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Full Length Article

Transcriptome Analysis of *Rhizoctonia solani* Anastomosis Group 5 Early Invasion in Potato

Shuai Yang[1], Fanxiang Min[1], Wenzhong Wang[1], Qi Wei[1], Xuezhi Dong[1], Xiaodan Wang[1], Ling Wang[2], Dianqiu Lyu[1*] and Jinghua Zhang[1]

[1]*Virus-free Seedling Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China*
[2]*Institute of Crops Tillage and Cultivation, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, China*
[*]For correspondence: smallpotatoes@126.com

Abstract

Rhizoctonia solani (*R. solani*) is a destructive soil-borne root infecting fungus which impaired quality and production of potato. This study was performed to investigate the response to *R. solani* AG-5 infection in potato. Minitubers (cv. Favorita) were inoculated by the *R. solani* AG-5 strain. Four days after emergence of *R. solani* AG-5 infection on the young stem of potato, samples were collected and prepared for the Illumina sequencing. The Gene Ontology (GO) and KEGG pathway enrichment analyses of differentially expressed genes (DEGs) between infected and control plants were performed. Functional analysis of these DEGs indicated a total of 338 DEGs, including 216 up-regulated (63.91%) and 122 downregulated DEGs (36.09%), were identified between the control and *R. solani* infected potato plants. These DEGs were associated with the plant growth, defense, fungal resistance by modulating the oxidative response (including NB-ARC domain-containing disease resistance proteins, ankyrin repeat-containing proteins, ANKs; and peroxidases), ion exchange/transportation (including calcineurin B-like proteins, CBLs; CBL-interacting protein kinase, CIPKs; calmodulin-like proteins, CMLs; and K^+ channel, KAT1) and plant hormone-mediated signaling (MYB44, ANKs). Some essential transcription factors including WRKY45 and MYB4 were significantly upregulated by *R. solani* infection. The *R. solani* infection triggered DEGs were related to plant growth, defense, oxidative response, and hormone-mediated signaling. The genes of CBL4, KAT1, ANKs, and MYBs might promote potato adjustment or resistance against *R. solani* infection.

Keywords: *Rhizoctonia solani*; Potato; Transcriptome; Differentially expressed genes

Introduction

Rhizoctonia solani (*R. solani*) is a widespread and destructive soil-borne root infecting fungus (Shamim *et al.*, 2014). *R. solani* causes stem canker and sheath blight diseases in various crops and vegetables including rice, maize, wheat, soybean, cauliflower, potato, and tomato (Anderson *et al.*, 2016).

R. solani is composed of 14 distinct anastomosis groups (AGs), that are AG-1 ~ 13 and the AG-BI (Zhu *et al.*, 2016). Six AGs including AG-2-1, AG-3PT, AG-4, AG-5, AG-7, and AG-8 are the pathogens of potato diseases (Muzhinji *et al.*, 2016). Among these 6 AGs, the AG 3-PT was reported to be the most common pathogen in potato diseases, and could causes stem canker, black scurf, growth cracking on potato, thus resulting into dramatic loss of potato yield and quality (Muzhinji *et al.*, 2014; Patil *et al.*, 2017). These 6 AGs cause potato lesions, canker symptoms or infections on different parts of potato plants. AG-2-1 isolates cause lesions on plants and tubers with different severities. The high level genetic diversity and the high evolutionary potential of AG-3PT gives it a high risk of fungicide resistance (Fiers *et al.*, 2011; Lin and Gudmestad, 2013). AG-3 PT mainly causes tuber black scurf and stem canker (Ferrucho *et al.*, 2012). Both AG-4 and AG-5 are commonly isolated from canker potatoes in warm environment, but not in black scurf tubers (Yang *et al.*, 2015). AG-7 infected potato stem, stolon, and tuber, whereas AG-8 only infected potato roots (Balali *et al.*, 1995; Carling *et al.*, 2007; Yang *et al.*, 2015).

The *R. solani* AG-5 was the most frequent AG (25%) in European soils (Goll *et al.*, 2014). *R. solani* AG-5 also is a frequent pathogen on oat, wheat, apple, and potato, and AG-5 induce stem canker and sheath blight on these plants (El-Sharouny, 2015; Hewavitharana and Mazzola, 2016; Zhang *et al.*, 2016). As reported, 4 AGs including AG-2-1, AG-3, AG-4 and AG-5 are main causes AGs for potato diseases in Heilongjiang Province of China (Yang *et al.*, 2017). Our previous works showed both AG-3 and AG-5 isolates from different areas in Heilongjiang Province exhibited the highest virulence on potato stems and roots, in comparison with AG-2-1 and AG-4 isolates (Yang *et al.*, 2017). The use of chemical fungicide and crop rotation or intercrop with disease resistant cultivars could partially

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control the prevalence of *R. solani* infection (Mazzola, 2007; Muzhinji *et al.,* 2016). However, the use of chemical fungicide is a severe environmental contaminate, and disease-resistant cultivars management are variable because of the wide host range long survival of *R. Solani* sclerotia in soil (Muzhinji *et al.,* 2016). Therefore, efficiency alternatives for improving crop resistance and controlling *R. solani* spread are in urgent need.

With the development and application of high throughout sequencing technology, the processes, differentially expressed genes (DEGs), and signaling which are related to plant defense, infection resistance, nutrient competition, and metabolism had been identified (Elsharouny, 2015; Chen *et al.,* 2016; Zhang *et al.,* 2016). Zhu *et al*. (2016) performed the *de novo* transcriptome analysis of *R. solani*AG-1 IA-invaded *Zoysia japonica*, and found most of the differentially expressed genes (DEGs) in *Zoysia japonica* root in response to infection were related to plant growth, oxidative response, and defense. Similar transcriptome analyses of *R. solani* AG-1 IA infected rice (resistant and susceptible) have been reported (Verwaaijen *et al.,* 2017; Zhang *et al.,* 2017). However, far less information is known on the responses of potato plant to *R. solani* AG-5 infection. The comprehensive understanding of the molecular processes in infected plants is direly needed for controlling *R. solani* infection in potato.

Heilongjiang is a major seed potato and primary potato production province in China (Yang *et al.,* 2017). With the spread of AGs infections and limitations of chemical fungicide, crop rotation or intercrop, the tuber black scurf and stem canker diseases are widely spreading on potato in Heilongjiang Province. The objective of this study was performed to investigate the responses of potato plants after being infected with one of the main infections in Heilongjiang Province, *R. solani* AG-5. Minitubers (cv. Favorita) were inoculated by the *R. solani* AG-5 isolate. This study would provide us with more information on the resistance against *R. solani* AG-5 infection in potato.

Materials and Methods

Plants, *R. solani* and Cultivation

R. solani AG-5 isolate was obtained from our lab and the standard strain of *R. solani* was a friend gift from professor Jun Hu, Inner Mongolia Agricultural University. *R. solani* AG-5 were cultured on the potato dextrose agar (PDA) plate for 3–4 days at 25℃. Then 8 mm agar disks were cut from the plates for inoculation. The comprehensive transcriptome analysis was performed using the infected potato plants and the health controls.

Inoculation and Sampling

The minitubers (cv. Favorita) were sterilized with 1% NaClO for 20 min, washed with sterile distilled water for 3 times, and sowed into sterile medium (Yang *et al.,* 2017). The agar disks were inoculated to the young stems after germination. When the infection was observed, the stems with typical infection were sampled and collected from 3 plants at 96 h after infection for RNA sampling. The normal stems from 3 health control plants were used as the experimental controls.

RNA Extraction, Quality Controlling, Library Preparation, and Illumina Sequencing

Stem from 3 infected and control plants were collected, total RNA was isolated using RNA prep Pure plant kit (Qiagen Biotech Co., Beijing, China) following the manufacturer's instruction. Agar gel electrophoresis sugar (AGE, 1%), K5500 spectrophotometer (Beijing Kaiao Technology Development Co., Ltd., Beijing, China) and the 2100 RNA Nano 6000 Assay Kit (Agilent Technologies, CA, USA) was used for the detection of integrity, concentration and purification, respectively. For library construction, the RNA (RNA Integrity Number ≥ 8.0, A260: A280 > 1.9 and A260: A230 >1.8) was enriched using the Oligo (dT) magnetic beads (Illumina Inc., San Diego, CA, USA), and fragmented using fragmentation buffer (Illumina). Then the cDNA was synthesized using a primer with six random bases, followed by purification using the QIAquick PCR purification kit (Qiagen), and then the adaptors ligation, AGE purification and PCR amplification. The PCR products of 6 libraries (3 treated and 3 controls) were subjected to the Illumina Hiseq PE150 sequencing (Illumina).

Data Processing, Sequencing Quality Control and Annotation

Original data (raw reads) was got after sequencing. Clean reads were obtained after filtering the low-quality sequences (≥ 15% bases with Q≤19), adaptor-pollution (≥ 5 bp adaptor bases in reads) and high content of unknown base reads (≥ 5%; CASAVA FASTQ files). The clean reads were used for the transcriptome assembly using Trinity (version trinityrnaseq-2.0.2) (Zerbino and Birney, 2008). The quality of sequenced reads (Qphred30, Q30), reads distribution, clean bases number, and base distribution were detected. Alignment to reference genome (ENSEMBL; http://www.ensembl.org/index.html) was performed using the TopHat v 2.0.12 (Trapnell *et al.,* 2009) and Bowtie v 1.0.1 softwares (Langmead *et al.,* 2009) with the default settings. The reads distribution in exon, intron and intergenic regions of reference genome was performed. Moreover, the single nucleotide polymorphysms (SNPs) in the sequences were identified using the Samtools-0.1.19 (Li *et al.,* 2009). All these alignment results were visualized using integrative genomics viewer (IGV) (Thorvaldsdóttir *et al.,* 2013).

Quantification of Gene Expression Levels and Alternative Splicing Detection

The RPKM of each gene was then calculated based on gene

length and mapped reads renumber using HTSeq v 0.5.4p3 and Cufflinks v 2.2.1 (Wagner *et al.,* 2012). The alternative splicing in the 6 plants was analyzed using the ASprofile 1.0 software.

DEGs Identification

The identification of the DEGs was performed using the DESeq R package (v 1.10.1, (Wang *et al.,* 2009). DESeq determined the DEGs based on the negative binomial distribution, followed by the Benjamini and Hochberg adjustment. DEGs with adjust p-value ≤ 0.05, and log2 (fold change) ≥ 1 (up) or ≤ -1 (down) were identified.

DEGs Annotation

Hierarchical clustering for DEGs was performed with pHeatmap. The aligned reads to reference genome were subjected the databases (all with default settings) of non-redundant protein (NR;http://www.ncbi.nlm.nih.gov/), non-redundant nucleotide database (NT;http://www.ncbi.nlm.nih.gov/), Uniprot (http://www.uniprot.org/), Gene Ontology (GO; http://www.geneontology.org/), Cluster of Orthologous Groups of proteins (COG;http://www.ncbi.nlm.nih.gov/COG/), Protein Family (Pfam; http://pfam.janelia.org/), Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/), to get the unigene annotation.

Interaction Analysis

To describe the interactive network of DEGs and to display the repeatedly occurring neighborhood of DEGs, STRING (Search Tool for the Retrieval of Interacting Genes) database (http://string-db.org/) was used to build the protein-protein interaction (PPI) network of encoding products of all of the DEGs (Von Mering *et al.,* 2007), with the default parameters. Cytoscape was used to visualize the PPI network (Shannon *et al.,* 2003).

GO and KEGG Enrichment Analysis

GO enrichment analysis of DEGs was performed using the Blast2GO. GO terms (molecular function, cellular component, and biological process) with corrected p value (q value) < 0.05 were considered as significant enriched. We used KEGG Orthology (KO) database to test the statistical enrichment of DEGs in KEGG pathways.

Novel Transcripts Prediction and Annotation

By comparing the Illumina sequences with the reference genome using the Cuffcompare program of Cufflinks, we identified the known and novel transcripts based on the TopHat v 2.0.12 alignment. Next, the novel transcripts were annotated by blasting against the NT database (http://www.ncbi.nlm.nih.gov/).

Results

Quality Control and Normalization of Sequencing Data

After sequencing, the raw data was quantified and the clean data quality was assessed. The Q30, alignment percentage (rate) to reference genome are showed in Table 1. The SNP and alternative splicing results are presented in Fig. 1. The most common alternative splicing types were TSS (alternative 5' first exon), and TTS (alternative 3' last exon), followed by AE (alternative 3' or 5' exon ends) and SKIP. In addition, some genes had various types of alternative splicing.

DEGs Analysis

To analyze the transcriptional changes associated with *R. solani* infection, we analyzed the DEGs between the *R. solani* AG-5infected potato and the control plants. A total of 338 DEGs, including 216 upregulated (63.91%) and 122 downregulated DEGs (36.09%), were identified between the control and *R. solani* AG-5 infected potato plants (Fig. 2a). Hierarchical clustering of the DEGs is shown in Fig. 2b.

GO and KEGG Enrichment Analysis

Bioinformatics analysis showed the identified DEGs were associated with plant defense, resistance, hormone and ion exchange/transportation. The GO analysis of the DEGs showed DEGs were significantly enriched into biological processes including "cellular process", "metabolic process", "response to stimulus"; cellular component including "cell part", "organelle", "membrane part", and "extracellular region"; molecular function such as "catalytic", "binding", "nucleic acid binding transcription factor", and "transporter" (Fig. 3).

The analysis of the KEGG pathway showed little DEGs enriched into significant pathways (Table 2). These pathways including "Long-term potentiation", "Natural killer cell mediated cytotoxicity", "Apoptosis", "Phenylalanine metabolism", "T cell receptor signaling pathway", and "Calcium signaling pathway".

Novel Transcript Prediction

Using the Cufflinks software, over thousands of novel transcripts were identified, with high lost-annotation rate (80 ~ 85%) against to NT database (Table 3), indicating most of these transcripts were noncoding RNAs.

Discussion

R. solani is a widespread fungus pathogen, which is seriously threating the production and quality of potato and various crops. In view of the complexity of genetic structure

Table 1: Summary of the sequencing data

Samples	Raw reads	Clean reads	Clean bases	Q30 (%)	Mapping rate (%)	Exon mapping rate
Treat - 1	49,369,678	41,992,978	6,298,946,700	93.72	67.77	93.08
Treat - 2	46,482,664	39,202,380	5,880,357,000	93.27	63.89	91.19
Treat - 3	51,814,342	44,419,914	6,662,987,100	94.07	63.18	90.06
Con - 1	49,094,376	41,614,938	6,242,240,700	93.49	66.21	93.39
Con - 2	47,548,578	40,296,052	6,044,407,800	93.32	62.81	93.41
Con - 3	47,091,702	38,116,086	5,717,412,900	92.72	64.85	93.34

Q30 (%) was the percentage of clean reads with Phred quality score > 30

Table 2: Top 30 enriched KEGG pathways of the differentially expressed genes (DEGs)

GO	Description	p	FDR	Genes
map04720	Long-term potentiation	0.001642071	0.052181358	3
map04650	Natural killer cell mediated cytotoxicity	0.001642071	0.052181358	3
map05031	Amphetamine addiction	0.000720328	0.052181358	3
map04210	Apoptosis	0.000975455	0.052181358	3
map05014	Amyotrophic lateral sclerosis (ALS)	0.001454776	0.052181358	3
map00360	Phenylalanine metabolism	0.001426697	0.052181358	6
map04380	Osteoclast differentiation	0.001454776	0.052181358	3
map04662	B cell receptor signaling pathway	0.001642071	0.052181358	3
map04660	T cell receptor signaling pathway	0.000610694	0.052181358	3
map04370	VEGF signaling pathway	0.001843813	0.052733047	3
map04360	Axon guidance	0.00280283	0.072873575	3
map00940	Phenylpropanoid biosynthesis	0.003351527	0.074326605	6
map04020	Calcium signaling pathway	0.003378482	0.074326605	3
map01110	Biosynthesis of secondary metabolites	0.004323206	0.088316922	17
map04724	Glutamatergic synapse	0.005118005	0.0975833	3
map04010	MAPK signaling pathway	0.006378404	0.114013978	3
map01062	Biosynthesis of terpenoids and steroids	0.00959041	0.161344538	1
map05152	Tuberculosis	0.013168531	0.209233321	3
map04626	Plant-pathogen interaction	0.021503281	0.295750383	6
map04146	Peroxisome	0.021715937	0.295750383	4
map04978	Mineral absorption	0.020045519	0.295750383	2
map04310	Wnt signaling pathway	0.027907725	0.362800421	4
map05166	HTLV-I infection	0.039393367	0.489847954	3
map00270	Cysteine and methionine metabolism	0.044579019	0.531233311	3
map00062	Fatty acid elongation	0.055775676	0.638073735	2
map01100	Metabolic pathways	0.059709264	0.656801906	22
map04712	Circadian rhythm - plant	0.06342062	0.671788792	2
map04070	Phosphatidylinositol signaling system	0.112353162	1	2
map04075	Plant hormone signal transduction	0.520529757	1	3
map05145	Toxoplasmosis	0.340408704	1	1

Table 3: Top 30 enriched KEGG pathways of the differentially expressed genes (DEGs)

Samples	Novel transcript	NT annotation	Lost annotation rate (%)
Treat -1	2,143	382	82.17
Treat -2	2,259	386	82.91
Treat -3	2,384	378	84.15
Con -1	2,188	381	82.59
Con -2	2,113	410	80.60
Con -3	1,968	375	80.95

of *R. solani*, there had no reports about the pathogenic mechanism. Digging out resistance genes in potato may be a way to study the mechanism of pathogenesis. At present, the pathogenesis of infection with *R. solani* in potato is not clear, so this study is of great significance for further research. Comparative analyses of transcriptome indicated the gene expression profiles in *R. solani* infected plants (Zhu *et al.*, 2016; Zhang *et al.*, 2017). After microbial infection, plants often show the defense and resistance responses. Plants develop various pathways or signals to response to fungus infections, including plant hormones, factors related to ion exchange/transportation, the transcription factors associated with various responses to biotic and abiotic stresses. In this present study, we infected the young stems of the potato using *R. solani* AG-5 and analyzed this infection induced mRNA expression profiles for the first time. The transcriptomic analysis showed the DEGs were associated with various functions, and were identified to be crucial factors for plant growth, development, and resistance to stresses.

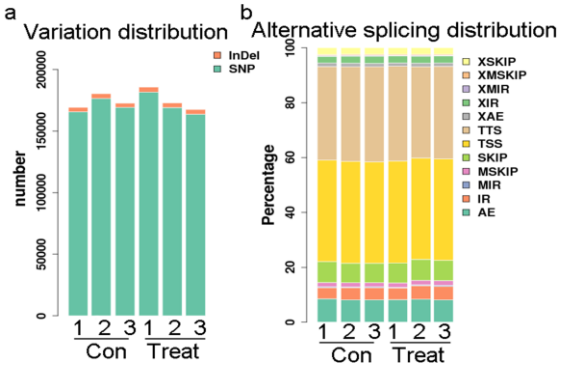

Fig. 1: The single nucleotide polymorphysms and alternative splicing distribution. a, the variation distribution. b, alternative splicing types and distribution in detected plants. SKIP, skipped exon; XSKIP, approximate SKIP; MSKIP, multi-exon SKIP; XMSKIP, approximate MSKIP; IR, intron retention; MIR, multi-IR; XMIR, approximate MIR; XIR, approximate IR; AE, alternative exon ends; XAE, approximate AE; TSS, alternative 5' first exon; TTS, alternative 3' last exon. Con, health control potato plants. Treat, the *R. solani* infected potato plants

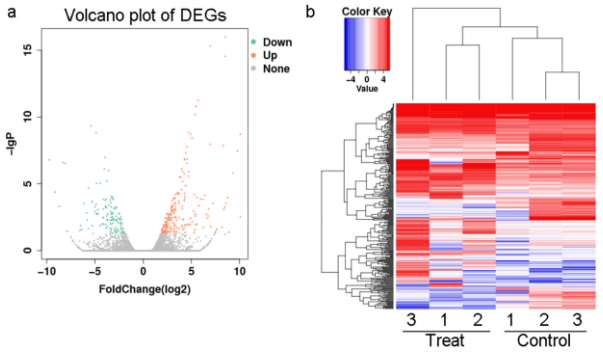

Fig. 2: Analysis of differentially expressed genes (DEGs). a, a volcano plot of DEGs between control and *R. solani* infected potato plants. b, the Hierarchical clustering of the DEGs between control and *R. solani* infected potato plants. Control, health control potato plants. Treat, the *R. solani* infected potato plants

Genes Related to Fungal Resistance, Plant Defense, and Oxidative Response

Among the DEGs between the *R. solani* infected potato and controls, some genes were associated with oxidative responses, such as the MYB44transcription factor, peroxidases, and ankyrin repeat-containing proteins (ANKs). The MYB superfamily is characterized by the presence of conserved MYB domains. MYBs involve in various processes and act as regulators for plant responses (Ambawat *et al.*, 2013). The *MYB44* is a member of R2R3-MYB family, which is the largest part of MYB family and responsible for biotic and abiotic stresses (Li *et al.*, 2014; Soler *et al.*, 2015).

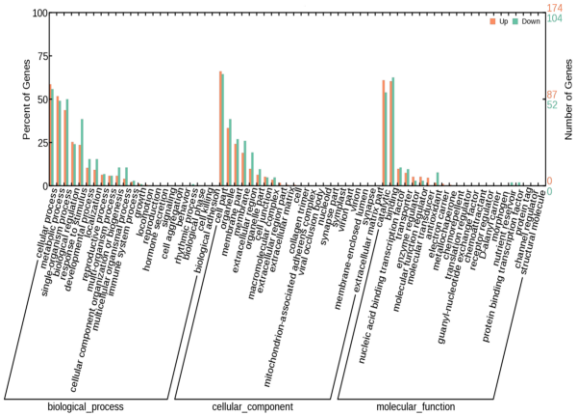

Fig. 3: The enriched GO classification analysis of differentially expressed genes (DEGs) Orange and green indicates up- or down-regulated DEGs number in the term, respectively

Peroxidases involves in the regulatory of various processes including oxidative response and adjustment (Yao *et al.*, 2013; Caverzan *et al.*, 2014; Xu *et al.*, 2014; Espinosa-Diez *et al.*, 2015). Various environmental stresses induce oxidative responses in plant (Das and Roychoudhury, 2016). After fungal pathogens, ROS is abnormally triggered and then acts as modulator of developmental signals, such as the programmed cell death (Lehmann *et al.*, 2015). The induced ROS by *R. solani* infection had been reported in *Zoysia japonica* roots (Zhu *et al.*, 2016). Besides, among the DEGs, the one gene encoding an ANK protein has been identified to take part in both antioxidation metabolism and disease resistance (Yan *et al.*, 2002). The ANK-encoding genes in other plants, such as the *Arabidopsis NPR1* gene and tobacco *NEIP2*, regulate plant response to stresses (Cao *et al.*, 1997; Yan *et al.*, 2002; Cao *et al.*, 2015). One rice ANK gene (*OsPIANK1*) was reported to be a positive regulator for infection of rice leaf blight pathogen (Mou *et al.*, 2013), suggesting the important roles of ANKs in plant defense.

Furthermore, we identified the NB-ARC domain-containing disease resistance proteins and several transcription factors are related to *R. solani* infection, such as the WRKY, MYB, and ABC. The expression changes of them in *R. solani* AG-5 infected rice and *Arabidopsis* had been identified in previous reports (Cordovez *et al.*, 2017; Zhu *et al.*, 2016). In our plants, we identified the upregulated transcription of *MYB4*, *MYB44*, *MYB48*, *WRKY45*, *WRKY72*, and ANKs encoding genes, and the down regulated transcription of peroxidases encoding genes, ABC transporters, revealing the *R. solani* AG-5 infection caused responses to ROS and pathogen resistance in potato plants.

Genes Associated with Plant Hormones

During the plant resistance to pathogen stress,

phytohormone dynamic profiling take great roles in balancing immune responses and adjustment (Verma *et al.*, 2016). For instance, abscisic acid (ABA) is essential for plant acclimation to salt and heat stress (Suzuki *et al.*, 2016). Also, ABA, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) take the major roles in responses to pathogens and abiotic stresses (Verma *et al.*, 2016). As reported, the expression of *MYB44* in plant was identified during treatment with ABA and JA (Li *et al.*, 2015). The *OsPIANK1* in rice was a positive regulator of rice leaf blight infection via mediating by the SA and JA pathways (Mou *et al.*, 2013). Also, the apoplastic peroxidases are required for SA-mediated defense against *Pseudomonas syringae* (Mammarella *et al.*, 2015). The octadecanoid pathway could regulate systemic cell death, and multiple biotic and abiotic stresses (Santino *et al.*, 2013; Sun *et al.*, 2014). We also identified the chloroplasticallene oxide synthase (AOS) encoding gene was significantly upregulated in *R. solani* infected potato plants in comparison with the health control. These results suggested these genes play important roles in resistance to *R. solani* AG-5 infection in potato plants.

Genes and Pathways Related to Cation Transportation and Cation-dependent Modulation

Among those mechanisms related to defense and metabolism responses, genes and pathways associated with cation transportation indeed and essentially modulate plant growth, response, and resistance. As reported, the calcium (Ca^{2+}) and potassium (K^+) signalings take basic part in plant physiology, biochemistry, immunity, and systemic balance. Calcineurin B contains 4 EF hand Ca^{2+} binding domains and serves as crucial switch for many molecular processes (Luan, 2009). The calcineurin B-like proteins (CBLs) act as Ca^{2+} sensors and regulate magnesium (Mg^{2+}) homeostasis, leaf transpiration and root K^+ uptake by interacting with the CBL-interacting protein kinase (CIPKs) in *Arabidopsis* (Cheong *et al.*, 2007; Tang *et al.*, 2015). Ca^{2+} could promote CBL–CIPK complex formation (Luan, 2009). As reported, the interaction of CBL4 (SOS3; a salt-overly-sensitive mutant gene mainly in roots) with CIPK24/SOS2 could directly regulate a downstream Na^+/H^+ antiporter (*SOS1*) and thus regulating salt tolerance (Luan, 2009). Besides, CBL1 also functions in various stress responses including salt tolerance, ABA response, and the CBL1-CIPK23 interaction can regulate the stomata actions and K^+ uptake via K^+ channel KAT1 (Luan, 2009; Liu *et al.*, 2015). Therefore, the CBLs, CIPKs, and their interactions modulate plant stress response, nutrient adjustment and salt adaption (Luan, 2009).

Moreover, the calmodulin-like proteins (CMLs), including CML42, CML44 and CML43, also showed the stress-responsive possibilities for enhancing plant tolerance to abiotic stresses and resistance to pathogens (Chiasson *et al.*, 2005; Bender *et al.*, 2014; Munir *et al.*, 2016). In our present study, we found the *R. solani* AG-5 infection induced DEGs associated with Ca^{2+} and K^+ signaling, such as the upregulated *CBL4*,*KAT1*, *CML44* and *CML1*, suggesting the potato plants had developed the resistance against *R. solani* AG-5 infection through factors or signalings associated with plant tolerance and nutrition adaption.

Novel Transcripts by *R. solani* Infection

At last, we analyzed the novel transcripts in *R. solani* AG-5 infected potato, which had no mapping onto the reference genome. About two thousands of novel transcripts were predicated in each plant, and only less of them (< 20%) were mapped to transcript in NCBI and were predicated with encoding products (putative proteins). These might reveal there were many non-coding RNAs, such as lncRNA and miRNA, had been deregulated by *R. solani* AG-5 infection. These suggested there might be more space to analyze the miRNA expression profiles related to *R. solani* infection.

Conclusion

Using the transcriptome analysis, we identified the DEGs associated with *R. solani* AG-5 invasion in potato (cv. Favorita). Functional annotation of these DEGs suggested plant resistance against *R. solani* AG-5 infection was mediated by various pathways and signalings which were associated with plant defense and fungal resistance. These DEGs were related to plant hormones signalings, oxidative responses, cation transportations and cation-dependent modulations, suggesting the potato plant developed responses and resistance against *R. solani* infection. However, more molecular experiments should be done to investigate whether these DEGs were essential for *R. solani* AG-5 resistance in potato.

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References

Ambawat, S., P. Sharma, N.R. Yadav and R.C. Yadav, 2013. MYB transcription factor genes as regulators for plant responses: an overview. *Physiol. Mol. Biol. Plant*, 19: 307–321

Anderson, J.P., J.K. Hane, T. Stoll, N. Pain, M.L. Hastie, P. Kaur, C. Hoogland, J.J. Gorman and K.B. Singh, 2016. Mass-spectrometry data for *Rhizoctonia solani* proteins produced during infection of wheat and vegetative growth. *Data Brief*, 8: 267–271

Balali, G.R., S.M. Neate, E.S. Scott, D.L. Whisson and T.J. Wicks, 1995. Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia. *Plant Pathol.*, 44: 1050–1057

Bender, K.W., S. Dobney, A. Ogunrinde, D. Chiasson, R.T. Mullen, H.J. Teresinski, P. Singh, K. Munro, S.P. Smith and W.A. Snedden, 2014. The calmodulin-like protein CML43 functions as a salicylic-acid-inducible root-specific Ca^{2+} sensor in *Arabidopsis*. *Biochem. J.*, 457: 127–136

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

Cao, Y.R., H.W. Chen, Z.G. Li, J.J. Tao, B. Ma, W.K. Zhang, S.Y. Chen and J.S. Zhang, 2015. Tobacco ankyrin protein NEIP2 interacts with ethylene receptor NTHK1 and regulates plant growth and stress responses. *Plant Cell Physiol.*, 56: 803–818

Carling, D.E., K.A. Brainard, G. Virgen-Calleros and V. Olalde-Portugal, 2007. First Report of *Rhizoctonia solani* AG7 on Potato in Mexico. *Plant Dis.*, 82: 127–127

Caverzan, A., A. Bonifacio, F.E. Carvalho, C.M. Andrade, G. Passaia, M. Schünemann, F. dos Santos Maraschin, M.O. Martins, F.K. Teixeira, R. Rauber and R. Margis, 2014. The knockdown of chloroplastic ascorbate peroxidases reveals its regulatory role in the photosynthesis and protection under photo-oxidative stress in rice. *Plant Sci.*, 214: 74–87

Chen, Z., A. Lin, W. Li, P. Yin, C. Liu, S. Li and H. Zeng, 2016. *De novo* Transcriptome Analysis of *Rhizoctonia solani* AG1 IA Strain Early Invasion in *Zoysia japonica* Root. *Front. Mol.*, 7: 77–82

Cheong, Y.H., G.K. Pandey, J.J. Grant, O. Batistic, L. Li, B.G. Kim, S.C. Lee, J. Kudla and S. Luan, 2007. Two calcineurin B-like calcium sensors, interacting with protein kinase CIPK23, regulate leaf transpiration and root potassium uptake in *Arabidopsis*. *Plant J.*, 52: 223–239

Chiasson, D., S.K. Ekengren, G.B. Martin, S.L. Dobney and W.A. Snedden, 2005. Calmodulin-like proteins from *Arabidopsis* and tomato are involved in host defense against *Pseudomonas syringae* pv. tomato. *Plant Mol. Biol.*, 58: 887–897

Cordovez, V., L. Mommer, K. Moisan, D. Lucas-Barbosa, R. Pierik, R. Mumm, V.J. Carrion and J.M. Raaijmakers, 2017. Plant Phenotypic and Transcriptional Changes Induced by Volatiles from the Fungal Root Pathogen *Rhizoctonia solani*. *Front. Plant Sci.*, 8: 1262

Das, K. and A. Roychoudhury, 2016. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Plant Sci.*, 2: 53

El-Sharouny, E.E., 2015. Effect of different soil amendments on the microbial count correlated with resistance of apple plants towards pathogenic *Rhizoctonia solani* AG-5. *Biotechnol. Biotechnol. Equip.*, 29: 463–469

Elsharouny, E.E., 2015. Effect of different soil amendments on the microbial count correlated with resistance of apple plants towards pathogenic AG-5. *Biotechnol. Biotechnol. Equip.*, 29: 463–469

Espinosa-Diez, C., V. Miguel, D. Mennerich, T. Kietzmann, P. Sánchez-Pérez, S. Cadenas and S. Lamas, 2015. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol.*, 6: 183–197

Ferrucho, R.L., J.M. Cifuentes, P. Ceresini and C. García-domínguez, 2012. *Rhizoctonia solani* GA-3PT is the major pathogen associated with potato stem canker and black scurf in Colombia. *Agron. Colomb.*, 30: 204–213

Fiers, M., V. Edel-Hermann, C. Héraud, N. Gautheron, C. Chatot, Y. Le Hingrat, K. Bouchek-Mechiche and C. Steinberg, 2011. Genetic diversity of *Rhizoctonia solani* associated with potato tubers in France. *Mycologia.*, 103: 1230–1244

Goll, M., A. Schade-Schütze, G. Swart, M. Oostendorp, J. Schott, B. Jaser and F. Felsenstein, 2014. Survey on the prevalence of *Rhizoctonia spp*. in European soils and determination of the baseline sensitivity towards sedaxane. *Plant Pathol.*, 63: 148–154

Hewavitharana, S.S. and M. Mazzola, 2016. Carbon source-dependent effects of anaerobic soil disinfestation on soil microbiome and suppression of *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans*. *Phytopathology*, 106: 1015–1028

Langmead, B., C. Trapnell, M. Pop and S.L. Salzberg, 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome. Biol.*, 10: 25

Lehmann, S., M. Serrano, F. L'Haridon, S.E. Tjamos and J.P. Metraux, 2015. Reactive oxygen species and plant resistance to fungal pathogens. *Phytochemistry*, 112: 54–62

Li, D., Y. Li, L. Zhang, X. Wang, Z. Zhao, Z. Tao, J. Wang, J. Wang, M. Lin, X. Li and Y. Yang, 2014. *Arabidopsis* ABA receptor RCAR1/PYL9 interacts with an R2R3-type MYB transcription factor, AtMYB44. *Int. J. Mol. Sci.*, 15: 8473–8490

Li, H.L., D. Guo and S.Q. Peng, 2015. Molecular and functional characterization of the JcMYB1, encoding a putative R2R3-MYB transcription factor in *Jatropha curcas*. *Plant Growth Regul.*, 75: 45–53

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis and R. Durbin, 2009. The sequence alignment/map format and SAMtools. *Bioinformatics*, 25: 2078–2079

Lin, H. and N.C. Gudmestad, 2013. Aspects of pathogen genomics, diversity, epidemiology, vector dynamics, and disease management for a newly emerged disease of potato: zebra chip. *Phytopathology*, 103: 524–537

Liu, W.Z., M. Deng, L. Li, B. Yang, H. Li, H. Deng and Y.Q. Jiang, 2015. Rapeseed calcineurin B-like protein CBL4, interacting with CBL-interacting protein kinase CIPK24, modulates salt tolerance in plants. *Biochem. Biophs. Res. Commun.*, 467: 467–471

Luan, S., 2009. The CBL–CIPK network in plant calcium signaling. *Trends. Plant Sci.*, 14: 37–42

Mammarella, N.D., Z. Cheng, Z.Q. Fu, A. Daudi, G.P. Bolwell, X. Dong and F.M. Ausubel, 2015. Apoplastic peroxidases are required for salicylic acid-mediated defense against *Pseudomonas syringae*. *Phytochemistry*, 112: 110–121

Mazzola, M., 2007. Manipulation of rhizosphere bacterial communities to induce suppressive soils. *J. Nematol.*, 39: 213

Mou, S., Z. Liu, D. Guan, A. Qiu, Y. Lai and S. He, 2013. Functional analysis and expressional characterization of rice ankyrin repeat-containing protein, OsPIANK1, in basal defense against *Magnaporthe oryzae* attack. *PLoS One*, 8: e59699

Munir, S., H. Liu, Y. Xing, S. Hussain, B. Ouyang, Y. Zhang, H. Li and Z. Ye, 2016. Overexpression of calmodulin-like (ShCML44) stress-responsive gene from *Solanum habrochaites* enhances tolerance to multiple abiotic stresses. *Sci. Rep.*, 6: 31772

Muzhinji, N., J.W. Woodhall, M. Truter and J.E. van der Waals, 2016. Population genetic structure of *Rhizoctonia solani* AG 3-PT from potatoes in South Africa. *Fungal Biol.*, 120: 701–710

Muzhinji, N., J. Woodhall, M. Truter and J.E. Van der Waals, 2014. Elephant hide and growth cracking on potato tubers caused by *Rhizoctonia solani* AG3-PT in South Africa. *Plant Dis.*, 98: 570–570

Patil, V.U., V. Girimalla, V. Sagar, V. Bhardwaj and S. Chakrabarti, 2017. Draft Genome Sequencing of *Rhizoctonia solani* Anastomosis Group 3 (AG3-PT) Causing Stem Canker and Black Scurf of Potato. *Amer. J. Potato Res.*, 5: 1–5

Santino, A., M. Taurino, S. De Domenico, S. Bonsegna, P. Poltronieri, V. Pastor and V. Flors, 2013. Jasmonate signaling in plant development and defense response to multiple (a) biotic stresses. *Plant Cell Rep.*, 32: 1085–1098

Shamim, M., D. Kumar, D. Srivastava, P. Pandey and K. Singh, 2014. Evaluation of major cereal crops for resistance against *Rhizoctonia solani* under green house and field conditions. *Ind. Phytopathol.*, 67: 42–48

Shannon, P., A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, N. Amin, B. Schwikowski and T. Ideker, 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome. Res.*, 13: 2498–2504

Soler, M., E.L.O. Camargo, V. Carocha, H. Cassan-Wang, H. San Clemente, B. Savelli, C.A. Hefer, J.A.P. Paiva, A.A. Myburg and J. Grima-Pettenati, 2015. The Eucalyptus grandis R2R3-MYB transcription factor family: evidence for woody growth-related evolution and function. *New Phytol.*, 206: 1364–1377

Sun, L., L. Zhu, L. Xu, D. Yuan, L. Min and X. Zhang, 2014. Cotton cytochrome P450 CYP82D regulates systemic cell death by modulating the octadecanoid pathway. *Nat. Commun.*, 5: 5372

Suzuki, N., E. Bassil, J.S. Hamilton, M.A. Inupakutika, S.I. Zandalinas, D. Tripathy, Y. Luo, E. Dion, G. Fukui and A. Kumazaki, 2016. ABA is required for plant acclimation to a combination of salt and heat stress. *PLoS One*, 11: e0147625

Tang, R.J., F.G. Zhao, V.J. Garcia, T.J. Kleist, L. Yang, H.X. Zhang and S. Luan, 2015. Tonoplast CBL–CIPK calcium signaling network regulates magnesium homeostasis in *Arabidopsis*. *Proc. Natl. Acad. Sci.*, 112: 3134–3139

Thorvaldsdóttir, H., J.T. Robinson and J.P. Mesirov, 2013. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform*., 14: 178–192

Trapnell, C., L. Pachter and S.L. Salzberg, 2009. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, 25: 1105–1111

Verma, V., P. Ravindran and P.P. Kumar, 2016. Plant hormone-mediated regulation of stress responses. *BMC Plant Biol*., 16: 86

Verwaaijen, B., D. Wibberg, M. Kröber, A. Winkler, R. Zrenner, H. Bednarz, K. Niehaus, R. Grosch, A. Pühler and A. Schlüter, 2017. The *Rhizoctonia solani* AG1-IB (isolate 7/3/14) transcriptome during interaction with the host plant lettuce (*Lactuca sativa* L.). *PLoS One*, 12: e0177278

Von Mering, C., L.J. Jensen, M. Kuhn, S. Chaffron, T. Doerks, B. Krüger, B. Snel and P. Bork, 2007. STRING 7—recent developments in the integration and prediction of protein interactions. *Nucl. Acids Res*., 35: 358–362

Wagner, G.P., K. Kin and V.J. Lynch, 2012. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. *Theor. Biosci.*, 131: 281–285

Wang, L., Z. Feng, X. Wang, X. Wang and X. Zhang, 2009. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics*, 26: 136–138

Xu, J., J. Yang, X. Duan, Y. Jiang and P. Zhang, 2014. Increased expression of native cytosolic Cu/Zn superoxide dismutase and ascorbate peroxidase improves tolerance to oxidative and chilling stresses in cassava (*Manihot esculenta* Crantz). *BMC Plant Biol.*, 14: 208

Yan, J., J. Wang and H. Zhang, 2002. An ankyrin repeat-containing protein plays a role in both disease resistance and antioxidation metabolism. *Plant J*., 29: 193–202

Yang, S., F. Min, W. Wang, Q. Wei, M. Guo, Y. Gao, X. Dong and D. Lu, 2017. Anastomosis Group and Pathogenicity of *Rhizoctonia solani* Associated with Stem Canker and Black Scurf of Potato in Heilongjiang Province of China. *Amer. J. Potato Res.*, 94: 95–104

Yang, Y., C. Zhao, Z. Guo and X. Wu, 2015. Anastomosis group and pathogenicity of *Rhizoctonia solani* associated with stem canker and black scurf of potato in China. *Eur. J. Plant Pathol*., 143: 99–111

Yao, P., W. Lu, F. Meng, X. Wang, B. Xu and X. Guo, 2013. Molecular cloning, expression and oxidative stress response of a mitochondrial thioredoxin peroxidase gene (AccTpx-3) from *Apis cerana cerana*. *J. Insect Physiol.*, 59: 273–282

Zerbino, D.R. and E. Birney, 2008. Velvet: algorithms for de novo short read assembly using de *Bruijn graphs*. *Genome. Res.*, 18: 821–829

Zhang, J., L. Chen, C. Fu, L. Wang, H. Liu, Y. Cheng, S. Li, Q. Deng, S. Wang and J. Zhu, 2017. Comparative transcriptome analyses of gene expression changes triggered by *Rhizoctonia solani* AG1 IA infection in resistant and susceptible rice varieties. *Front. Plant Sci.*, 8: 1422

Zhang, X., X.M. Zhang, H.H. Jiang and J. Hao, 2016. First Report of Rhizoctonia Solani AG-5 Causing Stem Canker on Buckwheat in Maine. *Plant Dis*., 100: 1241–1241

Zhu, C., L. Ai, L. Wang, P. Yin, C. Liu, S. Li and H. Zeng, 2016. De novo Transcriptome Analysis of *Rhizoctonia solani* AG1 IA Strain Early Invasion in *Zoysia japonica* Root. *Front. Microbiol.*, 7: 708–708

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