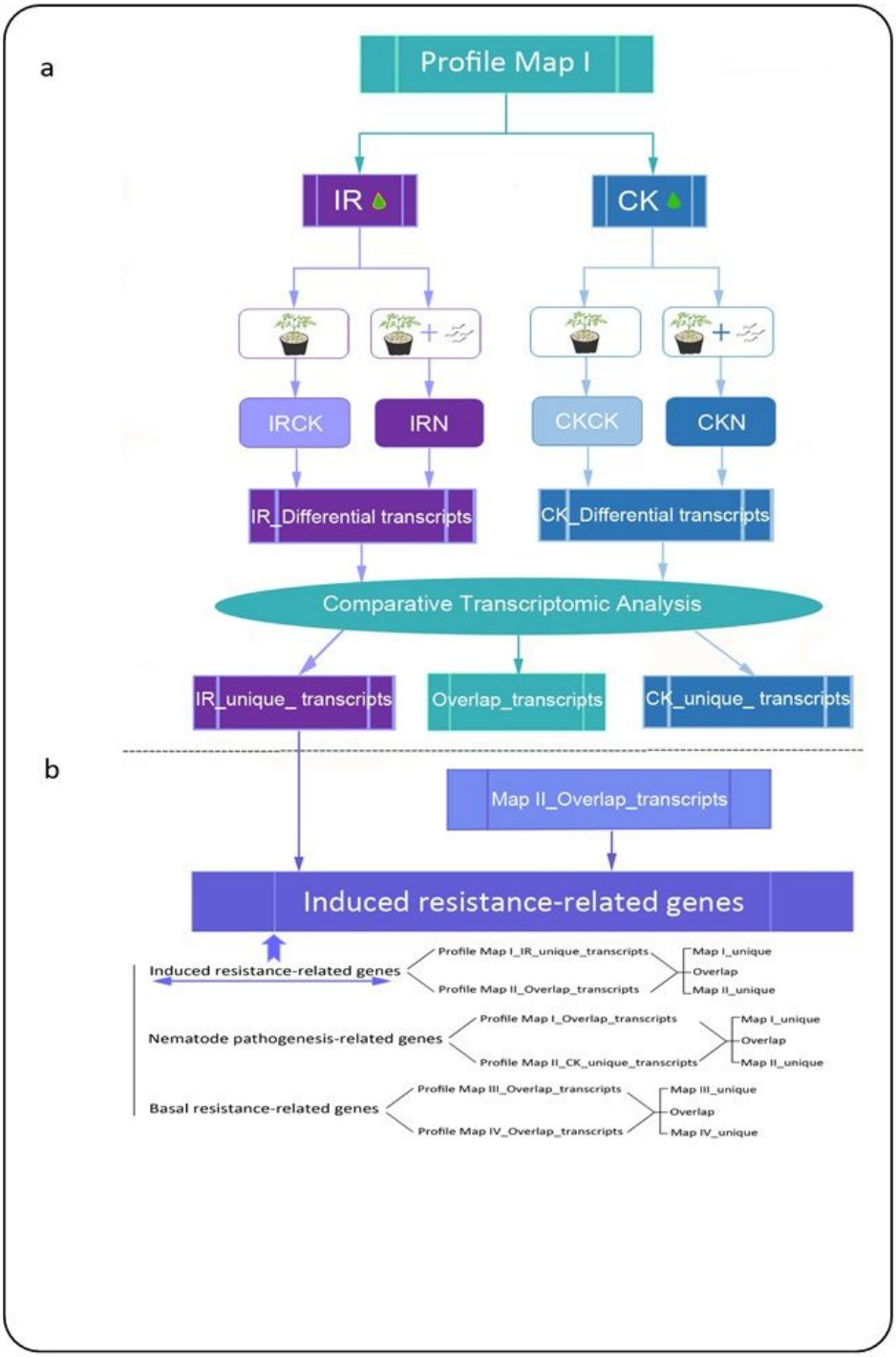

Figure 1

a. One of the profile maps for comparative transcriptomic analysis, namely profile map I— (IRN vs IRCK) vs (CKN vs CKCK). Firstly, constructed mRNA-seq libraries of different treatments; Secondly, generated transcriptome data of each mRNA library; Thirdly, obtained differential transcripts of induced resistant

(IR) group and control (CK) group, respectively; Finally, comparatively analyzed between the IR and CK groups, and obtained their own and shared transcripts. The remaining few were profile map II— (IRCK vs CKCK) vs (IRN vs CKN), profile map III— (IRCK vs CKCK) vs (CKN vs CKCK), profile map IV— (IRCK vs CKCK) vs (IRN vs IRCK), respectively. b. Graphic presentation of the mapping design of results analysis.

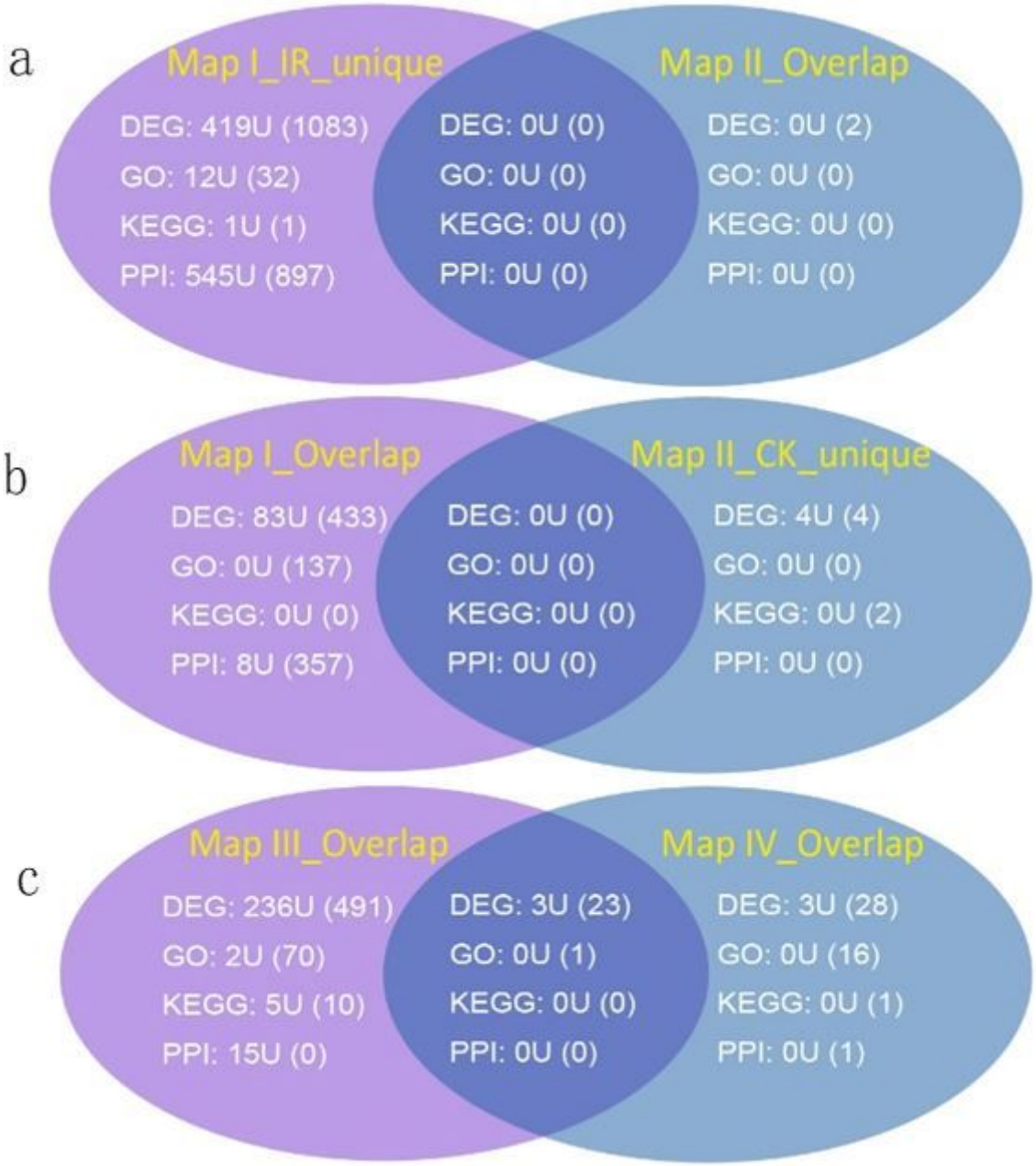

Figure 2

Venn diagrams of differential transcripts in (a) induced resistance-related, (b) nematode pathogenesis-related and (c) basal resistance-related groups following M. incognita inoculation at 15day post inoculation (dpi). Numbers in parentheses indicate down-regulated genes; U, upregulated genes.

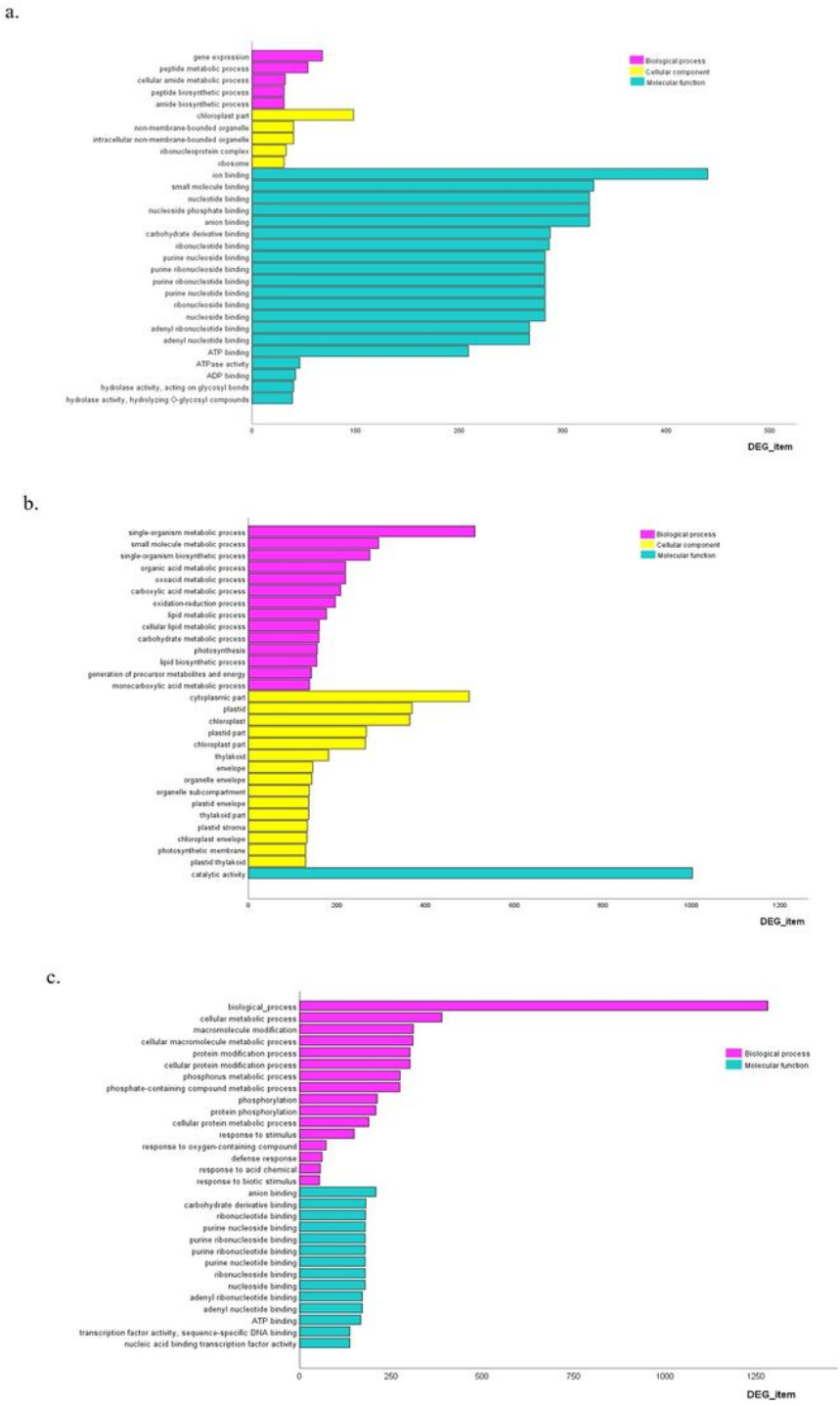

Figure 3

GO annotations of differentially expressed genes (a) GO annotation related to induced resistance, (b) GO annotation related to nematode pathogenesis and (c) GO annotation related to basal resistance.

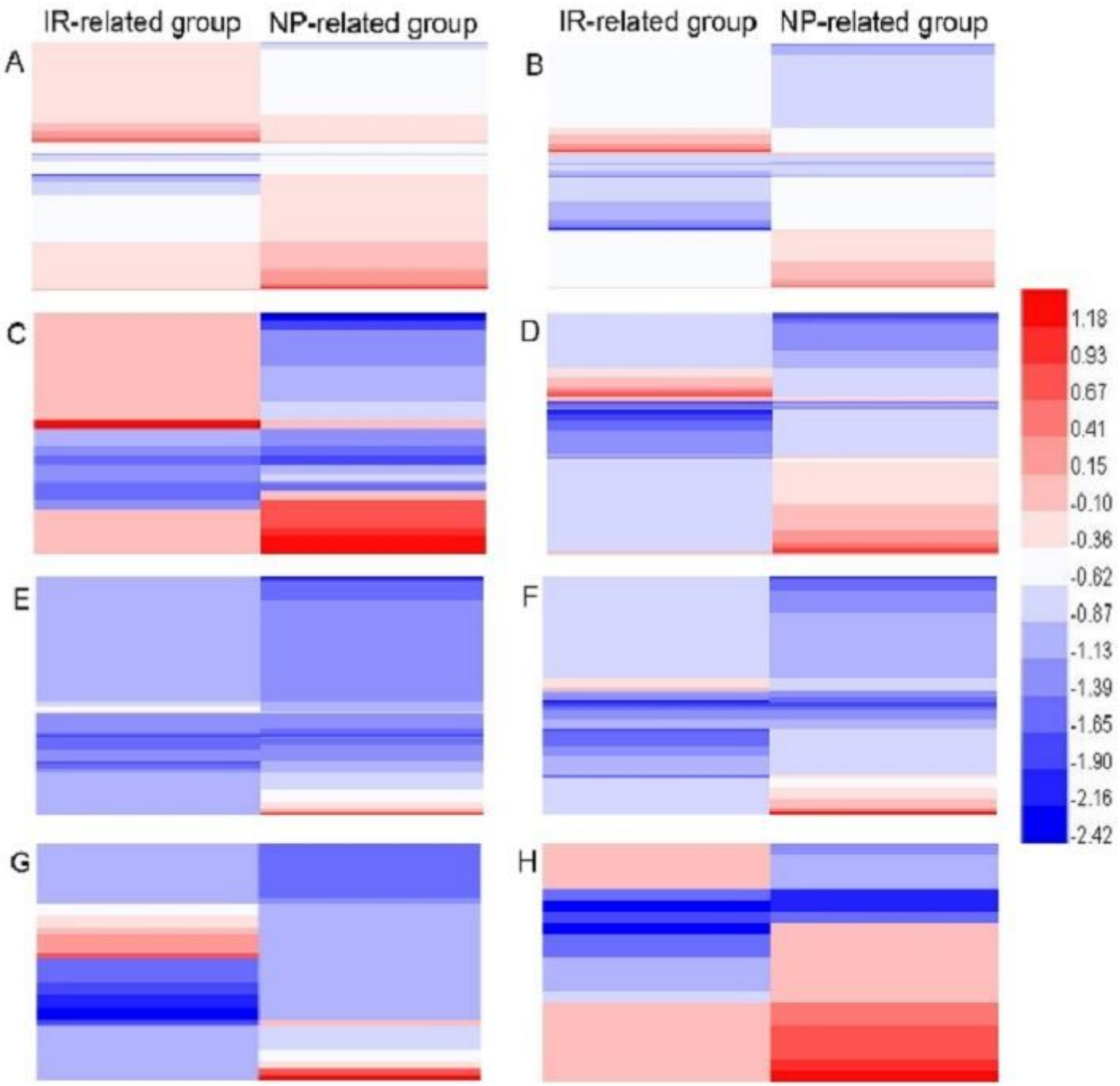

Figure 4

Expression pattern was represented by the heat map profile of IR-related group (resistance) versus NP-related group (pathogenicity). Color changes show the level of gene expression in their respective clusters. Red, highly expressed genes; blue, highly suppressed genes. The clusters are genes for (A) defense-related response, (B) signal transduction, (C) cell death, (D) transcription factor, (E) oxidoreductases, (F) defense enzymes activity, (G) protease inhibitor, and (H) phenylpropanoid metabolism.

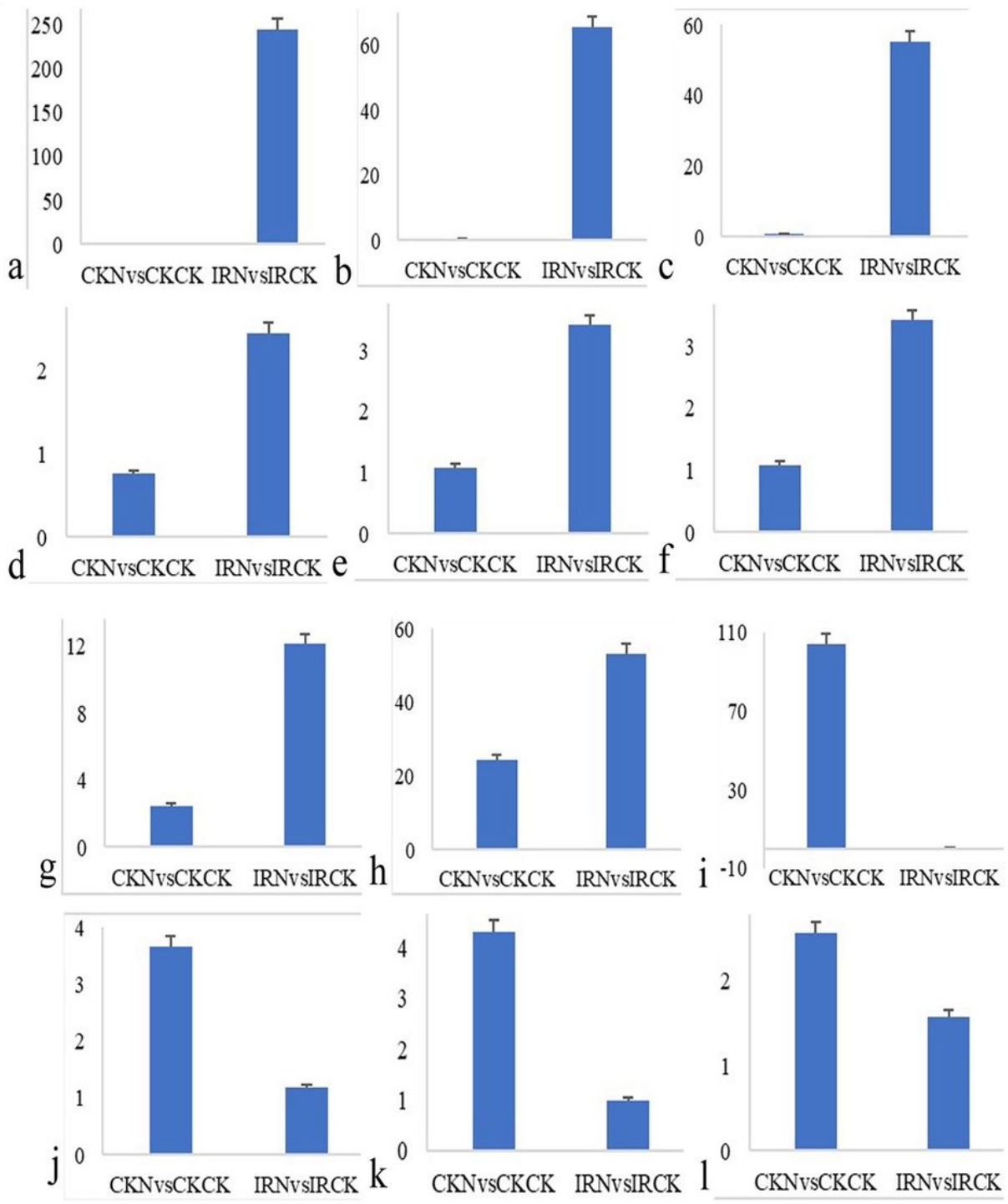

Figure 5

Validation of RNA-seq data by RT-qPCR of selected genes from tomato subjected to RKN (Meloidogyne incognita) infection with or without induction. The bars indicate relative gene expression of the control values (CKN vs CKCK) in non-induced plants with or without inoculation and the treatment values (IRN vs IRCK) in induced plants with or without inoculation, respectively. (a) myb-related protein 315-like, (b) peroxidase P7-like, (c) putative disease resistance protein RGA4, (d) disease resistance protein RPP13-

like, (e) auxin-responsive protein IAA, (f) 15.4 kDa class V heat shock protein, (g) apoptosis-inducing factor homolog A-like, (h) extra-large guanine nucleotide-binding protein 1-like, (i) protein BREAST CANCER SUSCEPTIBILITY 1 homolog, (j) peroxidase 3, (k) probable glycosyltransferase At3g07620, (l) transcription factor MYB48.

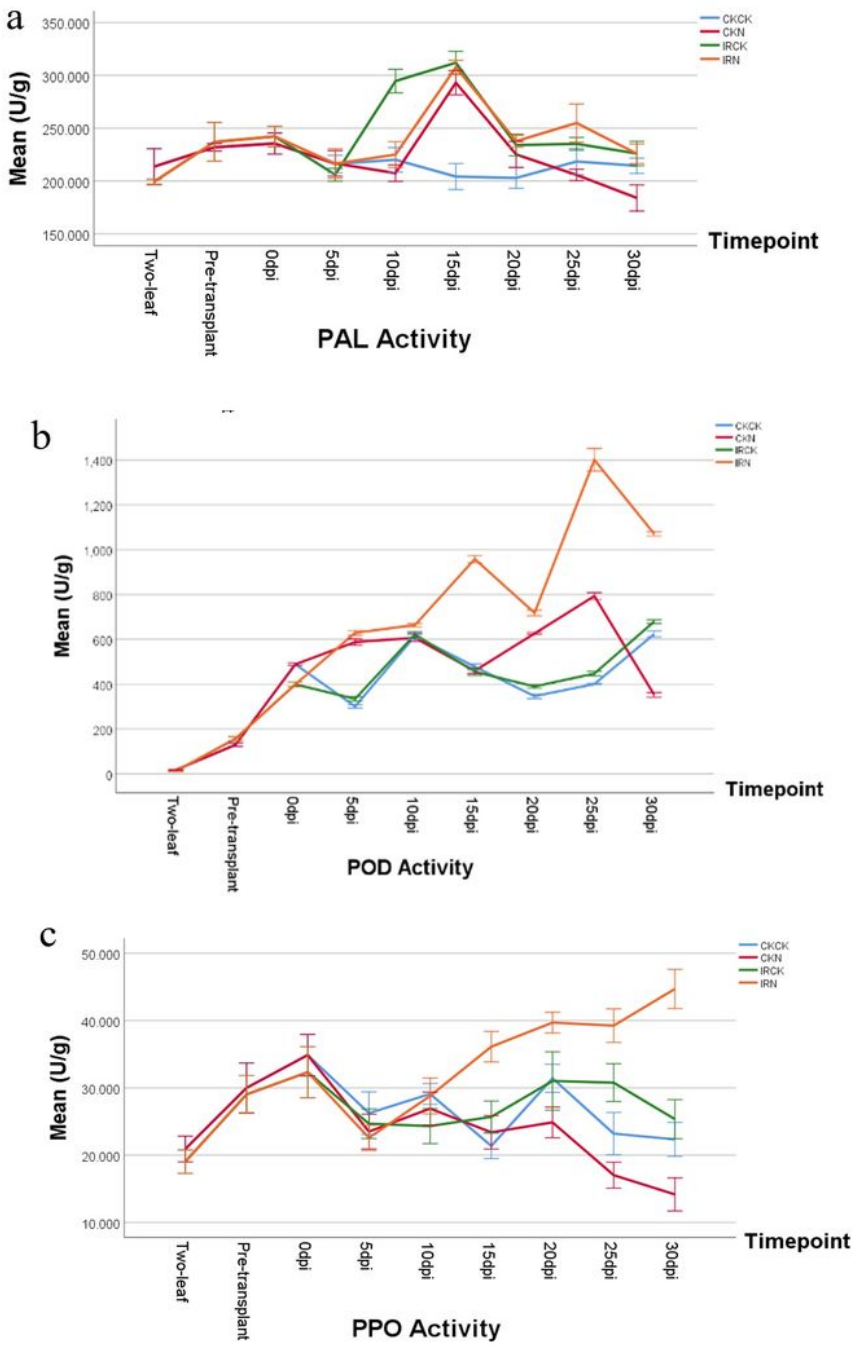

Figure 6

The activities of different enzymes, as measured in the crude extract prepared from roots harvested at various stages after M. incognita inoculation in Snef1216 induced plants (IRN), were compared with those in non-induced plants [1] and their respective mock-inoculated controls (IRCK and CKCK). (a) phenylalanine ammonia lyase (PAL), (b) Peroxidase [4], (c) polyphenol oxidase (PPO). Bars indicate the standard error at P-value=0.05.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.Inducedresistancerelatedgroups.xlsx
- SupplementaryTableS2.Nematodepathogenesisrelated.xlsx
- SupplementaryTableS3.Basalresistancerelatedgroups.xlsx
- SupplementaryTableS4.Hierarchicalclusteranalysis.xlsx
- SupplementaryTableS5.PrimersusedinRTqPCRvalidation.docx