Brief Communication

Tolerance to tomato yellow leaf curl virus in transgenic tomato overexpressing a cellulose synthase-like gene

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Received 20 August 2020; accepted 23 December 2020.

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Keywords: TYLCV, cellulose synthesis, cellulose synthase-like genes, disease tolerance, symptom suppression.

Resistance gene (R gene)-mediated plant immunity confers strong and specific protection against plant-invading pathogens but can be rapidly overcome by those with high evolutionary capacity, such as viruses (Fabre et al., 2012). In contrast, plant tolerance does not prevent pathogen propagation but reduces the detrimental effects of infection on host fitness (Pagan and Garcia-Arenal, 2020). As the severity of disease symptoms can represent the degree of plant tolerance, molecular approaches directed to reduce symptom severity can provide new insights for the development of disease-tolerant crops.

Tomato (Solanum lycopersicum L.) is an economically important vegetable crop worldwide. However, its productivity is challenged in many global areas by the disease caused by tomato yellow leaf curl virus (TYLCV) that induces severe symptoms (i.e. stunted growth, leaf size reduction and yellowing and curling of young leaves), because resistance-breaking variants continue to emerge despite the extensive efforts made to control the disease by deploying R gene-mediated resistance (Garcia-Andres et al., 2009).

Disease symptoms result from the complex transcriptomic reprogramming of numerous genes involved not only in defence responses, but also in plant growth, development and metabolism. In a previous transcriptome analysis, we showed that various genes associated with the cellulose biosynthesis pathway are significantly responsive to TYLCV infection (Seo et al., 2018). In particular, the cellulose synthase-like protein G2 gene (CslG2; Solyc07g043390) was extremely down-regulated upon TYLCV infection. Cellulose synthesis genes play critical roles in cell wall biosynthesis, cell elongation and plant growth (Richmond and Somerville, 2000). Thus, we hypothesized that TYLCV-induced symptoms leading to impairment of plant growth and development might be directly associated with the suppression of cellulose synthesis genes. Here, we show that the constitutive overexpression of Solyc07g043390 can significantly reduce the severity of TYLCV symptoms and increase disease tolerance and crop productivity in TYLCV-infected tomato.

A recent study in tomato identified 38 cellulose synthesis genes with in this gene family, including 16 cellulose synthase (CesA) and 22 cellulose synthase-like (Csl) genes (Song et al., 2019). Thus, we re-examined our previously reported RNA-seq data set obtained by transcriptomic analysis in healthy and TYLCV-infected tomato plants (Seo et al., 2018), focusing on the 38 CesA/Csl genes. Our RNA-seq analysis revealed that 14 CesA/Csl genes accumulated to significant levels in healthy tomato plants (Figure 1a). Through qRT-PCR, we verified that three Csl genes (Solyc07g043390, Solyc07g051820, and Solyc08g082650) were significantly down-regulated in TYLCV-infected tomato, while one CesA (Solyc12g056580) and two Csl (Solyc08g076320 and Solyc03g097050) genes were significantly up-regulated (Figure 1b).

Since no functional characterization of cellulose synthesis genes has been done so far in tomato, we first examined the loss-of-function phenotypes associated with the differential expression of five CesA/Csl genes upon TYLCV infection by employing a tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) system (Liu et al., 2002). Tomato plants inoculated with the TRV construct containing partial sequences of the β-glucuronidase (GUS) gene (pTRV-GUS) were used as negative controls. qRT-PCR confirmed the efficient silencing of each target gene, except for Solyc03g097050 (Figure 1c). We hypothesize that the unsuccessful silencing of Solyc03g097050 might be related to the dramatic transcripts’ level increase upon TRV infection itself. Solyc07g043390 (Csl) and Solyc12g056580 (CesA) silencing caused strong growth inhibition in tomato plants, whereas no distinguishable phenotypic differences were observed when the other three Csl genes (Solyc07g051820, Solyc08g076320, and Solyc03g097050) were silenced (Figure 1d).

Since TYLCV infection caused dramatic down-regulation of Solyc07g043390, and its silencing resulted in a stunted growth phenotype, a representative symptom of TYLCV infection, we further focused on the functional characterization of this gene. To see whether stem anatomy was affected in Solyc07g043390-silenced plants, thin transverse stem sections were stained with toluidine-blue-O (TBO) and histologically examined, as described previously (Chantreau et al., 2015). The stem sections of these plants contained thin cortex layers, where the parenchyma cells were significantly smaller than those observed in control plants (Figure 1e). A thin epidermis was another distinct feature of Solyc07g043390-silenced plants. The development of secondary xylem (blue-coloured parts) was highly inhibited in tomato stems by the silencing of Solyc07g043390 (Figure 1e). Besides, scanning electron microscopic observations of leaf epidermis revealed that Solyc07g043390 silencing resulted smaller pavement cells and severe wrinkling of cell walls (Figure 1e). Therefore, Solyc07g043390 may play a critical role in cell wall metabolism in various tomato tissues, including xylem and epidermis.
To investigate the gain-of-function phenotypes of Solyc07g043390 in tomato, we generated overexpressing transgenic (OE) plants using the 35S promoter. The transgenic plants had similar growth phenotypes, and here, we examined two representative independent, homozygous T2 lines (Figure 1f and g). Under normal conditions, the OE T2 lines exhibited a slightly

Figure 1 Constitutive overexpression of a Csl gene confers tolerance to TYLCV in tomato. (a) Expression levels of tomato CesA/Csl genes in healthy or TYLCV-infected plants. Normalized gene expression values expressed as FPKM were obtained by Illumina RNA-sequencing. ND, not detected (b) Relative expression levels of tomato CesA/Csl genes in healthy or TYLCV-infected plants. Relative transcript levels were quantified by qRT-PCR (Choi et al., 2019; Seo et al., 2018). The ubiquitin gene (UBQ10; Solyc07g064130) was used as internal reference for qRT-PCR experiments in this study. Expression of target genes was normalized to UBQ10. (c) Relative expression analysis of five selected CesA/Csl genes in gene-silenced plants by qRT-PCR. A TRV-based VIGS system was employed (Liu et al., 2002). (d) Phenotypes of gene-silenced plants. pTRV-GUS plants served as negative controls. (e) Anatomical observations of stems and leaves of control and Solyc07g043390-silenced plants. Stem sections (4th and 6th internodes from the bottom) were stained with TBO. Leaf epidermal cells were observed by scanning electron microscopy. Ep, epidermis; Co, cortex; Ph, phloem; Xy, xylem; Pi, pith. Bar: 100 µm. (f) Phenotypes of the transgenic T2 lines overexpressing Solyc07g043390 under the 35S promoter. NT, non-transgenic plant (g) Relative expression analysis of Solyc07g043390 in OE transgenic lines by qRT-PCR. Symptomatic phenotypes of NT and Solyc07g043390-OE plants upon TYLCV infection: (h) whole plants, (i) different-aged leaves. (j) Relative accumulation analysis of TYLCV in NT and OE plants by qRT-PCR. Phenotypic effects of Solyc07g043390 overexpression on growth (k), fruit yield (l) and fruit size (m) in TYLCV-infected tomato plants. Means ± SD from three independent experiments are shown and each column represents one group with 9 plants; paired Student’s t-tests were used to detect significant differences in the statistical analyses (*P < 0.05; **P < 0.01). Different letters above the bars indicate significant differences between samples (one-way ANOVA, P < 0.05).
slower growth rate and smaller leaf size compared with wild-type non-transgenic (NT) plants (Figure 1f). To test whether the constitutive overexpression of Solyc07g043390 could compensate for the decrease in the endogenous gene expression induced by TYLCV infection, leading thus to a decreased severity of symptoms, OE plants were inoculated with a TYLCV infectious clone via Agrobacterium-mediated infiltration (Kil et al., 2016). While NT plants showed the typical TYLCV symptoms at 25 days post-inoculation, only mild yellowing and curling of the upper leaves were observed in OE plants (Figure 1h and i). qRT-PCR revealed that, despite TYLCV infection, OE plants expressed Solyc07g043390 at a level similar to that of healthy NT plants (Figure 1j). However, viral accumulation was not suppressed in OE plants (Figure 1k). Therefore, tomato stunted growth seems to be directly associated with TYLCV-induced Solyc07g043390 down-regulation, and the constitutive overexpression of Solyc07g043390 could functionally compensate for that phenomenon. The reduction in the symptom severity was resulted in the improvement of plant growth and fruit size and yield in OE plants infected with TYLCV (Figure 1l-n).

The R gene-mediated resistance can be overcome in the short-term by viral pathogens that have great evolutionary capacity because host resistance itself acts as strong selective pressure in the virulence evolution (Fabre et al., 2012). In contrast, tolerance exerts a much weaker selective pressure on pathogens, and thus, the incidence of new virulent variants is usually very low in a tolerant plant (Pagan and Garcia-Arenal, 2020). Therefore, in the long-term, deploying tolerance in the crop fields may be advantageous in minimizing the emergence of resistance-breaking variants and preserving agricultural conditions in which disease causes only minimal damage. Here, we generated tomato plants highly tolerant to TYLCV infection by constitutively overexpressing the Csl gene Solyc07g043390, which is remarkably down-regulated upon TYLCV infection. Although OE plants were still susceptible to TYLCV, the major symptoms (stunted growth and leaf size reduction) significantly decreased. We also demonstrated that increased symptomatic tolerance resulted in increased crop productivity in the tomato-TYLCV pathosystem. Although increasing tolerance to viral infections is not a usual approach currently in transgenic researches, our study provides new insights into a molecular approach that could facilitate future plant immunity engineering.

Acknowledgements
This research was supported by a grant from the Agenda Program (PJ015308) funded by the Rural Development Administration of Korea.

Conflict of interest
The authors declare no conflict of interest.

Author contributions
JKS designed the experiments and supervised the project; SC, SJK and BC performed the experiments; SC, SJK and JHK analysed the data; SC and JKS wrote and revised the manuscript.

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