Involvement of the hexose transporter gene \textit{LeHT1} and of sugars in resistance of tomato to \textit{Tomato yellow leaf curl virus}

Dear Editor,

\textit{Tomato yellow leaf curl virus} (TYLCV) is a whitefly-transmitted geminivirus infecting tomato crops (Czosnek, 2007). TYLCV-resistant (R) and susceptible (S) lines with the same genetic background have been bred using \textit{Solanum habrochaites} as the resistance source. The gene(s) conferring resistance are unknown. Previously, we demonstrated that the hexose transporter gene \textit{LeHT1} is up-regulated upon infection in R plants and its silencing in R plants (RH) leads to the collapse of resistance (Eybishtz et al., 2010). To uncover the role of \textit{LeHT1} in resistance we (I) analyzed the transcriptome re-programming in leaves of S, R and RH plants using a home-designed microarray, before and 7 days after TYLCV inoculation (0, 7dpi), and (II) measured the concentration of sugars and their derivatives in S, R and RH leaves at 1 and 7dpi because \textit{LeHT1} is transporting both glucose and fructose (McCurdy et al., 2010).

Since a successful defense depends on the preparedness of the stress/immune responses and their fast activation, we first compared the transcriptome of S and R tomatoes before (So vs. Ro) and after infection (Si vs. Ri). Genes differentially expressed upon infection (fold-change ≥3, \(p \leq 0.01\)) are shown in a hierarchical cluster (Figure 1A). The genes in each cluster and their functional characterization are listed (Supplementary Table 1). Upon infection, the genes differentially expressed in S vs. R plants, are also those differentially expressed in Si vs Ri plants. In Ro plants - the highly expressed genes were related to biotic stress, jasmonic acid and ethylene biosynthesis, signal transduction, and RNA regulation and processing (Supplementary Table 2). These genes may contribute to the rapid establishment of resistance in response to TYLCV infection. So plants showed a significant enrichment (Fisher exact test \(P=0.0004084\)) of transcripts encoding the Bzip transcription factor family, which is involved in abiotic stress tolerance (Supplementary Table 2). As the crosstalk between biotic and abiotic stress response is usually antagonistic, this may induce the balance to lean towards resistance in R plants. In Ri plants (vs. Si), RNA regulation and processing-related transcripts are over-represented, including a significant enrichment (Fisher exact test, \(p=2.068\times10^{-6}\)) in Dicer-like (DCL) and Ribonuclease III-like protein genes (Supplementary Table 3). These genes may be involved in infection response through mRNA decay or gene silencing via sequence-specific mRNA degradation (Blevins et al., 2006). In Si plants (vs. Ri), Bzip remains, as before infection, one of the major transcripts (Fisher exact test \(P=0.0002031\)) (Supplementary Table 3).

\textit{LeHT1}, which is expressed at very low levels in Ro and So plants, is strongly induced upon infection of R tomatoes (Ri). \textit{LeHT1} was silenced in R plants (RH) which were subsequently infected (RHi). As a result RHi plants acquired a Si phenotype. At 7dpi, while Si plants contained \(~1,700\) times more virus than Ri plants, RHi plants contained \(~40\) times more virus than Ri tomatoes (Figure 1B). Hierarchical clustering of the raw expression data shows that \textit{LeHT1} silencing has only a minor effect on R plant gene expression (RHo is similar to Ro). Most of the genes upregulated in R plants...
upon LeHT1 TRV-mediated silencing are involved in response to pathogens; nonetheless, induction of these genes did not prevent collapse of resistance of the RH plants upon TYLCV infection (Eybishtz et al., 2010; Lozano-Duran et al. 2011; Supplementary Table 4). Hence the comparison between RHi, Ri and Si, which shows that following infection RHi gene expression profile is usually similar to that of Ri but not to Si, is valid (Supplementary Figure 1).

TYLCV infection significantly modified gene expression in S, R and RH tomatoes (Si vs. So, Ri vs. Ro, RHi vs. RHo). TYLCV induced different responses in the three plant types, both by the number of genes differentially expressed and by their regulation pattern (fold-change ≥3, p≤ 0.01; Supplemental Tables 5 and 6). Upon infection of R plants (Ro vs. Ri), 345 genes were differentially expressed, compared to only 110 genes upon infection of S tomatoes (So vs. Si) and 220 genes upon infection of RH plants (RHo vs. RHi). These results point to a strong response of R plants to the virus, which may be related to the resistance phenotype. A similar phenomenon was described in Arabidopsis ecotypes resistant and susceptible to Cucumber mosaic virus (Ishihara et al., 2004). Among the 214 R-unique genes, 82% were down-regulated (divided into 24 different functional categories); among the 26 S-unique genes, 84% were up-regulated (in 6 different functional categories) and among the 58 RH-unique genes, 82% were down-regulated after infection (in 14 different functional categories) (Supplement table 5). Thus, at 7dpi, the transcriptional pattern of RHi plants resembled more that of Ri than that of Si. These results might indicate that these Ri-specific genes are not involved in general stress response, but rather contribute to TYLCV resistance. The Ri vs. RHi comparison showed that 37% of the down-regulated genes in RHi plants are involved in protein post translational modification (PTM) and degradation by E3 ubiquitination (Supplement Table 7). PTMs play a role in increasing plant immunity while ubiquitination is important for resistance of plants to pathogens (Stulemeijer and Joosten, 2008). Hence, the changes in gene expression upon RH plant infection possibly occurred via a decrease in control by PTM. Therefore, in R plants, gene expression seems to be regulated at the mRNA level (see above).

The level of sugars and their derivatives was measured in Si, Ri and RHi tomatoes at 1 and 7dpi (Figure 1C). At 1dpi, when LeHT1 is strongly induced in Ri plants (Figure 1D), the sugar content of Si and Ri plants was similar, but different from that of RHi plants. In RHi plants, where the amount of LeHT1 transcripts decreased by 75-80% compared to Ri (Figure 1D) reaching levels present in Ro and So plants (Supplementary Figure 2A), the concentrations of monosaccharides, mostly hexoses (glucose, fructose and mannose), also decreased. These results demonstrate the cardinal role of LeHT1 in regulating sugar content in R plants. In all tomato plants, the hexose content correlated with the expression level of LeHT1.

At 7dpi, the amounts of ~80% of the sugars increased in the Ri plants, compared to a ~60% decrease in Si plants; the sugars profile of RHi plants were closer to Si than to Ri plants (Figure 1E). Hence, even though the transcription pattern showed that RH plants are closer to R than to S plants (Supplementary table 5), the sugar content of RH tomatoes was closer to that of the S plants than to the original R genotype. The amounts of disaccharides (sucrose, sorbitol) decreased upon infection in Si and RHi plants, but not in Ri
tomatoes. Therefore, in the plant sugar pool, the cellular sucrose:hexose ratio is emerging as an important parameter determining cellular responses.

Since both the enzyme invertase and the rate of photosynthesis influence the mono-disaccharide ratio (Kocal et al., 2008), we monitored invertase expression and rate of photosynthesis upon infection. Contrary to Si plants where the cell wall invertase was not up-regulated, in Ri tomatoes there was an increase in the expression of the gene LIN6 (but not LIN8) at 1dpi (Figure 1F, Supplementary Figure 2B), inducing the cleaving of sucrose to glucose and fructose. LIN6 an extracellular apoplast invertase, controls the sucrose supply from source to sink organs in tomato and plays a role in plant defense (http://solgenomics.net/locus/763/view). Ro and So differ by their photosynthetic activity before infection; upon infection, at 7dpi as at 1dpi, photosynthesis decrease was more pronounced in Si than in Ri plants (Figure 1G).

Our results are in accordance with the model of Berger et al. (2007) describing changes in sugar metabolism in response to pathogen infection. Accordingly, when a pathogen such as TYLCV attacks a tomato plant, it initiates rapid changes resulting in the decline in photosynthesis accompanied by an increase in invertase expression, release of hexose that activates defense response or, if failing, promoting pathogen replication and disease expression (Figure 1H). In S plants the reduction in photosynthesis (Figure 1G) was accompanied by a reduction in sucrose amounts, changing the ration mono/disaccharides (Figure 1C and 1E), which likely de-activates the stress-response signaling, lowering the plant defenses and allowing virus replication (Figure 1B), spread and symptom development. In R plants, the reduction in photosynthesis is less pronounced than in S plants. Contrary to S plants where the cell wall invertase is not upregulated, in R tomatoes there is an increase in the expression of LIN6 already at 1dpi (Figure 1F), inducing the cleaving of sucrose to glucose and fructose. In parallel to LIN6 upregulation, LeHT1 is upregulated in infected R plants (Figure 1D). Therefore, larger amounts of hexose could be internalized into the cells. The enhanced amounts of internal hexose (Figure 1C and 1E), which may activate phytohormone-mEDIATE responses, osmoregulates the cell homeostasis and efficiently activates the plant defense responses, amplifying resistance leading to TYLCV containment, perhaps via a RNAi silencing pathway (Supplementary Table 3). In R plants where LeHT1 has been silenced (Figure 1D), the hexose transporter is not expressed, and hence hexoses cannot be internalized into the cell in order to act as defense signaling molecules, likely decreasing the PTM effect, and inducing the collapse of resistance (Moghaddam and Van den Ende, 2012). In this respect, sugar metabolism in RH and S plants are similar, but remarkably differ from R plants (Figure 1C and 1E).

SUPPLEMENTARY DATA

Supplementary Data are available at Molecular Plant Online.
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LEGENDS OF FIGURES

Figure 1.
(A) Hierarchical clustering analysis of differentially expressed genes between resistant (R) and susceptible (S) tomato plants before and at 7dpi, showing genes up- (yellow) or down- (blue) regulated (P ≤ 0.01 and fold change ≥ 3, ≤ -3); no significant change is in black. - So vs. Ro: comparison of non-infected plants; Si vs. Ri: comparison of infected plants. Complete linkage and Euclidean distance methods were used to compute the clustering.
(B) Relative virus amounts in infected R, RH, S and R:TRV-PDS (vector with PDS marker only, RPi), at 7dpi as measured by qPCR. Six plants for each genotype were assayed. Values represent means ±SE.
(C) Hierarchical clustering analysis of differential sugar and sugar derivatives in infected leaves from R, RH and S tomatoes at 1 and 7dpi, quantified by GC-MS. Each cell represents the log2 mean values of five independent biological repetitions of each time point and is relative to control collected in each of the time points. Black: no significant difference (P>0.05), Yellow: up regulation, Blue: down regulation significantly different from control (P<0.05). Euclidean distance and average linkage were used to compute the clustering.
(D) Time course of LeHT1 expression (qPCR) upon TYLCV infection in Ri, Si and RHi plants. Values represent means ±SE from six biological replicates for each plants.
(E) Distribution (%) of decrease and increase amounts of sugar and sugar derivatives in Ri, Si and RHi plants upon infection at 7dpi, compared to non-infected plants.
(F) Invertase (LIN6) expression during TYLCV infection in Ri and Si plants. Value represent means ±SE of six biological replicates for each genotype.
(G) Photosynthesis rate in infected R (white rectangle) and S (black rectangle) at 0, 3 and 7dpi.
(H) Model describing changes in carbohydrate metabolism in response to TYLCV infection of S, R and RH plants. Each feature may can be referred to the relevant Figure or Table.

REFERENCES


