Diverse Regulations of Viral and Host Genes in Tomato Germplasms Responding to Tomato Yellow Leaf Curl Virus Inoculation

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Abstract Tomato yellow leaf curl virus (TYLCV) is the dominating pathogen of tomato yellow leaf curl disease that caused severe loss to tomato production in China. In this study, we found that a TYLCV-resistant tomato line drastically reduced the accumulation of viral complementary-sense strand mRNAs but just moderately inhibited that of viral DNA and virion-sense strand mRNAs. However, two other resistant lines did not have such virus inhibition pattern. Analysis of differential expressed genes showed that the potential host defense-relevant processes varied in different resistant tomatoes, as compared to the susceptible line, suggesting a diversity of tomato TYLCV-resistance mechanisms.

Key words Tomato yellow leaf curl virus; Virus replication; Gene expression

Tomato yellow leaf curl virus (TYLCV) is the dominant species among the more than ten *Bemisia tabaci*-transmitted begomoviruses that cause tomato yellow leaf curl disease widely occurring in China since 2006[1-3]. Currently, application of antiviral germplasm is the effective way to control this devastating pathogen in the tomato growing areas and a number of TYLCV-resistant tomato varieties have been developed in China[4-5]. To seek antiviral germplasm befitting the cultivation conditions in southern China, in our preliminary work we screened more than 100 tomato materials from the germplasm bank of Guangzhou Academy of Agricultural Sciences (Guangzhou, China) via resistance identification and obtained three lines (R1, R2 and R3) with different extents of resistance. Both lines R1 and R2 were tested positive by conventional PCR detection at 4 weeks post inoculation (wpi) with the TYLCV infectious clone, and the R1 plants showed leaf curl symptom at 4 wpi but the R2 plants did not until at 6 wpi. Line R3 remained symptomless and negative in PCR detection at 6 wpi. In this study, to investigate the performance of TYLCV in tomato hosts of different resistance levels, we examined the dynamics of TYLCV accumulation and gene expression in the three lines R1 - R3, with a susceptible line (S) as the control, which became infected at 2 wpi.

Materials and Methods

The tomato seeds were germinated on wet towels and sowed in flower pots (9 cm in diameter, each pot with one plant) kept in a growth chamber (25℃, relative humidity 60% - 70%, 12 h light : 12 h dark). When they were in a 4-leaf stage, the seedlings with uniform growth were selected for inoculation of the TYLCV infectious clone, which was kindly offered by Dr. Zifu He from Guangdong Academy of Agricultural Sciences, Guangzhou, China. For each material, thirty plants as three replicates were subject to the inoculation. The viral infectious clone-containing agrobacterium was propagated in LB medium (with 50 μg/ml kanamycin, 10 μg/ml tetracycline and 50 μg/ml rifampicin) under 28℃, 150 rpm shaking for 48 h, resuspended in an inoculation solution (500 μM acetosyringone and 10 mM), and inoculated to the leaf surfaces of the experimental seedlings (2 leaves inoculated for each plants) using a 2 ml syringe. The inoculated plants were kept in the growth chamber under the aforementioned conditions. The leaves of these plants were sampled at 14, 28, and 42 d post inoculation (dpi), respectively, for total DNA/RNA extraction and (RT-)qPCR analyses. The DNA extraction, RNA extraction, qPCR and RT-qPCR were conducted respectively using EasyPure Plant Genomic DNA kit, EasyPure Plant RNA kit, TransStart Tip Green qPCR SuperMix, and TransScript II Green One-Step qRT-PCR SuperMix purchased from TransGen Biotech Co., Ltd. (Beijing, China), according to the manufacturer's protocols. The RT-qPCR primers reported in the reference[6] were used for the six viral genes (V1, V2, C1 - C4), and the same C1 primers were used for qPCR. The ubiquitin (UBI) gene of the host served as an internal control in (RT-)qPCR analyses with primers 5'-TCGTAAGGAGTGCCCTAATGCTGA-3' and 5'-CAATCGCCTCCAGCCTTGTTGTAA-3'. In addition, to explore the patterns of TYLCV infection-induced host gene regulation in different tomato lines, a RNA-Seq-based analysis of differential gene expression was conducted. The leaf samples of the lines S, R1, R2 and R3 at 28 dpi were collected (each line with three biological replicates and 10 plants for each replicate), and total RNA extraction and transcriptomic

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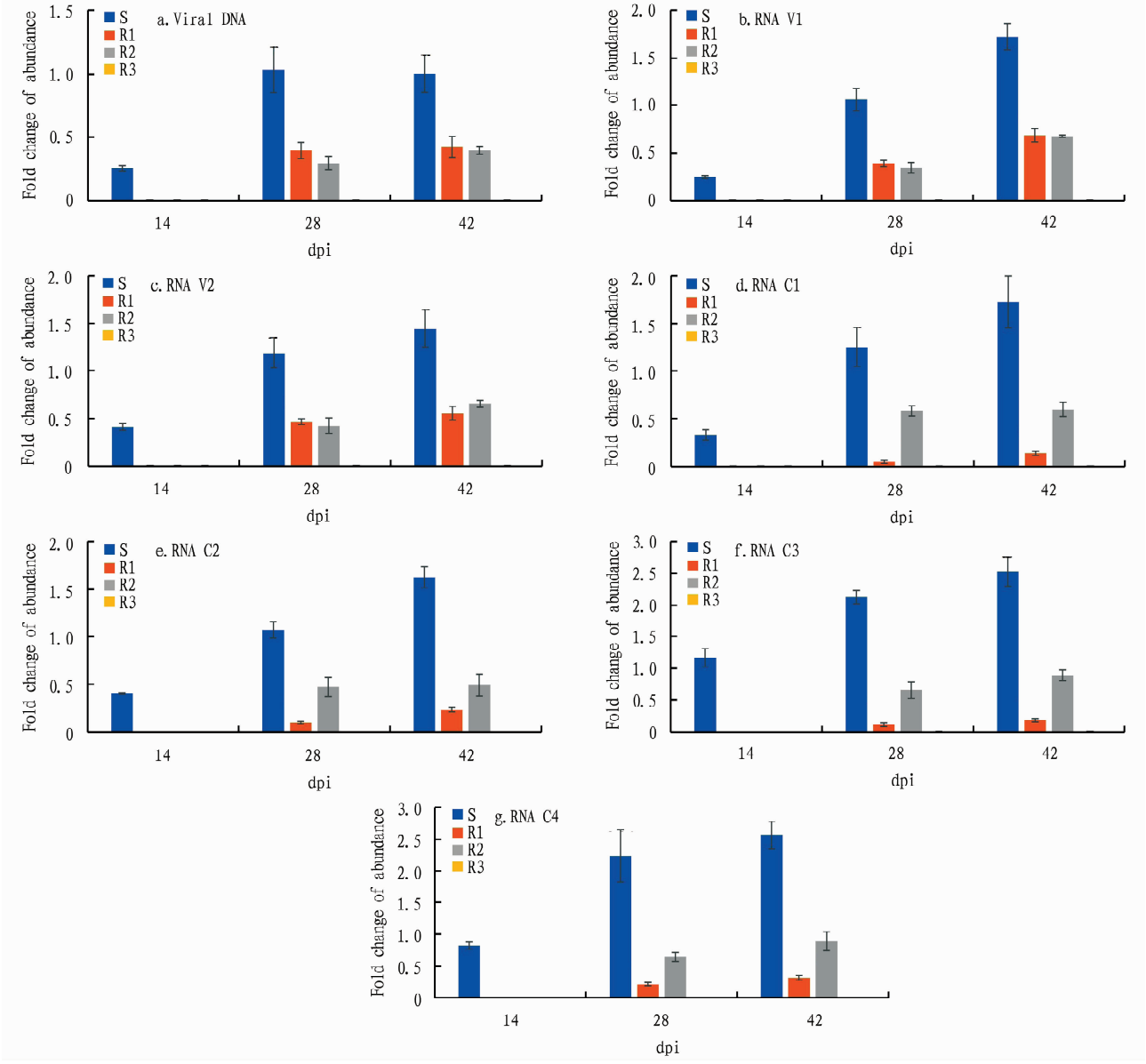
sequencing on the Illumina platform (sequencing mode: paired-end, 2 * 150 bp) were done by Personalbio Technology Co., Ltd. (Nanjing, China) using standard-experiment, quality-control and data-processing procedures.

Results and Analysis

Neither viral DNA nor mRNAs could be detected by (RT-) qPCR in the plants of line R3 from 14 to 42 dpi. Both lines R1 and R2 became detectable with TYLCV at 28 dpi, but the viral DNA abundances in the two were lower than one half of that in the susceptible line at 28 and 42 dpi (Fig. 1 a). Similar expression patterns were observed for the mRNAs of V1 and V2, the two viral genes on the virion-sense strand (Fig. 1 b and c). However, the expression of the viral mRNAs of C1-C4, the four genes on the complementary-sense strand, was at the drastically low levels in R1 plants but at the moderate levels similar to the expression of V1 and V2 mRNAs in line R2, compared with in the susceptible plants, at 28 dpi and 42 dpi (Fig. 1 d, e, f, g). The begomoviral C1, C2, C3 and C4 proteins involve in viral replication, transcription activation, replication enhancement, and suppressing of RNAi[7-9]. Our findings suggested that tomato resistance to TYLCV may be conferred by different mechanisms in different host lines, including expression inhibition of the viral complementary-sense genes that are required for virus propagation.



The relative abundances represented by the y-axes were obtained from $2^{(-\Delta Ct)}$, where $\Delta Ct = Ct\ (target\ gene) - Ct\ (UBI)$.

Fig. 1 Abundances of viral DNA (a) and mRNAs of the six viral genes (b-g) relative to the internal control, the host UBI gene (whose abundance is normalized to be 1.0) in the susceptible (S) and three resistant (R1, R2 and R3) tomato lines at 14, 28, and 42 d post inoculation, respectively, of the TYLCV infectious clone

The RNA-Seq generated 40.1 – 41.3 M of clean reads, which consisted of 6.02 – 6.19 G base pairs (bp), averagely, from the S, R1, R2 and R3 samples, and over 96% of their clean reads were mapped to the tomato chromosomes documented by Solanaceae Genomics Network (https://solgenomics.net/). The mapped sequences were annotated to certain functional genes in the GO, KEGG and Swissprot databases, and based on their expression levels normalized by Fragments Per Kilo bases per Million fragments, the differential expressed genes (DEGs) were identified between the resistant and the susceptible lines using the criterion |log2FoldChange| >1 with adjusted *P* value <0.05. The results showed that compared with the susceptible line, both lines R1 and R2, whose virus resistance lasted relatively shorter, had near-equal numbers of up-regulated and down-regulated genes (949 up-regulated vs. 903 down-regulated ones in R1 plants; 808 up-regulated vs. 793 down-regulated ones in R2 plants); whereas the R3 plants, showing longer resistance, had only 678 up-regulated genes but as many as 1 143 down-regulated ones. This quantity difference of DEGs between the hosts of longer and shorter resistance suggested that these two types of anti-TYLCV tomatoes may have distinct host-virus interaction-mediated defense mechanisms. The regulation orientations of DEGs revealed by RNA-Seq were confirmed by RT-qPCR for ten randomly selected DEGs using their sequence-based specific primers and the 28 dpi RNA samples as templates. According to the Swissprot database, 10 up-regulated and 5 down-regulated genes in R1 plants, whereas 4 up-regulated and 10 down-regulated genes in line R3, and only two down-regulated ones in line R2, were annotated as disease resistance-related genes; and some types of resistance-related DEGs encoding heat shock proteins, Cytochrome P450, E3 ubiquitin-protein ligases, WRKY transcription factors, mitogen-activated protein kinases (which regulate the salicylic acid and jasmonic acid pathways) and auxin signaling proteins were identified in each of the three lines, whereas two lipocalin genes found down regulated in R2 and R3 lines, also suggesting the diversity and complexity of the potential antiviral networks in tomatoes reported previously[10-13]. However, we did not identified the regulation of autophagy-related genes in the three lines, a recently-found mechanism conferring the TYLCV resistance[14].

GO enrichment analysis showed that the DEGs in R1 plants annotated to the top 10 GO terms of significant enrichment (*P* <0.05) largely were associated with DNA binding and transcription (with 146 ones down-regulated), potentially accounting for the low mRNA abundances of the viral genes on the complementary-sense strand found in this study; in R2 plants the DEGs of significant enrichment largely were photosynthesis-related genes (all up-regulated); in R3 plants, most of the DEGs with GO enrichment involved in DNA binding, transcription, and RNA metabolic and biosynthetic processes (largely down-regulated). KEGG enrichment analysis indicated that upon TYLCV infection, the "plant-pathogen interaction" and "phenylpropanoid biosynthesis" pathways commonly in the three resistant lines, the "MAPK signaling" pathway in lines R1 and R2, the "galactose metabolism" pathway in lines R2 and R3, and the "plant hormone signal transduction" pathway in lines R1 and R3, were regulated; whereas each line altered a number of specific pathways responding to the virus infection. These findings further suggest different mechanisms potentially underlying host resistance to the same virus in different tomato materials, and confirmed the intricate defensive networks used by the hosts to counteract the virus. In this study, the results of comparative analysis of DEGs between more than one antiviral tomato lines (as well as between lines of longer and shorter resistance) can be a good complement to the previous research on TYLCV-modulated plant transcriptomes using just one resistant and one susceptible host line, or an infected line and an uninfected control[13,15-16]. Further studies may be carried out to explore how the virus-host interactions result in different resistance levels againstthe virus in various tomato lines.

Conclusions and Discussion

In summary, we discovered the near-complete expression inhibition of mRNAs of the TYLCV genes on the complementary-sense strand, to our knowledge for the first time, and found different patterns of virus infection-responding gene regulation, which were associated with distinct host defense mechanisms, in tomato lines with different extents of resistance. The findings in this study are helpful to better understand the TYLCV-host interactions relevant to the virus resistance, and provide insight into strategy development of disease resistance-oriented tomato breeding and the control of this economically significant virus.

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"grey-matter" content of Zhenliangyou 8612 was 25%, the seedling percentages after treatment with mixed seed coating agents A, B, and C were, respectively, (75 ± 0.58)%, (80 ± 0.33)% and (80 ± 0.33)%, which were higher than their corresponding germination rates of (74 ± 0.58)%, (78 ± 0.33)% and (78 ± 0.33)%. It might be due to the formation of a large amount of hyphae during the soaking and germination stage of "grey-matter" seeds, which affected other seeds and led to the production of abnormal seedlings; and during the field seedling cultivation process, the hyphae could not affect other normal seeds, so the seedling percentage of seeds with a "grey-matter" content greater than 15% was greater than the germination rate.

No yield-related measurement was carried out in this study, because previous studies showed that the impact of "grey-matter" of hybrid rice seeds on rice seeds was mainly manifested in the stage of seed soaking and germination. The activities of microorganisms related to "grey-matter" led to the generation of abnormal seedlings, which affected the germination rate, thus affecting the seed quality. Whether "grey-matter" affected the yield remains to be further explored and studied.

Conclusions

In this study, the seeds of two different varieties, Zhenliangyou 8612 and Taiyou 390, with different "grey-matter" contents, were treated with different mixed seeding coating agents, to explore the effects of different combinations on germination rate, seedling quality and seedling percentage. The main conclusions were described as below.

(1) The combinations of treatment B (seed coating agent A + Linong) and treatment C (Manshijin + seed coating agent A) could significantly improve the seed quality of the two varieties with a "grey-matter" content greater than 15%.

(2) The combinations of treatment B (seed coating agent A + Linong) and treatment C (Manshijin + seed coating agent A) could significantly improve indexes including seedling height, fibrous roots and fresh weight of the two varieties, but there were no significant effects on the main root length, dry weight, leaf number, and tiller number.

(3) When the "grey-matter" content was lower than 15%, the seed quality and field seedling quality were in a good state.

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