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Molecular dissection of distinct symptoms induced by tomato chlorosis virus and tomato yellow leaf curl virus based on comparative transcriptome analysis

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ABSTRACT

The viral infection of plants may cause various physiological symptoms associated with the reprogramming of plant gene expression. However, the molecular mechanisms and associated genes underlying disease symptom development in plants infected with viruses are largely unknown. In this study, we employed RNA sequencing for in-depth molecular characterization of the transcriptional changes associated with the development of distinct symptoms induced by tomato chlorosis virus (ToCV) and tomato yellow leaf curl virus (TYLCV) in tomato. Comparative analysis of differentially expressed genes revealed that ToCV and TYLCV induced distinct transcriptional changes in tomato and resulted in the identification of important genes responsible for the development of symptoms of ToCV (i.e., chlorosis and anthocyanin accumulation) and TYLCV (i.e., yellowing, stunted growth, and leaf curl). Our comprehensive transcriptome analysis can provide molecular strategies to reduce the severity of disease symptoms as well as new insights for the development of virus-resistant crops.

1. Introduction

The severity of disease symptoms is directly correlated with crop productivity (Gaunt, 1995). Plant viruses induce various symptoms in their host plants, such as mosaic, yellowing, chlorosis, stunting, and necrosis. In plants, the expression of symptoms is a complex physiological process and relates to a large variety of genes that are involved in not only defense responses, but also in plant growth, development, and metabolism. In many cases, each virus can induce specific symptoms in a host plant and the same virus can induce different symptoms in different host plants, indicating that the symptoms are the results of transcriptome reprogramming upon specific host–virus molecular interactions. Thus, examination of the differential regulation of genes involved in symptom development and identification of the critical regulatory components can provide molecular understanding of disease symptoms and a roadmap to explore the resistance mechanisms and molecular strategies in reducing the severity of disease symptoms.

Tomato (*Solanum lycopersicum*) is an economically important vegetable crop worldwide and it has long served as a model system for plant development, genetics, pathology, and physiology. Tomato growth and productivity are often challenged by various biotic and abiotic factors (Kissoudis et al., 2016). Viral diseases caused by tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) are some of the most serious biotic factors limiting tomato production in many countries (Moriones and Navas-Castillo, 2000; Navas-Castillo et al., 2000). TYLCV, a member of the genus *Begomovirus* in the family *Geminiviridae*, is a DNA virus with a single-stranded circular DNA genome of approximately 2.7–2.8 kb. The symptoms of TYLCV infection in tomato include stunted growth, marked reduction of leaf size, and yellowing and curling of young leaves, which cause severe yield loss (Navot et al., 1991). ToCV, a member of the genus *Crinivirus* in the family *Closteroviridae*, is an RNA virus comprising two segments of positive-sense single-stranded RNAs, RNA1 (8594–8595 nt) and RNA2 (8242–8247 nt) (Wintermantel et al., 2005). ToCV infection causes serious leaf chlorosis symptoms in tomato plants. In some tomato cultivars, ToCV induces increased anthocyanin accumulation together with chlorosis symptoms in the infected leaves. Initially, the symptoms appear in the lower leaves of the ToCV-infected tomato plants, and then progress toward the upper leaves (Wintermantel and Wisler, 2006). As expressed in symptoms, tomato responds to these two viruses with quite

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different physiological and developmental changes. Thus, in-depth characterization of the molecular mechanisms involved in the development of distinct symptoms can provide further understanding of plant physiology and metabolism. In particular, yellowing and chlorosis, which are common symptoms of virus infection in leaves, have been considered to be regulated by leaf senescence processes (Espinoza et al., 2007a, 2007b; Pageau et al., 2006), but the molecular mechanisms and genes involved are still largely unknown.

The completion of the tomato genome sequence and recent advances in next-generation RNA sequencing have allowed high-throughput analysis and comparison of differential transcriptome profiles and have aided in the identification of important genes in the family *Solanaceae*. In this study, we employed RNA sequencing and comparative transcriptome analysis to gain better insights into the molecular mechanisms underlying the development of distinct symptoms induced by viruses in tomato. Using this approach, we comparatively analyzed the transcriptome profiles of tomato plants presenting the typical symptoms of infection with ToCV, TYLCV or ToCV + TYLCV (co-infection of ToCV and TYLCV). Our analyses revealed that ToCV and TYLCV induced distinct transcriptional changes in tomato and that these transcriptional changes are highly correlated with symptom development. Analysis of differentially expression genes (DEG) resulted in the identification of important genes that function in the development of the symptoms of ToCV (i.e., chlorosis and anthocyanin accumulation) and TYLCV (i.e., yellowing, stunted growth, and leaf curl) and possible molecular mechanisms are discussed in explaining symptom development.

2. Results and discussion

2.1. Transcriptome analysis of distinct symptoms induced by ToCV and TYLCV in tomato

The development of viral symptoms results from complex molecular and physiological processes. Thus, investigation of transcriptome profiles of distinct symptoms could provide important insights for an integrative molecular understanding of various physiological processes in plants. In this study, we investigated the infection of tomato by two different viruses, ToCV and TYLCV, to examine symptom-specific host transcriptome responses because ToCV and TYLCV induce very distinct symptoms in tomato (Fig. 1A); whereas ToCV induced chlorosis and anthocyanin accumulation in the infected leaves of tomato (Fig. 1B), TYLCV infection resulted in stunted growth, severe leaf size reduction, and the yellowing and curling of upper young leaves. In addition, the tomato plants co-infected with ToCV and TYLCV showed integrative symptoms of those induced by each virus.

To examine the changes in the tomato transcriptomes associated with symptom expression when infected with ToCV, TYLCV, or ToCV + TYLCV, we performed Illumina RNA sequencing. Tomato seedlings (*Solanum lycopersicum* cv. Tenten) were infected with ToCV, TYLCV, or ToCV + TYLCV by single-leaflet grafting inoculation (Lee et al., 2017). Eight weeks after inoculation, total RNA was isolated from upper symptomatic leaves and the infection with each virus was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) using specific primers (data not shown). Total RNA extracted from uninoculated plants was used as a healthy control. A total of eight cDNA libraries (two libraries for each sample) was sequenced by Illumina RNA sequencing. For mapping, we used a total of 34,725 reference transcripts of *S. lycopersicum* (iTAG2.3) (Sato et al., 2012). The raw HiSeq reads were filtered and trimmed by the Illumina pipeline and approximately 54 to 72 million clean pair-end reads were obtained from each of the eight libraries (Table 1). The obtained reads were then mapped on the reference tomato transcripts; this resulted in the mapping of approximately 82.40–84.13% of the nucleotides (Table 1). Mapping results showed that the nucleotide coverage ranged from 49.76 times (TYLCV infection) to 70.77 times (healthy) (Table 1). The nucleotide coverage for the healthy sample was significantly higher than all other virus-infected samples, indicating that the entire level of tomato gene expression decreased upon virus infection (Table 1).

2.2. Identification of DEGs in response to infection with ToCV and/or TYLCV

The expression levels of mapped genes were normalized with a value of FPKM (fragments per kilobase of exon per million fragments mapped). Normalized read counts (FPKM values) between two samples were statistically compared to identify DEGs. In this study, we identified DEGs in response to infection with ToCV, TYLCV, or ToCV + TYLCV by comparing the virus-infected samples to the healthy sample using a two-fold change in expression with a false discovery rate (FDR) ≤ 0.01 and mean of read count ≥ 1000 as cutoffs. Our analysis resulted in identification of 461, 1142, and 1568 DEGs in response to infection with ToCV, TYLCV, and ToCV + TYLCV, respectively, compared with healthy tomato (Fig. 1C and Supplementary Tables S1–S3). To examine global gene expression patterns in each sample, the magnitude distribution of the DEGs was illustrated by MA plot analysis (Fig. 1D). The MA plots showed that tomato gene expression pattern was more affected by TYLCV infection than ToCV infection and that co-infection of two viruses enhanced changes in the gene expression pattern (Fig. 1D).

We then compared the DEGs among three infection conditions to identify the genes specifically expressed in response to each virus infection. A total of 1840 genes was differentially expressed when we combined the DEG data from three infection conditions (up-regulation of 814 genes and down-regulation of 1024 genes) (Supplementary Table S4). Many genes were specifically regulated in response to each virus. For example, 456 and 353 genes were up- and down-regulated, respectively, by TYLCV infection when compared with ToCV infection (Fig. 2A). A relatively small number of genes were specifically affected by ToCV infection (up-regulation of 61 genes and down-regulation of 67 genes), when it is known that 93 and 240 genes are commonly up- and down-regulated, respectively, by ToCV infection and TYLCV infection (Fig. 2A). Interestingly, 204 and 364 genes were specifically up- and down-regulated, respectively, by co-infection of ToCV and TYLCV (Fig. 2A), suggesting that co-infection by two different viruses might induce synergistic effects on the physiology of host plants in ways different from single infections. In addition, a hierarchical clustering of the total DEGs identified six groups according to the expression patterns in each infection condition (Fig. 2B and Supplementary Table S4). For example, cluster 4 contained 16 genes whose expression was down-regulated by infection with ToCV or ToCV + TYLCV but up-regulated by TYLCV infection. Alternatively, cluster 6 offers the reverse in which expression of 13 genes was up-regulated by infection with ToCV or ToCV + TYLCV but down-regulated by TYLCV infection.

2.3. Gene ontology analysis of identified DEGs

For a better understanding of the DEGs involved in response to infection with ToCV and/or TYLCV, the functional classes of DEGs were subjected to gene ontology (GO) analysis. A total of 92, 161, and 197 GO terms were significantly enriched by infection with ToCV, TYLCV, and ToCV + TYLCV, respectively (Fig. 3A and Supplementary Tables S5-S7). Among three infection conditions, 36 and 51 GO terms were commonly identified for up- and down-regulated DEGs, respectively (Fig. 3B). The commonly enriched GO terms for up-regulated DEGs included defense response (GO:0006952), innate immune responses (GO:0045087), response to stimulus (GO:0050896), cell communication (GO:0007154), cytoplasm (GO:0005737), and intracellular membrane-bound organelle (GO:0043231) (Fig. 3C and Supplementary Tables S5-S7). In particular, many of the DEGs identified that were related to plant immunity were commonly upregulated when infected with ToCV, TYLCV, or ToCV + TYLCV (Table 2). The GO terms enriched in common for the down-regulated DEGs contained photosynthesis (GO:0015979), thylakoid