Microbiological Research 227 (2019) 126294

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Comparative analysis of biocontrol agent *Trichoderma asperellum* ACCC30536 transcriptome during its interaction with *Populus davidiana* × *P. alba* var. *pyramidalis*

Shida Ji[a], Zhihua Liu[b], Bin Liu[b], Yucheng Wang[a,b,*]

[a] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, 830011, China
[b] State Key Laboratory of Tree Genetics and Breeding (Northeast Forestry University), 26 Hexing Road, 150040, Harbin, China

ARTICLE INFO

Keywords:
Trichoderma asperellum
Transcriptome
Biocontrol
Alternative splicing

ABSTRACT

After exposure to with *Populus davidiana* × *P. alba* var. *pyramidalis*, the expression of genes in *Trichoderma asperellum* were compared in four transcriptomes. The top 20 high expression genes included six heat shock proteins and three hydrophobins, indicating that *Trichoderma* can rapidly adapt to environment stresses and elicit a plant defense response. The genes, involved in the interaction between *Trichoderma* and plant, showed an increasing expression level, for example sugar transporters, EPL1s, endoxylanases, pectin lyases, and nitrilases. Interestingly, sugar transporters also showed high expression when *T. asperellum* was cultured on medium lacking a carbon substrate, which would contribute to *T. asperellum*'s survival and domination in ecological niche competition. And the genes related to mycoparasitism were expressed abundantly following *T. asperellum*'s interaction with PdPap, indicating the PdPap induction could enhance the mycoparasitic ability of *T. asperellum*. Twelve chitinases and five glucanases showed higher expression in transcriptome Cs, indicating that *T. asperellum* secretes both types of enzyme before interacting with pathogens, allowing *T. asperellum* to implement mycoparasitism and obtain more energy. Many novel transcripts were obtained in each transcriptome, which may play important roles in the biocontrol process of *T. asperellum*. Interestingly, *T. asperellum* undergo constitutive alternative splicing in the biocontrol process: Seven biocontrol genes were alternative spliced via intron retention. qRT-PCR analysis proved that intron retention is negatively associated with the expression of chitinase, oligopeptide transporters, and beta-lactamase. However, the percentage of MAPK intron retention was quite low, suggesting that intron retention has little effect on the function of MAPK.

1. Introduction

Trichoderma spp., as an excellent biocontrol agent, plays important roles in agriculture, including promoting plant growth, inducing plant systemic resistance, and killing phytopathogens (Lea et al., 2018; He et al., 2019; Yuan et al., 2019). Many *Trichoderma* transcriptome analyses have focused on the *Trichoderma*-pathogen interaction. Mycoparasitism of *Trichoderma* against *Botrytis cinerea* and *Phytophthora capsica* revealed the synergistic transcription of various genes involved in cell wall degradation (Barbara et al., 2011). In the presence of the cell wall of *Cronartium ribicola*, the expressions of two main glycoside hydrolase gene families in the *T. atroviride* transcriptome (i.e. chitinase and glucosidase) were induced (Liu et al., 2016a). During its interaction with *Rhizoctonia solani*, *T. atroviride* expressed an array of genes involved in the production of secondary metabolites, GH16 ß-glucanases, various proteases, and small secreted cysteine rich proteins (Atanasova et al., 2013). However, few transcriptome studies of *Trichoderma* have examined the mechanism of growth promotion and the induction of plant systemic resistance.

When interacting with plants, *Trichoderma* spp. can secret elicitors to promote plant growth and induce plant systemic resistance. First, *Trichoderma* is established in the plant rhizosphere. Monosaccharides and disaccharides excreted by plant roots into the rhizosphere provide an important carbon substrate for mycorrhizae (Nehls et al., 2010) and sucrose has a similar role in the establishment of *Trichoderma* in the rhizosphere (Vargas et al., 2009a). Further sucrose is actively transferred from the plant to the fungus by sucrose permeases and sucrose transporters, which are induced in the early stages of root colonization (Vargas et al., 2011), and other root exudates are assimilated by a large number of major facilitator solute transporters (Chaudhary et al., 2016;

* Corresponding author at: Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, 818 Beijing South Road, Urumqi, China.
E-mail address: wangyucheng@ms.xjb.ac.cn (Y. Wang).

https://doi.org/10.1016/j.micres.2019.126294
Received 15 April 2019; Received in revised form 13 June 2019; Accepted 18 June 2019
Available online 19 June 2019
0944-5013/

Nygren et al., 2018). In addition, plants can also sense the bioactive metabolites excreted by *Trichoderma*, including volatile and nonvolatile substances, such as auxins, 6-pentyl-2*H*-pyran-2-one (6-PP), sesquiterpenes, gliotoxin, viridin, harzianopyridone, harziandione, and peptaibols (Reino et al., 2008; Vinale et al., 2008). These can increase photosynthesis and biomass production and elicit developmental programs via the regulation of gene expression (Studholme et al., 2013; Rubio et al., 2014). *Trichoderma* spp. produces the auxin indole-3-acetic acid (IAA) (Li et al., 2017; Nieto-Jacobo et al., 2017). IAA stimulates cell division, elongation, and differentiation processes, ultimately increasing the growth and yield of the host plant. Fungal colonization of *Arabidopsis thaliana* roots induced the expression of the auxin-inducible marker gene DR5: uidA and increased development of lateral roots and root hairs (Contreras-Cornejo et al., 2015). Mutations in genes involved in auxin transport or signaling, including *AUX1*, *BIG*, *EIR1*, and *AXR1*, reduced the beneficial effects of *Trichoderma* on biomass production and root branching (Contreras-Cornejo et al., 2009). 6-PP is a major volatile organic compound (VOC) biosynthesized by *T. atroviride* and is involved in plant–fungus cross-kingdom signaling. 6-PP modulates the expression of PIN-FORMED (PIN) auxin-transport proteins in a specific and dose-dependent manner in primary roots, further promoting plant growth, regulating root architecture, inhibiting primary root growth, and inducing lateral root formation (Garnica-Vergara et al., 2016; Contreras-Cornejo et al., 2018)

Second, plants respond to the presence of other organisms by activating potential defense mechanisms. Pathogens can elicit a two-branched innate immune defence, including MAMP (microorganism-associated molecular patterns)-triggered immunity (PTI) and effector-triggered immunity (ETI) (Druzhinina et al., 2011). Similar to other non-plant pathogenic microorganisms, *Trichoderma* spp. triggers induced systemic resistance (ISR), which culminates in the accumulation of components of the associated jasmonate and ethylene signaling pathways, such as hydroperoxide lyase, peroxidase, and phenylalanine ammonia lyase. The hormone interactions within the plant immune signaling network determine the specific nature of the defense response that is triggered (Lu et al., 2015). *T. asperellum* SKT-1 is a microbial pesticide of seedborne diseases, and is capable of inducing systemic resistance in rice. The salicylic acid (SA) signaling pathway is important in inducing systemic resistance to colonization by *T. asperellum* SKT-1, and both the SA and jasmonic acid–ethylene (JA–ET) signaling pathways combine in the signaling of induced resistance by its cell-free culture filtrate (Yoshioka et al., 2015). ISR by beneficial microbes is characterized by priming of the plant for a more efficient activation of defense responses upon subsequent encounters with pathogens or insects (Conrath et al., 2015; Martinez-Medina et al., 2016). Beneficial root-associated microbes induce plant resistance in systemic tissues against a wide range of pathogens that attack the roots or the shoots (Pieterse et al., 2014).

In the present study, after interacting with *Populus davidiana* × *P. alba* var. *pyramidalis* (PdPap), the expression of biocontrol genes of *T. asperellum* were studied by comparing four newly constructed transcriptomes. To evaluate the changes in biocontrol genes expression following the Trichoderma–PdPap interaction, the top 20 highly expressed genes in the PdPap transcriptome were analyzed. In addition, the genes involved in the *Trichoderma*-plant interaction, the *Trichoderma*-pathogen interaction, and the *Trichoderma* defence response were also assessed. Besides, the identified novel transcripts are in addition to previously identified *Trichoderma* biocontrol genes. To determine the impact of alternative splicing on the biocontrol process, the patterns of alternative splicing of *Trichoderma* functional genes was also determined, and the expression levels of four randomly selected biocontrol genes was assessed using quantitative real-time reverse transcription PCR (qRT-PCR) analysis. The transcriptomes of *T. asperellum* ACCC30536 will increase our understanding of the evolution of *Trichoderma* mycoparasitism and environmental opportunism.

2. Materials and methods

2.1. Strains and plant material

Trichoderma asperellum ACCC30536 (CBS433.97) was obtained from the Agricultural Culture Collection of China. The stems, roots, and leaves of aseptic *Populus davidiana* × *P. alba* var. *pyramidalis* Loucne (PdPap poplar) seedlings were used as inducing substrates for *T. asperellum*.

2.2. Induced assays for C-starvation, N-starvation, PdPap, and minimal medium stresses

The 1×10^6 spores of the biocontrol agent *T. asperellum* ACCC30536 were cultured in ¼ PD medium at 28 °C for 48 h under continuous shaking at 180 rpm. After the spores germinated into mycelia, they were filtered, washed, and transferred into minimal medium (MM) (15 g/L NaH_2PO_4, 5 g/L $(NH_4)_2SO_4$, 600 mg/L $CaCl_2{\cdot}2H_2O$, 600 mg/L $MgSO_4{\cdot}7H_2O$, 5.0 mg/L $FeSO_4$, 2.0 mg/L $CoCl_2$, 1.6 mg/L $MnSO_4$ and 1.4 mg/L $ZnSO_4$, pH = 5.0) at 28 °C in shaker at 200 rpm for 48 h. The mycelia were then transferred into four kinds of inducing culture medium: C-starvation (Cs), MM without a carbon source; Nstarvation (Ns), MM without a nitrogen source; PdPap, carbon source in MM as follows: 1% (w/v) root powder, 1% (w/v) stem powder, or 1% (w/v) leaf powder from PdPap poplar; and MM, MM with 0.5% glucose and 0.5% ammonium sulfate. The mycelia were incubated at 25 °C for 72 h under the same conditions. The mRNA was isolated with magnetic beads and fragmented into short fragments, and cDNA is synthesized. The library is sequenced using Illumina HiSeqTM 2000. After filtering of raw data to decrease data noise and filter out “dirty” raw reads, the remaining reads are called "clean reads", which were aligned to reference genes and genome file with SOAPaligner/SOAP2, and the alignment data is utilized to calculate distribution of reads on reference genes and perform coverage analysis. Then QC of alignment is performed to determine if resequencing is needed. If alignment result passes QC, we will proceed following analysis including gene expression, gene structure refinement, alternative splicing, novel transcript prediction and annotation and SNP detection. Finally, four transcriptome libraries were constructed (Wang et al., 2019). The PdPap and Ns treatments simulate Trichoderma’s interaction with the plant and pathogen, respectively (Druzhinina et al., 2011). The MM and Cs transcriptomes served as controls.

2.3. Gene expression difference analysis

The gene expression level was calculated using the reads per kilobase of transcript per million mapped reads (RPKM) method (Mortazavi and Williams, 2008), which can eliminate the influence of different gene lengths and sequencing discrepancies on the calculation of gene expression. Therefore, the calculated gene expression can be directly used to compare the difference in gene expression among samples. This analysis identifies genes with different expression levels among samples, and then carries out gene ontology (GO) functional analysis. Referring to ‘The significance of digital gene expression profiles’ (Audic and Claverie, 1997), we developed a strict algorithm to identify differentially expressed genes (DEGs) between two samples. DEG analysis generates a large multiplicity problem in which thousands of hypotheses are tested simultaneously, in which correction for false positive and false negative errors are performed using the false discovery rate (FDR) method, which is a threshold for judging the significance of gene expression differences. More stringent criteria with smaller FDRs and larger fold-change values can be used to identify DEGs.

2.4. Top 20 high expression

Following *T. asperellum*’s interaction with PdPap, many genes are

expressed in *T. asperellum*. To further understand the genes related to the *Trichoderma*-plant interaction, these genes were ranked by their expression levels (RPKM), and the top 20 genes were obtained and classified by gene function. The expression levels of these genes in the other transcriptomes are also listed.

2.5. Statistical analysis of biocontrol genes

Following *T. asperellum* induction by four kinds of medium (MM, Cs, Ns, and PdPap), many biocontrol genes are expressed in *T. asperellum*, and their expression levels were determined. These genes were classified into three categories by their function, including those related to Trichoderma–plant interaction, Trichoderma–pathogen interaction, and the defense response of *Trichoderma*. The distribution of each category of genes was illustrated using heatmaps that were drawn using the Origin 2018 program (OriginLab Corporation, Northampton, MA, USA).

2.6. Novel transcripts

We assembled transcripts from the reads using Cufflink (Roberts et al., 2011). To discover novel transcribed regions, we compared our assembled transcripts and annotated genomic transcripts from reference sequences. To be reported as a novel transcript, an assembled transcript must meet three requirements: the transcript must be at least 200bp away from an annotated gene, the transcript must be over 180 bp, and the sequencing depth must be no less than 2. To study the function of novel transcripts, distinguished protein-coding RNAs from noncoding RNAs using a Support Vector Machine-based classifier, named Coding Potential Calculator (CPC http://cpc.cbi.pku.edu.cn/).

2.7. Detecting alternative splicing in Trichoderma

Alternative splicing makes it possible for a gene to generate different mRNA transcripts that are ultimately translated into distinguishable proteins. There are seven main types of alternative splicing, including exon skipping, intron retention, alternative 5′ splice sites, alternative 3′ splice sites, alternative first exons, alternative last exons, and mutually exclusive exons. To select software to detect alternative splicing events, we have evaluated four pieces of software based on 50 nt reads simulated by Maq (Li and Ruan, 2008). The results showed that SOAPsplice (Huang et al., 2011) was the best, we then used in our analysis pipeline. Furthermore, we verified the alternatively spliced genes related to biological control using PCR, in which one of the two primers is designed against an intron region. The primers are listed in Supplementary Table 1. To determine the function of alternative splicing, the proportions of alternatively spliced transcripts were counted using qRT-PCR. The treatment method was the same as that used to generate the four transcriptomes. For each inducing condition, mycelia were collected at 0, 3, 6, 12, 24, 48, and 72 h post-induction. At each time point, the biomass was obtained as three replicates. The samples are stored at −80 °C. Total RNA was extracted using the CTAB method, digested with DNaseI (Promega, USA), and reverse-transcribed into cDNA using a PrimeScript RT Kit (Takara, Japan) according to the manufacturer's instructions. The transcription of these genes in *T. asperellum* was detected using qRT-PCR and was calculated according to the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008), using cDNA as template, and *actin* as reference gene. Three qRT-PCR replicates were performed per cDNA sample, and the primers are listed in Supplement Table 1.

2.8. Data availability

Strains and plasmids are available upon request. Supplemental files available at FigShare. File S1 contains primers used to verify alternatively spliced genes. File S2 shows data quality of four different transcriptomes. File S3 shows Gene ontology of differentially expression genes (DEGs) from *T. asperellum.* File S4 contains the top 20 highly expressed genes under PdPap stress. File S5 contains the expression levels of genes related to the biocontrol mechanism of *T. asperellum* in the four transcriptomes.

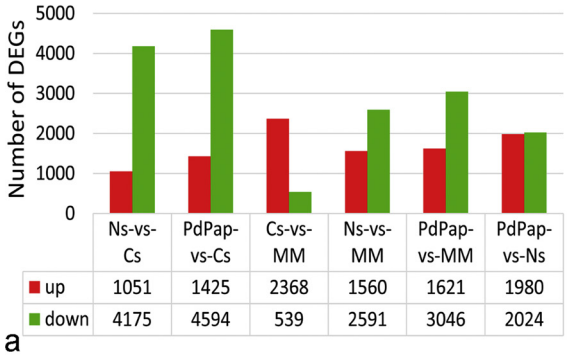
3. Results

3.1. The characteristics of transcriptome resequencing

After quality control and filtering of raw reads, "Total clean reads" for the four samples (including MM, Cs, Ns and PdPap) were obtained, which were then aligned to the genome file. The results showed the quality of the sequenced transcriptomes was sufficient for our analyses (Supplementary Table 2).

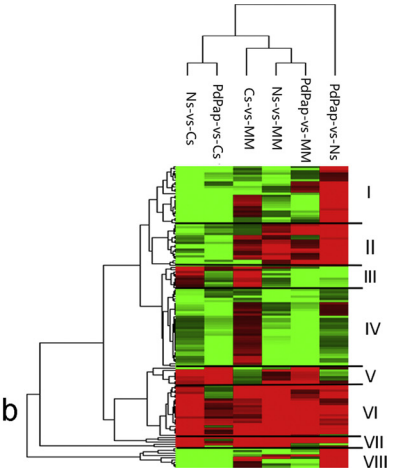
3.2. Identification of differentially expressed genes in four inducing conditions

After the identification of differentially expressed genes (DEGs) of *T. asperellum* in the four transcriptomes (MM, Cs, Ns and PdPap), compared with the Cs library, there are 2368 (MM), 4175 (Ns), and 4594 (PdPap) downregulated DEGs in the other libraries, respectively. Following MM induction, there were 2591 and 3046 upregulated DEGs in the MM library compared with those in the Ns and PdPap libraries, respectively. In addition, comparing the PdPap with Ns libraries identified 1980 upregulated genes and 2024 downregulated genes (Fig. 1a).



	Ns-vs-Cs	PdPap-vs-Cs	Cs-vs-MM	Ns-vs-MM	PdPap-vs-MM	PdPap-vs-Ns
up	1051	1425	2368	1560	1621	1980
down	4175	4594	539	2591	3046	2024

a



b

Fig. 1. The differential expression of genes in four transcriptomes.
a: The differential expression of genes in each pairwise comparison. b: Pattern clustering analysis of differentially expressed genes in four transcriptomes. The heatmap of gene clusters is shown as log2 ratio values of fold change in each pairwise comparison. Roman numerals specify clusters characterized by consistent expression patterns. Log2 fold changes of gene expression are scaled, ranging from +3 (red) to −3 (green). Red represents upregulated genes, green represents downregulated genes, and black indicates no significant difference in gene expression. The hierarchical clustering method in T-MeV 4.9 software is used.

3.3. Pattern clustering analysis of differentially expressed genes

The four transcriptomes shared 135 DEGs. Compared with their expression in transcriptomes MM and Cs, the genes in Clades I and II were upregulated in transcriptome Ns. The genes in both clades were closely related to mycoparasitism of *Trichoderma*, and mainly included glycoside hydrolase genes, oligopeptide transporter genes, and oxidoreductase genes (Fig. 1b). The genes in Clade III were upregulated in the PdPap transcriptome compared with that the other transcriptomes, indicating that these genes (including a PAS sensor, zinc finger protein, and regulator of G protein signaling) may play important roles in interaction between *Trichoderma* and PdPap. The genes in Clade IV were associated with establishing *Trichoderma* in the rhizosphere and developing interactions with plant roots; they were upregulated in the PdPap transcriptome compared with that in transcriptomes MM and Cs (Fig. 1b), for example sugar transporter, MFS transporters, G-protein coupled receptor, and fungal transcriptional regulatory protein. The genes in Clades V, VI, and VII were downregulated in the PdPap and Ns transcriptomes compared with those in the MM and Cs transcriptomes, indicating that these genes contributed little to the biocontrol process of *Trichoderma*. The genes in Clade VIII were upregulated in the PdPap and Ns transcriptomes compared with those in the MM and Cs transcriptomes (Fig. 1b), and were closely related to establishing *Trichoderma* in the plant rhizosphere. These genes were upregulated to a greater degree in the Ns transcriptome compared with those in the MM and Cs transcriptomes; however, the reason remains unknown.

3.4. Gene ontology (GO) analysis of differential expression genes (DEGs)

To better understand the functions of genes regulated during the interaction between *Trichoderma* and PdPap, GO analysis was carried out. The DEGs were assigned into three categories according their functions, including biological process, molecular function, and cellular component, and the total number of DEGs was counted (Supplementary Table 3). In the PdPap and Ns transcriptomes, there were seven, eight, and four GO term annotations showing significant enrichment in biological process, molecular function, and cellular component, respectively (Fig. 2). In biological process, compared with the Ns transcriptome, more upregulated genes (n = 132) were related to the oxidation-reduction process than downregulated genes (n = 52) in the PdPap transcriptome (Fig. 2). The biological process category also contained many carbohydrate metabolism-related processes, including polysaccharide catabolic process, carbohydrate metabolic process, polysaccharide metabolic process, single-organism carbohydrate catabolic process, glucan metabolic process, and carbohydrate catabolic process, which were associated with rhizosphere establishment and mycoparasitism. In terms of molecular function, compared with that in the Ns transcriptome, many catalytic activity-related genes (n = 571) were upregulated, and 171 oxidoreductase genes were upregulated in the PdPap transcriptome (Fig. 2). Hydrolase activity related genes (n = 134) associated with rhizosphere establishment and mycoparasitism were also upregulated and most transmembrane transporter related genes were activated in response to PdPap stress. In the category "cellular component", the expression levels of genes related to extracellular region, membrane part, intrinsic to membrane, integral to membrane, and membrane increased, possibly because the structure or formation of the cell membrane changes during the interaction between *Trichoderma* and PdPap (Fig. 2). In addition, compared with that in the MM transcriptome, more oxidoreductase activity-related genes in PdPap transcriptome were upregulated; however, in the other GO categories, most genes were downregulated (Fig. 2).

3.5. The top 20 high expression genes following PdPap induction

The expression levels of the top 20 highly expressed genes under PdPap stress were compared with their expression in the other three

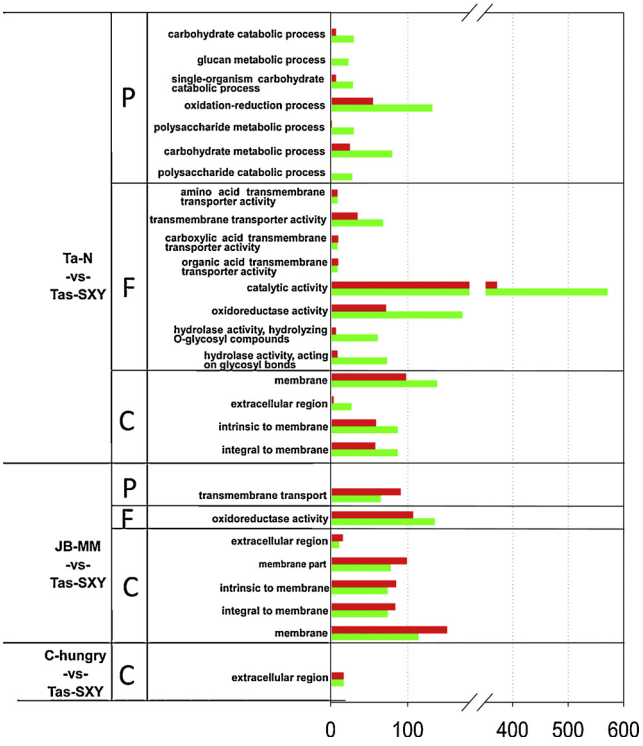


Fig. 2. Functional classes of differentially expressed gene in response to four different stresses.
The interval of omission ranged from 180 to 350. P: biological process, F: molecular function, C: cellular component. MM: *T. asperellum* was exposed to minimal medium; Cs: *T. asperellum* was exposed to MM without sucrose; Ns: *T. asperellum* was exposed to MM without $(NH_4)_2SO_4$; PdPap: *T. asperellum* was exposed to MM with powder with PdPap.

libraries (Supplementary Table 4), and mainly included six heat shock protein genes, three hydrophobin genes, two AAA ATPase genes, two oxidation-reduction genes, one protease inhibitor, one fatty acid synthase, one oligopeptide transporter, one collagen protein, and three genes of unknown function. However, the 20 genes showed relatively lower expression levels in the MM, Cs, and Ns transcriptomes (Fig. 3), indicating these genes may play important roles in the interaction between *Trichoderma* and PdPap. The three unknown-function genes were upregulated strongly, including e_gw1.5.1082.1, gm1.9173_g, and gm1.3584_g, their RPKM values were 32142, 188016, and 40218 respectively (Supplementary Table 4). These results suggested that the three genes are closely related to *Trichoderma*'s response to PdPap stress

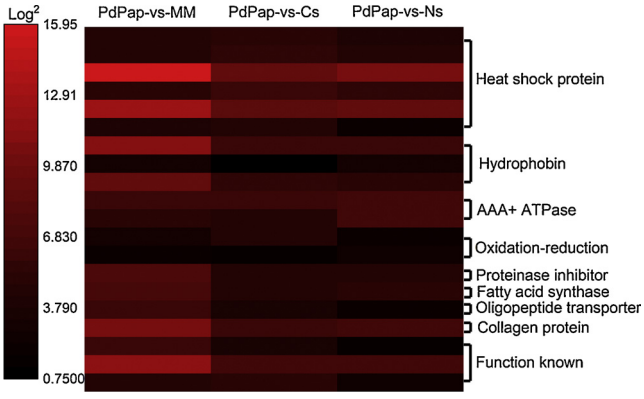


Fig. 3. The top 20 highly expressed genes under PdPap stress.
MM: *T. asperellum* was exposed to minimal medium; Cs: *T. asperellum* was exposed to MM without sucrose; Ns: *T. asperellum* was exposed to MM without $(NH_4)_2SO_4$; PdPap: *T. asperellum* was exposed to MM with powder with PdPap.

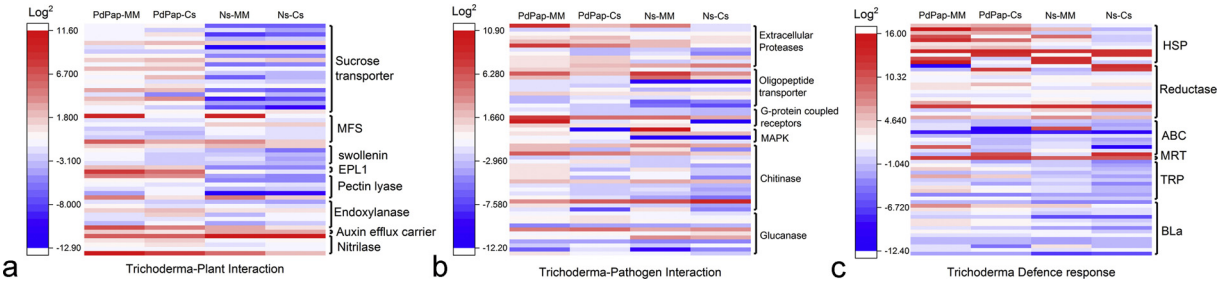


Fig. 4. Heatmaps of the expression levels of genes related to the biocontrol mechanism of *T. asperellum* in the transcriptomes.
a: Genes related to the Trichoderma-plant interaction. b: Genes related to the Trichoderma-pathogen interaction c: Genes related to the Trichoderma defense response.

and need to be studied further.

3.6. Statistical analysis of biocontrol genes in T. asperellum

When *Trichoderma* is established in the rhizosphere of the plants, it can kill pathogens and promote plant growth and stress responses. Therefore, to further determine the expression patterns of biocontrol genes, their expression levels in the four transcriptomes were assessed (Fig. 4; Supplementary Table 5). The genes related to rhizosphere establishment and inducing plant resistance were upregulated in the PdPap transcriptome. Interestingly, most sugar transporters showed lower expression levels in the Ns transcriptome compared with that in the Cs and MM transcriptomes. In addition, pectin lyases, EplIs, and swollenin gene expression levels were also downregulated in the Ns transcriptome (Fig. 4a). However, the genes related to promoting plant growth had higher expression levels in the Ns transcriptome compared with those in the Cs and MM transcriptomes (Fig. 4a). Following simulation of *T. asperellum* interacting with pathogens (Ns), the expression levels of some genes related to mycoparasitism were upregulated; however, other genes related to mycoparasitism had higher expression in Cs and MM transcriptomes (Fig. 4b), indicating that genes related to mycoparasitism are expressed before *T. asperellum* interacts with pathogens. In addition, many of these genes also had higher expression levels in PdPap transcriptome compared with that in the Cs and MM transcriptomes (Fig. 4b), indicating that plants also can induce the *T. asperellum* to parasitize the pathogens. Many defense response genes were expressed abundantly after *T. asperellum* interacting with PdPap, showing that the early stage of the interaction is stressful to *T. asperellum*. The expression levels of multidrug resistance transporter genes (*MRT*) were upregulated in the Ns and PdPap transcriptome, but most beta-lactamase genes (*BLAC*) and tetracycline resistance protein genes (*TRP*) had low expression levels, especially in the Ns transcriptome (Fig. 4c).

3.7. Prediction and annotation of novel transcripts from the four transcriptomes

The prediction of novel transcripts indicated that 2478 novel transcripts were obtained in the MM transcriptome, 2914 in the Cs transcriptome, 2401 in the Ns transcriptome, and 2191 in the PdPap transcriptome (Table 1). These genes may play important roles in the defense response of *T. asperellum* during its interaction with plants or pathogens. These novel transcripts contained not only coding genes, but also noncoding genes. There were many more coding genes than noncoding genes and the average length of the coding genes was also longer. The most abundant group of coding genes were 3000–3500 bp (Fig. 5). These coding genes may encode proteins involved in catalysis and signal transduction. Among the non-coding genes, the 500–1000 bp group was the most abundant. These noncoding genes may bind with transcription factors or be transcribed as functional RNAs, thus participating in the regulation of physiological activities and pathological processes. Thus, these non-coding genes require further study.

Table 1
Novel transcripts in the four transcriptomes MM: *T. asperellum* was exposed to minimal medium; Cs: *T. asperellum* was exposed to MM without sucrose; Ns: *T. asperellum* was exposed to MM without $(NH_4)_2SO_4$; PdPap: *T. asperellum* was exposed to MM with powder with PdPap.

	MM	Cs	Ns	PdPap
Novel transcripts	2478	2914	2401	2191
The average length of novel transcripts	3285	3374	3226	3810
Number of coding genes	1983	2200	1934	1835
The average length of coding genes	3748	3923	3677	4239
Number of noncoding genes	495	714	467	356
The average length of noncoding genes	1430	1682	1359	1598

MM: *T. asperellum* was exposed to minimal medium; Cs: *T. asperellum* was exposed to MM without sucrose; Ns: *T. asperellum* was exposed to MM without (NH4)2SO4; PdPap: *T. asperellum* was exposed to MM with powder with PdPap.

3.8. Alternative splicing of T. asperellum ACCC30536 genes and their relationship to biocontrol

According to junction reads (reads crossing exon-exon boundaries) in the alignment result, we could identify alternative splicing events among the combined collection of annotated genes. The statistical analysis found that intron retention was most frequent alternative splicing formulation, involved in 2527, 2959, 2696 and 4374 genes in MM, Cs, Ns and PdPap transcriptomes, respectively (Fig. 6a–d). Some genes showed alternative splicing that resulted from alternative 5′ splicing, alternative 3′ splicing, or exon skipping. Meanwhile, only a few genes used an alternative first exon, an alternative last exon, and a mutually exclusive exon among the four transcriptomes (Fig. 6a–d). In addition, among 33 verified biocontrol genes, seven showed alternative splicing via intron retention, including major facilitator superfamily (*MFS*) (gm1.7255_g), beta-lactamase (*BLAC*) (fgenesh1_pg.10_#_214), oligopeptide transporter protein (*OTP*) (estExt_Genewise1-Plus.C_180277 and fgenesh1_pm.4_#_101), mitogen-activated protein kinase (*MAPK*) (MIX7687_32006_31), protease (fgenesh1_kg.2_#_641_#_Locus1210v1rpkm119.33), and chitinase (fgenesh1_pm.1_#_1180) genes (Fig. 6f).

To determine the relationship between the alternative splicing events and the stress response, the expression level of the alternatively spliced transcripts and normally spliced transcripts were assessed using qRT-PCR. The protein encoded by the *BLAC* gene is commonly used in bacterial resistance mechanisms against beta-lactam antibiotics, and its expression level was upregulated undr Ns stress, up to $2^{10.2}$-folds (Fig. 7a). However, the percent of alternative splicing events was lower undr Ns stress compared with that under other stresses (Fig. 7e). MAPKs play an important role in cellular signal transduction when *T. asperellum* interacts with the environment. *T. asperellum* expressed more MAPKs following induction with by Ns and PdPap (Fig. 7b). The percentage of *MAPK* alternative splicing events was quite low and was

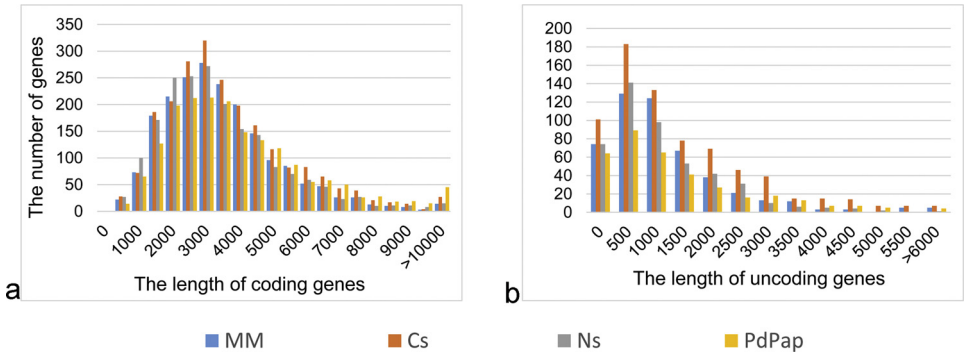


Fig. 5. The number and length of coding and noncoding novel transcripts.
a: The number and length of coding novel transcripts; b: The number and length of noncoding novel transcripts.

negatively correlated with expression levels (Fig. 7f). Chitinase can hydrolyse the fungal cell wall and is essential in the mycoparasitism process of *Trichoderma*. Chitinase expression levels were upregulated after induction by Cs, Ns, and PdPap (Fig. 7c). As the expression levels of the chitinase genes increased, the proportion of alternative splicing events decreased (Fig. 7g). *OTP2* is closely related to *Trichoderma* pathogen sensing. Under Ns stress, the *OTP2* expression level was higher than that under the other induction conditions (Fig. 7d). At 12 and 24 h post-induction, the expression levels of *OTP2* were higher under Ns stress than that of under MM and Cs; however, the proportion of alternative splicing events was lower (Fig. 7h). These results suggested that alternative splicing acts as a negative regulator of stress-response genes in *Trichoderma*.

4. Discussion

Trichoderma spp. can reduce the severity of plant diseases by inhibiting plant pathogens in the soil via their potent antagonistic and mycoparasitic activity. *Trichoderma* spp. can interact directly with plant roots, further increasing plant growth potential, resistance to disease, and tolerance to abiotic stresses (Hermosa et al., 2012). To gain a deeper understanding of the biocontrol mechanism of *T. asperellum*, four transcriptomes were obtained, sequenced, and analyzed. The PdPap and Ns treatments simulate *Trichoderma*'s interaction with the plant and pathogen, respectively (Druzhinina et al., 2011). The MM and Cs transcriptomes served as controls.

Compared with the controls, the abundant DEGs in the PdPap and Ns transcriptomes (Fig. 1a) play important roles in the biocontrol process of *T. asperellum*. Pattern clustering analysis of DEGs indicated

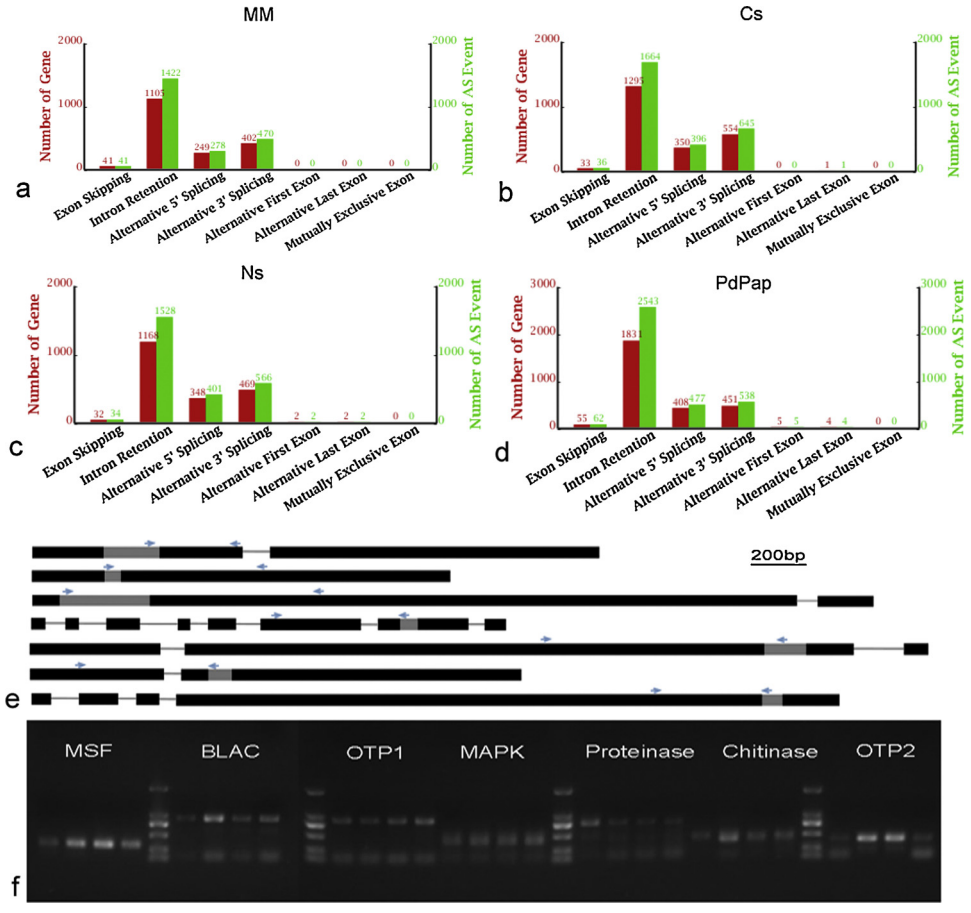


Fig. 6. The alternative splicing events of 7 biocontrol genes from *T. asperellum*.
a–d: The number and types of alternative splicing genes detected in the MM, Cs, Ns, and PdPap transcriptomes. e: The structures of alternative spliced genes. The arrows indicate the location of the PCR primers used to identify alternative splicing events. f: The result of PCR tests for alternative splicing events. From left to right for each gene: MM, Cs, Ns, and PdPap.

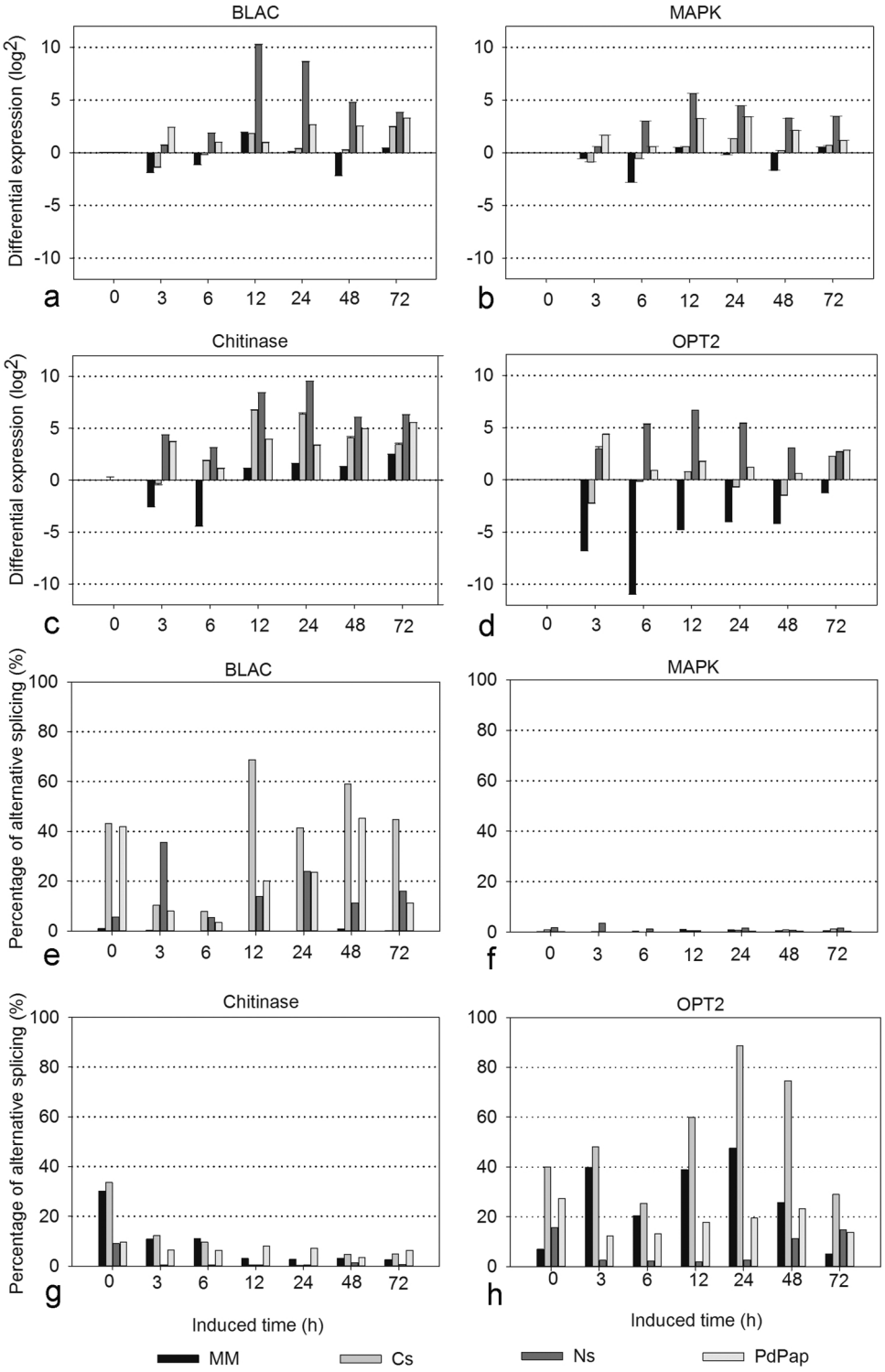


Fig. 7. The percentage of alternative splicing events for four biocontrol genes in the four transcriptomes.
a–d: The expression levels of BLAC, MAPK, Chitinase and OPT2 following exposure to MM, Cs, Ns, and PdPap. e–h: The percentage of alternative splicing events of BLAC, MAPK, Chitinase and OPT2 following exposure to MM, Cs, Ns, and PdPap.

that, compared with their expression in the MM and Cs transcriptomes, the genes related to mycoparasitism, rhizosphere colonization, and stress response showed increased expression levels in the Ns and PdPap transcriptomes (including those encoding glycoside hydrolase, oligopeptide transporter, PAS sensor, G-protein coupled receptor, Regulator of G protein signaling, Fungal transcriptional regulatory protein, Sugar transporter, MFS transporters, Zinc finger protein, and oxidoreductase) (Fig. 1b).

In the GO analysis, the PdPap and Ns transcriptome showed significant enrichment GO terms (Fig. 2), which indicated that many of the DEGs in both transcriptomes share similar functions. This suggested that both induction conditions activated similar biological processes in *T. asperellum*. Both transcriptomes shared 184 oxidation-reduction genes, which may be because plants and phytopathogens are potential competitors and excrete certain antibiotic substances into the environment, and when *T. asperellum* senses other organisms, it will

express more oxidation-reduction enzymes to reduce the levels of reactive oxygen species (ROS) and avoid cell injury (Choudhury et al., 2017). In addition, both transcriptomes shared many genes related to the polysaccharide catabolic process, carbohydrate metabolic process, and glucan metabolic process (Fig. 2), which are closely related to rhizosphere establishment (Vargas et al., 2009b; Nehls et al., 2010), mycoparasitism (Wu et al., 2017; Elangovan et al., 2018), and inducing plant defense responses (Moran-Diez et al., 2009; Sarrocco et al., 2017).

The relationship between *Trichoderma* and plants is considered as symbiosis (Vargas et al., 2009a); however, interestingly, stress-response genes (including those encoding six heat shock proteins and two reductases) were abundantly expressed in the PdPap transcriptome (Fig. 3), which indicate PdPap is a kind of stress to *T. asperellum* in the process of Trichoderma-plant interaction, However *T. asperellum* could counteract the effects of stress or remove it altogether by expressing stress-response genes to maintain the *Trichoderma*-plant symbiosis system. In addition, following the interaction with PdPap, *T. asperellum* also expressed more Hydrophobins, which are extracellular proteins involved in the plant–ectomycorrhiza interaction (Yu et al., 2019). Several hydrophobic proteins isolated from *Trichoderma* affect plant development (QID74) (Samolski et al., 2012) and resistance to diseases (Sm1, EPL1, and EPLT4) (Huang et al., 2015; Wang et al., 2013). Type II hydrophobin secreted by *T. longibrachiatum* displayed a direct antifungal ability, induced plant systemic resistance by microbe-associated molecular patterns, and promoted plant growth (Roberto et al., 2018). Proteinase inhibitors also play important roles in plant resistance to biotic or abiotic stresses (Khadeeva et al., 2009; Li et al., 2015); therefore, we speculated that proteinase inhibitors from *T. asperellum* share similar functions with those of plants. The expression of these genes mentioned above may exert a beneficial preconditioning effect on the *Trichoderm*a-plant symbiosis system

To more comprehensively understand the biocontrol mechanisms of *T. asperellum*, the expression levels of genes involved in responding to environment stresses were assessed in the four transcriptomes. Compared with transcriptomes Cs and MM, many genes related to the biocontrol functions of *T. asperellum* had higher expression levels in transcriptomes Ns and PdPap. The proteins encoded by these genes play vital roles in promoting plant growth, inducing plant resistance (Fig. 4a), mycoparasitism (Fig. 4b), secreting secondary metabolites, and stress-responses (Fig. 4c). Compared with those in transcriptomes Cs and MM, the expression levels of most genes related to the *Trichoderma*-plant interaction were upregulated; however, notably, many genes encoding sugar transporters showed increasing expression in transcriptome Cs, compared with that in transcriptome Ns (Fig. 4a). The explanation may be that to obtain enough energy, *T. asperellum* absorbs sugars from the soil via sugar transporters, and barren soil might inhibit other microorganisms from obtaining energy, thus allowing *T. asperellum* to dominate the ecological niche. Competition mechanisms play important roles in the inhibition of soil-borne diseases and leaf pathogens by limiting space and nutrients, thereby inhibiting their growth (Mac as-Rodr guez et al., 2018). In the Cs transcriptome, certain genes related to the *Trichoderma*-pathogen interaction had higher expression levels compared with those in Ns (Fig. 4b). Extracellular proteases (n = 3), oligopeptide transporters (n = 5), G-protein coupled receptors (n = 3), and MAPKs (n = 4), which play vital roles in *Trichoderma*'s sensing of pathogens (Druzhinina et al., 2011; Li et al., 2017; Zhang et al., 2017), showed higher expression levels in the Cs transcriptome compared with that in the Ns transcriptome. A previous study showed that many genes encoding proteases and oligopeptide transporters were expressed before and during *Trichoderma*'s contact with phytopathogens (Steindorff et al., 2014). Before the *Trichoderma*-pathogen interaction, high expression levels of signal transduction genes may promote *T. asperellum*'s ability to sense pathogens quickly. Chitinases and glucanases, as cell wall degrading enzymes, are essential for mycoparasitism (Kowsari et al., 2014). Most of them had higher expression levels in the Cs transcriptome (Fig. 4b); therefore, we speculated that *T. asperellum* secretes abundant chitinases and glucanases when lacking carbon source nutrients to implement mycoparasitism and obtain energy. In addition, the defense response genes of *T. asperellum* were abundantly expressed in the PdPap and Ns transcriptome, for example those encoding heat shock proteins (HSPs) (Jacob et al., 2016), reductase (Choudhury et al., 2017), and metarhizium raffinose transporter (MRT) (Mazurkiewicz et al., 2005). This observation indicated that *T. asperellum* could reduce or counteract the negative effects of its interaction with pathogens or plants.

Alternative splicing generates multiple transcripts from the same gene, occurs in thousands of genes, and plays a crucial role in plant development and stress responses (Thatcher et al., 2016; Laloum et al., 2017). In recent years, high-throughput sequencingbased analysis of plant transcriptomes has suggested that up to 60% of plant gene loci encode alternatively spliced mature transcripts (Yamile et al., 2012; Filichkin et al., 2015). Transcriptome analysis identified many alternative splicing events in *T. asperellum*, for example intron retention, alternative 5′, and alternative 3′ splicing (Fig. 6a–d). Among 33 verified biocontrol genes, seven were alternatively spliced via intron retention (Fig. 6f), and intron retention was ubiquitous among the four different induction conditions. Four genes were selected for qRT-PCR analysis. The expression levels of *BLAC*, *MAPK*, chitinase, and *OTP2* gene were upregulated under Ns and PdPap stresses (Fig. 7a–d), which indicated that the four genes play positive roles in *T. asperellum*'s interaction with pathogens or plants. Interestingly, that the percent of alternative splicing events decreased with the upregulation of gene expression levels. For example, at 12 and 24 h post-induction, the expression levels of *BLAC* were highest under Ns stress; however, the percent of alternative splicing events was lower than that under Cs and PdPap stresses (Fig. 7a, e). The percent of alternative splicing events of *MAPK* and *OTP2* decreased with increasing expression levels of the genes (Fig. 7c, d, g, h). So, it's speculated that alternative splicing is a kind of negative regulation to the expression of the four stress response genes. OsWRKY62 and OsWRKY76 from *Oryza sativa*, as transcriptional repressors, undergo constitutive and inducible alternative splicing. The short alternative OsWRKY62.2 and OsWRKY76.2 isoforms could interact with each other and with full-length proteins. OsWRKY62.2 showed a reduced repressor activity in planta. The amino termini of OsWRKY62 and OsWRKY76 splice variants also showed reduced binding to the canonical W box motif (Liu et al., 2016b). The *TvCyt2* gene from *T. virens*, encoding a member of the p450 family, comprises 1680 bp and contains two introns. The first intron (33 bp) of TvCyt2 is retained and interrupts the region that encodes the putative signal peptide, causing the loss of protein function (Ram rez-Valdespino et al., 2017). However, we could not conclude that alternative splicing is negative related to expression levels for all genes. Thousands of genes can be alternatively spliced, and the mechanism is quite complex; thus, determining the regulatory effects of alternative splicing events for individual genes cannot be generalized for all genes. In addition, the percentage of intron retention events in MAPK expression was quite low; therefore, we speculated that intron retention does not have a large effect on the function of MAPK in signal transduction.

The results of the present study showed *T. asperellum*, as an excellent biocontrol agent, could promote plant growth, trigger plant immunity, and kill pathogens via mycoparasitism, by excreting antibiotics and exerting niche competition. However, *T. asperellum* expressed genes related to mycoparasitism before interacting with pathogens, which effectively contributed to survival and mycoparasitism. In addition, alternative splicing is involved in the biocontrol function of *T. asperellum*, and may negatively regulate the expression of biocontrol genes. However, the relationship between alternative splicing and the biocontrol function of *T. asperellum* requires further study.

Acknowledgments

This work is supported by grants from the National Natural Science

Foundation of China(NSFC: 31870627) and the National High Technology Research and Development Program (the 13th Five-Year Plan Program; grant number 2016YFC0501505).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2019.126294.

References

Atanasova, L., Crom, S.L., Gruber, S., Coulpier, F., Seidl-Seiboth, V., Kubicek, C.P., Druzhinina, I.S., 2013. Comparative transcriptomics reveals different strategies of Trichoderma mycoparasitism. BMC Genom. 14 (1), 121.

Audic, S., Claverie, J.M., 1997. The significance of digital gene expression profiles. Genome Res . 7 (10), 986–995.

Barbara, R., Enrique, I.L., Mach, R.L., Alfredo, H.E., 2011. Identification of mycoparasitism-related genes in Trichoderma atroviride. Appl. Environ. Microbiol. 77 (13), 4361–4370.

Chaudhary, N., Kumari, I., Sandhu, P., Ahmed, M., Akhter, Y., 2016. Proteome scale census of major facilitator superfamily transporters in Trichoderma reesei using protein sequence and structure based classification enhanced ranking. Gene 585 (1), 166–176.

Choudhury, F.K., Rivero, R.M., Blumwald, E., Mittler, R., 2017. Reactive oxygen species, abiotic stress and stress combination. Plant J. 90 (5), 856.

Conrath, U., Beckers, G.J.M., Langenbach, C.J.G., Jaskiewicz, M.R., 2015. Priming for enhanced defense. Annu. Rev. Phytopathol. 53 (1), 97.

Contreras-Cornejo, H.A., Del-Val, E., Mac as-Rodr guez, L., Alarc n, A., Gonz lez-Esquivel, C.E., Larsen, J., 2018. Trichoderma atroviride, a maize root associated fungus, increases the parasitism rate of the fall armyworm Spodoptera frugiperda by its natural enemy Campoletis sonorensis. Soil Biol. Biochem. 122, 196–202.

Contreras-Cornejo, H.A., L pez-Bucio, J.S., M ndez-Bravo, A., Mac as-Rodr guez, L., Ramos-Vega, M., Guevara-Garc, A.A., L pez-Bucio, J., 2015. Mitogen-activated protein kinase 6 and ethylene and auxin signaling pathways are involved in arabidopsis root-system architecture alterations by Trichoderma atroviride. Mol. Plant Microbe Interact. 28 (6) MPMI01150005R.

Contreras-Cornejo, H.A., Macias-Rodriguez, L., Cortes-Penagos, C., Lo Pez-Bucio, J., 2009. Trichoderma virens, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. Plant Physiol. 149 (3), 1579–1592.

Druzhinina, I.S., Verena, S.S., Alfredo, H.E., Horwitz, B.A., Kenerley, C.M., Enrique, M., Mukherjee, P.K., Susanne, Z., Grigoriev, I.V., Kubicek, C.P., 2011. Trichoderma: the genomics of opportunistic success. Nat. Rev. Microbiol. 9 (10), 749–759.

Elangovan, E., Palaniyandi, M., Rasappa, V., 2018. Expression analysis on mycoparasitism related genes during antagonism of Trichoderma with Colletotrichum falcatum causing red rot in sugarcane. J. Plant Biochem. Biotechnol. 3 (27), 351–361.

Filichkin, S., Priest, H.D., Megraw, M., Mockler, T.C., 2015. Alternative splicing in plants: directing traffic at the crossroads of adaptation and environmental stress. Curr. Opin. Plant Biol. 24 (6), 125–135.

Garnica-Vergara, A., Barrera-Ortiz, S., Mu oz-Parra, E., Raya-Gonz lez, J., M ndez-Bravo, A., Mac as-Rodr guez, L., Ruiz-Herrera, L.F., L pez-Bucio, J., 2016. The volatile 6-pentyl-2H-pyran-2-one from Trichoderma atroviride regulates Arabidopsis thaliana root morphogenesis via auxin signaling and ETHYLENE INSENSITIVE 2 functioning. New Phytol. 209 (4), 1496–1512.

He, A., Liu, J., Wang, X., Zhang, Q., Song, W., Chen, J., 2019. Soil application of Trichoderma asperellum GDFS1009 granules promotes growth and resistance to Fusarium graminearum in maize. J. Integr. Agric. 3 (18), 599–606.

Hermosa, R., Viterbo, A., Chet, I., Monte, E., 2012. Plant-beneficial effects of Trichoderma and of its genes. Microbiology 158 (1), 17–25.

Huang, S., Zhang, J., Li, R., Zhang, W., He, Z., Lam, T., Peng, Z., Yiu, S., 2011. SOAPsplice: genome-wide ab initio detection of splice junctions from RNA-Seq data. Front. Genet. 2, 46.

Huang, Y., Mijiti, G., Wang, Z., 2015. Functional analysis of the class II hydrophobin gene HFB2-6 from the biocontrol agent Trichoderma asperellum ACCC30536. Microbiol Res. 171, 8–20.

Jacob, P., Hirt, H., Bendahmane, A., 2016. The heat shock protein/chaperone network and multiple stress resistance. Plant Biotechnol. J. 4 (15), 405–414.

Khadeeva, N.V., Kochieva, E.Z., Tcherednitchenko, M.Y., Yakovleva, E.Y., Sydoruk, K.V., Bogush, V.G., Dunaevsky, Y.E., Belozersky, M.A., 2009. Use of buckwheat seed protease inhibitor gene for improvement of tobacco and potato plant resistance to biotic stress. Biochemistry (Mosc.) 74 (3), 260.

Kowsari, M., Motallebi, M., Zamani, M., 2014. Protein engineering of Chit42 towards improvement of Chitinase and antifungal activities. Curr. Microbiol. 68 (4), 495–502.

Laloum, T., Guiomar, M.N., Duque, P., 2017. Alternative splicing control of abiotic stress responses. Trends Plant Sci. 2 (23), 140.

Lea, A., Sabine, G., Alexander, L., Theresa, R., Leoni, A., Martin, M.N.T., Nancy, S.P., Kreil, D.P., Susanne, Z., 2018. The Gpr1-regulated Sur7 family protein Sfp2 is required for hyphal growth and cell wall stability in the mycoparasite Trichoderma atroviride. Sci. Rep.-UK 1 (8), 12064.

Li, H., Ruan, J.R., 2008. Mapping short DNA sequencing reads and calling variants using mapping quality scores. Genome Res. 18 (11), 1851–1858.

Li, R., Wang, W., Wang, W., Li, F., Wang, Q., Xu, Y., Wang, S., 2015. Overexpression of a cysteine proteinase inhibitor gene from Jatropha curcas confers enhanced tolerance to salinity stress. Electron. J. Biotechnol. 18 (5), 368–375.

Li, Y.T., Hwang, S.G., Huang, Y.M., Huang, C.H., 2017. Effects of Trichoderma asperellum on nutrient uptake and Fusarium wilt of tomato. Crop Prot. 100, 275–282.

Liu, J., Chen, X., Liang, X., Zhou, X., Yang, F., Liu, J., He, S.Y., Guo, Z., 2016a. Alternative splicing of rice WRKY62 and WRKY76 transcription factor genes in pathogen defense. Plant Physiol. 171 (2) pp.01921.2015.

Liu, N.Y., Bao, Z.R., Li, J., Ao, X.Y., Zhu, J.Y., Chen, Y.H., 2016b. Identification of differentially expressed genes from Trichoderma atroviride strain SS003 in the presence of cell wall of Cronartium ribicola. Genes Genom. 39 (5), 1–12.

Lu, J., CALM, Robert, Michael, R., Marco, C., Laurent, M.N.S., Josep, M., Michael Joseph, S., Yonggen, L., Jonathan, G., Matthias, E., 2015. Induced jasmonate signaling leads to contrasting effects on root damage and herbivore performance. Plant Physiol. 167 (3), 1100–1116.

Mac as-Rodr guez, L., Guzm n-G mez, A., Garc a-Ju rez, P., 2018. Trichoderma atroviride promotes tomato development and alters the root exudation of carbohydrates, which stimulates fungal growth and the biocontrol of the phytopathogen Phytophthora cinnamomi in a tripartite interaction systemda. FEMS Microbiol. Ecol. 9 (94) fiy137.

Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C.M.J., Pozo, M.J., Ton, J., Dam, N.M.V., Conrath, U., 2016. Recognizing plant defense priming. Trends Plant Sci. 21 (10), 818–822.

Mazurkiewicz, P., Sakamoto, K., Poelarends, G.J., Konings, W.N., 2005. Multidrug transporters in lactic acid bacteria. Mini Rev. Med. Chem. 5 (2).

Moran-Diez, E., Hermosa, R., Ambrosino, P., Cardoza, R.E., Gutierrez, S., Lorito, M., Monte, E., 2009. The ThPG1 endopolygalacturonase is required for the Trichoderma harzianum-plant beneficial interaction. Mol. Plant Microbe Interact. 22 (8), 1021–1031.

Mortazavi, A., Williams, B.A., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat. Methods 5 (7), 621–628.

Nehls, U., G Hringer, F., Wittulsky, S., Dietz, S., 2010. Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. Plant Biol. 12 (2), 292–301.

Nieto-Jacobo, M.F., Steyaert, J.M., Salazar-Badillo, F.B., Nguyen, D.V., Rost s, M., Braithwaite, M., De Souza, J.T., Jimenez-Bremont, J.F., Ohkura, M., Stewart, A., 2017. Environmental growth conditions of Trichoderma spp. affects indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. Front. Plant Sci. 8, 102.

Nygren, K., Dubey, M., Zapparata, A., Iqbal, M., Karlsson, M., 2018. The mycoparasitic fungus Clonostachys rosea responds with both common and specific gene expression during interspecific interactions with fungal prey. Evol. Appl. 11 (6).

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Wees, S.C.M.V., Bakker, P.A.H.M., 2014. Induced systemic resistance by beneficial microbes. Annu. Rev. Phytopathol. 52 (1), 347–375.

Ram rez-Valdespino, C.A., Porras-Troncoso, M.D., Corrales-Escobosa, A.R., Wrobel, K., Mart Nez-Hern, N.P., Olmedo-Monfil, V., 2017. Functional characterization of TvCyt2, a member of the p450 monooxygenases from Trichoderma virens relevant during the association with plants and Mycoparasitism. Mol. Plant Microbe Interact. 31 (3) MPMI-01-17-0015.

Reino, J.L., Guerrero, R.F., Hern ndez-Gal, n R., Collado, I.G., 2008. Secondary metabolites from species of the biocontrol agent Trichoderma. Phytochem. Rev. 7 (1), 89–123.

Roberto, M., Simone, S., Michelina, R., Ani, B., Enrico, C., Sebastiano, N., Barbara, B., Maurizio, C., Paola, M., Matteo, L., 2018. The hydrophobin HYTLO1 secreted by the biocontrol fungus Trichoderma longibrachiatum triggers a NAADP-mediated calcium signalling pathway in Lotus japonicus. Int. J. Mol. Sci. 9 (19), 2596.

Roberts, A., Pimentel, H., Trapnell, C., Pachter, L., 2011. Identification of novel transcripts in annotated genomes using RNA-Seq. Bioinformatics 27 (17), 2325–2329.

Rubio, B.N.M., Quijada, N.M., P rez, E., Dom nguez, S., Monte, E., Hermosa, R., 2014. Identifying beneficial qualities of Trichoderma parareesei for plants. Appl. Environ. Microbiol. 80 (6), 1864–1873.

Samolski, I., Rincón, A.M., Pinzón, L.M., 2012. The qid74 gene from Trichoderma harzianum has a role in root architecture and plant biofertilization. Microbiology 158 (1), 129–138.

Sarrocco, S., Matarese, F., Baroncelli, R., Vannacci, G., Seidl-Seiboth, V., Kubicek, C.P., Vergara, M., 2017. The constitutive endopolygalacturonase TvPG2 regulates the induction of plant systemic resistance by Trichoderma virens. Phytopathology 107 (5), 537–544.

Schmittgen, T., Livak, K., 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. (3), 1101–C1108.

Steindorff, A.S., Ramada, M.H.S., Coelho, A.S.G., Miller, R.N.G., Pappas, G.J., Ulhoa, C.J., Noronha, E.F., 2014. Identification of mycoparasitism-related genes against the phytopathogen Sclerotinia sclerotiorum through transcriptome and expression profile analysis in Trichoderma harzianum. BMC Genom. 15 (1), 204.

Studholme, D.J., Harris, B., Le, C.K., Winsbury, R., Perera, V., Ryder, L., Ward, J.L., Beale, M.H., Thornton, C.R., Grant, M., 2013. Investigating the beneficial traits of Trichoderma hamatum GD12 for sustainable agriculture-insights from genomics. Front. Plant Sci. 4 (4), 258.

Thatcher, S.R., Danilevskaya, O.N., Meng, X., 2016. Genome-wide analysis of alternative splicing during development and drought stress in maize. Plant Physiol. 1 (170), 586.

Vargas, W.A., Crutcher, F.K., Kenerley, C.M., 2011. Functional characterization of a plant-like sucrose transporter from the beneficial fungus Trichoderma virens. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. New Phytol. 189 (3), 777–789.

Vargas, W.A., Mandawe, J.C., Kenerley, C.M., 2009a. Plant-derived sucrose is a key element in the symbiotic association between Trichoderma virens and maize plants. Plant Physiol. 151 (2), 792–808.

Vargas, W.A., Mandawe, J.C., Kenerley, C.M., 2009b. Plant-derived sucrose is a key

element in the symbiotic association between Trichoderma virens and maize plants. Plant Physiol. 151 (2), 792–808.
Vinale, F., Sivasithamparam, K., Ghisalberti, E., 2008. A novel role for Trichoderma secondary metabolites in the interactions with plants. Physiol. Mol. Plant Pathol. 72 (1), 80–86.
Wang, M., Wang, Y., Zhang, Y., 2019. Comparative transcriptome analysis of salt-sensitive and salt-tolerant maize reveals potential mechanisms to enhance salt resistance. Genes Genom . 41 (7), 781–801.
Wang, Y., Bouillon, C., 2013. Interfacial study of class II hydrophobin and its mixtures with milk proteins: Relationship to bubble stability. J. Agr. Food Chem. 61 (7), 1554–1562.
Wu, Q., Sun, R., Ni, M., Yu, J., Li, Y., Yu, C., Dou, K., Ren, J., Chen, J., 2017. Identification of a novel fungus, Trichoderma asperellum GDFS1009, and comprehensive evaluation of its biocontrol efficacy. PLoS One 12 (6), e0179957.
Yamile, M., Brown, J.W.S., Craig, S., Andrea, B., Maria, K., 2012. Transcriptome survey reveals increased complexity of the alternative splicing landscape in Arabidopsis. Genome Res. 22 (6), 1184–1195.
Yoshioka, Y., Ichikawa, H., Naznin, H.A., Kogure, A., Hyakumachi, M., 2015. Systemic resistance induced in Arabidopsis thaliana by Trichoderma asperellum SKT-1, a microbial pesticide of seedborne diseases of rice. Pest Manage. Sci. 68 (1), 60–66.
Yu, C., Dou, K., Wang, S., Wu, Q., Ni, M., Zhang, T., Lu, Z., Tang, J., Chen, J., 2019. daElicitor hydrophobin Hyd1 interacts with Ubiquilin1-like to induce maize systemic resistance. J. Integr. Plant Biol doi: 10.1111/jipb.12796.
Yuan, M., Huang, Y., Ge, W., Jia, Z., Song, S., Zhang, L., Huang, Y., 2019. daInvolvement of jasmonic acid, ethylene and salicylic acid signaling pathways behind the systemic resistance induced by Trichoderma longibrachiatum H9 in cucumber. BMC Genom. 20, 144.
Zhang, Y., Bingfa, S., Feng, D., Hu, H., Matthew, C., Qu, Q., 2017. Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein. Nature 546 (7657).