SKIP Silencing Decreased Disease Resistance Against *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* DC3000 in Tomato

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INTRODUCTION

The splicing process is completed by spliceosome, which can be classified as two types, including major spliceosome and minor spliceosome (Moore and Proudfoot, 2009; Turunen et al., 2013). SKIP, one of the splicing factors and important components of spliceosome, has possessed several conserved domains (including the SNW/SKI-interacting protein, SKIP) and the specific motifs (Folk et al., 2004; Bres et al., 2009; Chen et al., 2011; Wang et al., 2012). The C terminal plays a vital role in protein stability, while the SNW domain is necessary for the biochemical activity. In Arabidopsis, the SNW domain can integrate into spliceosome in the meantime of interacting with the Paf1 complex (Li et al., 2016). The interaction of SMP1/2 with SKIP facilitates the recruitment of second-step splicing factors into the Arabidopsis spliceosome (Liu et al., 2016).

SKIP is involved in splicing in an either direct or indirect way. In mammals and yeast, SKIP is a component of the 35S-U5snRNP complex, which participates in the common RNA splicing directly (Albers et al., 2003). Growing evidence shows that SKIP is involved in transcription regulation and RNA splicing through interacting with different proteins and thus takes parts in regulating several
signaling pathways. For example, SKIP protein participates in at least five signaling pathways in human, including the steroid hormone (Zhang et al., 2001, 2003), TGF-β (Barry et al., 2003), MyoD (Kim et al., 2001), Notch (Zhou et al., 2000; Laduron et al., 2004), and E2F/pRb (Prathapam et al., 2002) pathways.

In addition to RNA splicing, SKIP exerts its functions in numerous steps, such as transcription elongation and transport of mature mRNA. Many species harbor the homologous SKIP protein in nucleus that is between 60 and 80 kDa. However, SKIP in different species has different functions. For instance, in yeast, the weak mutation of the SKIP homolog Prp45 has defects in the splicing of ACTIN and other genes, which leads to the fatal potent mutation of Prp45 and the growth with temperature sensitivity (Figueroa and Hayman, 2004; Bessonov et al., 2008; Gahura et al., 2009). In yeast, SKIP, one of the transcription factors (TFs), helps to modulate gene expression patterns (Lim et al., 2010). In drosophila, Bsx42 involved in transcription regulates edcyson, the Notch signaling pathway, nervous system development, and organ formation (Wieland et al., 1992; Negeri et al., 2002; Ivanov et al., 2004). In nematode, CeSKIP exerts an essential role in individual survival and embryonic development (Kostrouchova et al., 2002; Piano et al., 2002; Kamath et al., 2003; Simmer et al., 2003; Rual et al., 2004; Sonnichsen et al., 2005). Recently, the SKIP functions have been gradually explored, but mainly in Arabidopsis. SKIP shows physical interaction with SR45 (an SR protein specific to plant) for regulating the biological clock. Mutations of the skip-1 gene will lead to a phenotype of a prolonged clock period through changing alternative splicing (AS) in PSEUDO-RESPONSE REGULATOR 7 (PRR7) together with PRR9, both of which are related to the oscillator morning loop. As indicated by this result, SKIP participates in regulating the genes associated with the biological clock of Arabidopsis at the post-transcription level (Wang et al., 2012). Apart from the defects of the biological clock, the skip-I plant also exhibits the pleiotropic phenotype, such as the early blossoming. Additionally, it is still unclear about how SKIP suppresses floral transition at a molecular or biochemical level, thereby attenuating B. cinerea together with stress resistance of Arabidopsis by regulating the scavenging activity for reactive oxygen species (ROS) (Zhang et al., 2013).

This research aimed to analyze the functions of SKIP genes in tomato to resist against Botrytis cinerea as well as Pseudomonas syringae pv. tomato (Pst) DC3000, the necrotrophic fungal and the (hemi)-biotrophic bacterial pathogens, separately, using virus-induced gene silencing (VIGS). It was shown in this study that the VIGS-mediated silencing of SlSKIP1b resulted in the accumulation of more ROS, but decreased the levels of defense-related genes (DRGs) in the case of pathogen infection, thereby attenuating B. cinerea and Pst DC3000 resistance. These findings demonstrated that the SKIP genes play vital parts in regulating the anti-pathogen response of tomato.

**MATERIALS AND METHODS**

**Plant Cultures as Well as Treatments**

Two tomato varieties (Solanum lycopersicum), Suhong2003 as well as MicroTom, were employed in this research. Suhong2003 was used in all experiments except for the whole plant disease assays against B. cinerea that used the cultivar MicroTom. Tomato seedlings were grown in a material mixture within a greenhouse. Besides, seedlings of 2 and weeks old were utilized to carry out VIGS assays and to analyze gene expression after pathogen inoculation and treatments with defense-related signal molecules, respectively. Typically, the treatments with defense-related signal molecules were done by spraying MeJA, ACC, and SA (all at 100 µM and from Sigma-Aldrich), and water was used as control. At the designated time points after treatment, the leaves were collected.

**Pathogen Infection Together With Disease Assays**

In this study, B. cinerea infection in tomato plants was completed by two approaches (Li et al., 2014; Zhang et al., 2020). In brief, after collecting spores, their densities were tuned to $1 \times 10^5$ spores/mL. For the detached leaf disease assays, leaf samples with full expansion were collected from the 6-week-old VIGS agroinfiltrated plants, and put on the cheesecloth pre-immersed within the distill sterilized water in trays. Each side of the leaves was inoculated by a drop of 2.5 µL spore suspension, followed by disease development in high humidity. At 4 days...
later, the lesion size in those infected leaf samples was recorded. In the whole plant disease assays, sporulation was sprayed onto tomato plants until it evenly covered the leaf surface. Afterward, those infected plants were then put into a high-humidity environment. At 4 days after inoculation, photographs were taken for the phenotype. Then, after collecting leaves at the designated time points, the fungus quantity and gene levels were analyzed. qRT-PCR was adopted to define fungal growth by *B. cinerea BcActin* gene expression.

Plants were inoculated with *Pst* DC3000 according to the following steps (Li et al., 2014): after harvesting and resuspending bacteria into MgCl₂ (10 mM) to OD₆₀₀ = 0.0002, all leaves were immersed into the bacterial suspension using the 0.04% Silwet L-77, followed by 1.5 min of negative pressure treatment at −40 kPa. The phenotype was photographed at 4 days following infection. Besides, leaves were harvested to analyze specific gene levels as well as bacterial growth. To measure the bacterial growth, 70% ethanol was used to sterilize leaf discs for 10 s, then sterile water was utilized to wash them for thrice, followed by grinding within the 10 mM MgCl₂ solution (200 µL) until a homogenate was obtained. Later, the homogenate was diluted with 10 mM MgCl₂ at a ratio of 1:10 to different concentrations, cultured in the King’s B solid medium for 3 days, and the colonies were recorded.

**Characterization of SISKIP Genes**

Using the BlastP program, the tomato genome database was searched at http://solgenomics.net using those featured Arabidopsis ATSKIP as queries. Afterward, those obtained sequences of SISKIPs nucleotides and amino acids (AAs) were downloaded.

**RNA Extraction Along With qRT-PCR**

The Trizol reagent (Invitrogen, Shanghai, China) was used to extract total RNA according to specific protocol. The PrimeScript RT reagent kit (TaKaRa, Dalian, China) was used for reverse transcription following specific instructions to synthesize cDNAs, which served as the templates for PCR and qRT-PCR. In this study, the CFX96 real-time PCR assay system (Bio-Rad, Hercules, CA, United States) was used for qRT-PCR. Dissociation curves were used to verify that the amplified production was single in PCR. Target gene transcript expression was shown as relative transcript expression to an Actin gene in tomato. The 2⁻ΔΔCT approach was applied in calculating the relative gene expression level according to previous description. Table 1 lists those gene-specific primers adopted for qRT-PCR.

**Construction of the VIGS Vector and Agroinfiltration**

Fragments of 300–400 bp in sizes for SISKIPs were amplified by PCR with respective pairs of gene-specific primers (Table 1). The amplified PCR products were digested with corresponding restriction enzymes (XbaI/Xhol) and cloned into TRV2, yielding recombinant plasmids TRV-SISKIP1a and TRV-SISKIP1b. After confirmation by sequencing, the correct recombinant plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101 by electroporation and positive clones were selected by colony PCR for VIGS assays. Agrobacteria carrying TRV-SISKIP1a or TRV-SISKIP1b were grown in YEP liquid medium with 50 µg/mL kanamycin, 50 µg/mL rifampicin, and 25 µg/mL gentamicin in a shaker until OD₆₀₀ reached to 0.8–1.0. Agrobacterial cells were collected by centrifugation and resuspended in infiltration buffer containing 10 mM MgCl₂, 10 mM MES (pH 5.7) and 200 µM acetylsyringone, and the bacterial concentrations in suspensions were adjusted to OD₆₀₀ = 1.5. The agrobacteria carrying TRV-SISKIP1a or TRV-SISKIP1b were mixed with the same volume of agrobacteria carrying TRV1, and the mixtures were incubated for 3 h at room temperature. The mixed agrobacterial suspension was separately infiltrated into the abaxial surface of the 2-week-old seedlings using a 1-mL needleless syringe (Liu et al., 2002). A group of tomato seedlings were infiltrated with agrobacteria harboring a construct of TRV-PDS (*Phytoene desaturase*) and used as positive controls for silencing evaluation of the VIGS procedure (data showed in Supplementary Material). The agroinfiltrated plants were allowed to grow for 4 weeks in a growth room under the same conditions as mentioned above and then used for different experiments.

**Detection and Measurement of H₂O₂**

The DAB staining method was utilized to detect H₂O₂ accumulation within the leaf tissues. After *B. cinerea* (0 and 24 h) and *Pst* DC3000 (0 and 48 h) inoculation, leaves were harvested, respectively. After 3 h of immersion into the 1-mg/mL DAB solution (pH 3.8), the leaf samples were boiled in 95% ethanol until the chlorophyll was completely removed. Finally, a digital camera was utilized to visualize H₂O₂ accumulation in those stained leaf samples. The H₂O₂ measurement was done by an

| Primers          | Sequences (5’–3’)              | Size (bp) |
|------------------|--------------------------------|-----------|
| SKIP1a-vigs-F    | GTC TCTAGA TGCGATCTGCAAGAAGGCT | 351       |
| SKIP1a-vigs-R    | GTC TCTAGA GGTTGAGACCAACACGCT  | 349       |
| SKIP1b-vigs-F    | GTC TCTAGA GTTACTGTGGAGACCAACGCT | 349       |
| SKIP1b-vigs-R    | AGT CTGGAG GGTTCTTGGTGTCTGAACCT | 113       |
| SKIP1a-RT-F      | TACTGCGAGAGCACTGAGGGTG         | 113       |
| SKIP1a-RT-R      | CCTGCGATCTGGAAGACCAATT         | 112       |
| SKIP1b-RT-F      | AAGCTCAATCTTCAGAAGGTTGTA      | 107       |
| SKIP1b-RT-R      | CTGGCACTTCTCCTAATGCTCACT      | 107       |
| SKIP1a-qRT-F     | CGGAGATACCTGGTGACTAGT         | 113       |
| SKIP1a-qRT-R     | CAAGGATTGTCTCGTAAATGAG         | 96        |
| SKIP1b-qRT-F     | TGGAAACAAAGAAGGTAACAG         | 116       |
| SKIP1b-qRT-R     | CGATCTTAATTAGTTCATGATG         | 116       |
| SKIP2-qRT-F      | GTGTTGAGGATATAACAAATACGA     | 109       |
| SKIP2-qRT-R      | TTGCTGTTAATTATATACAGCA        | 109       |
| SKIP1a-vigs-R    | GTC TCTAGA TGGCATCTGCAAGAAGGCT | 351       |
| SKIP1b-vigs-R    | GTC TCTAGA GTTACTGTGGAGACCAACGCT | 349       |
| SKIP1a-RT-F      | TACTGCGAGAGCACTGAGGGTG         | 113       |
| SKIP1a-RT-R      | CCTGCGATCTGGAAGACCAATT         | 112       |
| SKIP1b-RT-F      | AAGCTCAATCTTCAGAAGGTTGTA      | 107       |
| SKIP1b-RT-R      | CTGGCACTTCTCCTAATGCTCACT      | 107       |
| SKIP1a-qRT-F     | CGGAGATACCTGGTGACTAGT         | 113       |
| SKIP1a-qRT-R     | CAAGGATTGTCTCGTAAATGAG         | 96        |
| SKIP1b-qRT-F     | TGGAAACAAAGAAGGTAACAG         | 116       |
| SKIP1b-qRT-R     | CGATCTTAATTAGTTCATGATG         | 116       |
| SLAP1-F          | TTGGAAACAAAGAAGGTAACAG         | 116       |
| SLAP1-R          | CGATCTTAATTAGTTCATGATG         | 116       |

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### Table 1

**The list of primer sequence of the genes in this article.**
H$_2$O$_2$ Kit (Jiancheng, Nanjing, China). The content of H$_2$O$_2$ was calculated by the formula: (OD value of the measured sample – OD value of the blank)/(OD value of a standard solution – OD value of the blank × 163 mmol/L)/concentration of the protein of tissue.

Experiment Design and Data Analysis
All experiments were repeated independently three times. More than 10 plants were used in each of independent experiments such as disease assay with *B. cinerea* or *Pst DC3000*. Data obtained from three independent experiments were subjected to statistical analysis according to the Student’s *t*-test. The probability values of *p* < 0.05 were considered as significant difference between treatments and their corresponding controls.

RESULTS
Characterization of SISKIP Genes in Tomato
Using the characterized Arabidopsis *AtSKIP* genes as queries, a tomato genomic database was searched by Blastp analysis, and two loci were identified in tomato genome, which were named as *SISKIP1a* (XM_004251580.4) and *SISKIP1b* (XM_004250540.4) (the information about *SISKIPs* can be seen in Supplementary Data). Moreover, ESTs together with the potential full-length cDNAs of *SISKIPs* were discovered against tomato genomic database as well as NCBI GenBank database, separately, which indicated the constitutive expression of *SISKIPs* in tomato. The ORFs of *SISKIPs* were cloned and sequenced, which found the totally same *SISKIPs* ORF sequences with those predicted ORF sequences.

Expression Models of SISKIPs With Pathogen Inoculation and Treatments With Defense-Related Signal Molecules
The function analysis of *SISKIPs* was completed to reveal their probable biological roles in resisting against disease. First, this study investigated the *SISKIP1a* and *SISKIP1b* expression models responding to the inoculation of *Pst DC3000* and *B. cinerea*, together with the treatment of defense-related signal molecules, including 1-amino cyclopropane-1-carboxylic acid (ACC, the ET precursor), methyl jasmonate (MeJA), and salicylic acid (SA) in tomato plants. After 72 h of *Pst DC3000* infection, the *SISKIP1b* level was notably upregulated by about 6.3 times relative to that in control plants with mimic inoculation, whereas the *SISKIP1a* level showed no difference after *Pst DC3000* infection (Figure 1A). In the case of inoculation with *B. cinerea*, the condition was very similar to that in inoculation with *Pst DC3000*. After 48 h of *B. cinerea* infection, the *SISKIP1b* level prominently increased by about 7.1-folds relative to that in control plants with mimic inoculation, but the *SISKIP1a* level showed no dramatic difference (Figure 1B). Moreover, none of

![FIGURE 1](https://www.frontiersin.org) | SISKIP expression responding to *B. cinerea* together with *Pst DC3000* infection. (A) SISKIP gene expression patterns responding to *Pst DC3000* infection. (B) SISKIP gene expression patterns responding to *B. cinerea* infection. Data presented are the means ± SD from three independent experiments with biological distinct samples and * above the columns indicate significant differences at *p* < 0.05 level.
Our selected signal molecules associated with defense affected the SlSKIP1a level, while all of those signal molecules triggered SlSKIP1b expression (Figures 2A, B). In addition, the SlSKIP1b level significantly increased at 12 h after ACC and JA treatments, and its expression was the maximal at 24 h after SA treatment (Figure 2B). As suggested by these data, SlSKIP expression might be triggered via B. cinerea and Pst DC3000 as well as the defense-related signal molecules.

**SlSKIP Silencing of Tomato**

For analyzing SlSKIP effects on the resistance against disease, the VIGS approach was utilized to manage the endogenous SlSKIP expression. Therefore, the SlSKIP gene silencing efficiency was checked at first. Then, the normal VIGS protocol was adopted for the 2-week-old tomato seedlings. At 4 weeks later, the silencing efficiency was measured, with plants transfected using the TRV-PDS construct being the positive controls. The silencing efficiency of SlSKIP genes was evaluated to be 65%, which was used for further functional studies (Figures 3A, B).

**Silencing of SlSKIP1b Led to Reduced B. cinerea Tolerance**

For studying those potential SlSKIP genes’ functions to resist B. cinerea, this study applied two distinct approaches, namely, detached leaf and whole plant disease assays for preliminary and further confirmation, respectively. The seedlings of TRV-SlSKIPs- and TRV-GUS-infiltrated plants were compared for their disease phenotypes and fungal quantity, for the sake of confirming disease phenotype. As obtained from detached leaf disease assays, the leaf lesion size in TRV-SlSKIP1b-infiltrated plants prominently elevated by about 54% at 3 days after infection (dpi) (Figure 4A), compared with that in TRV-GUS-infiltrated counterparts (Figure 4B). Meanwhile, the leaf lesion size in TRV-SlSKIP1a-infiltrated plants (3 dpi) was not significantly different from that of the TRV-GUS-infiltrated counterparts (Figures 4A, B).

For further confirming the above finding, whole-plant disease assays were conducted to estimate disease phenotype and test...
FIGURE 3 | SlSKIP gene silencing efficiency along with specificity of plants inoculated with VIGS. (A) The SlSKIP1a and SlSKIP1b silencing efficiency of plants inoculated with TRV-SlSKIP1a and those inoculated with TRV-SlSKIP1b, respectively. (B) SlSKIP1a silencing specificity of plants inoculated with TRV-SlSKIP1a. (C) SlSKIP1b silencing specificity of plants inoculated with TRV-SlSKIP1b. Data presented are the means ± SD from three independent experiments with biological distinct samples and * above the columns indicate significant differences at $p < 0.05$ level.

FIGURE 4 | SlSKIP1b silencing led to the weakened B. cinerea tolerance verified through detached leaf disease assays. Agrobacteria that carried TRV-SlSKIPs and TRV-GUS constructs were transfected into the tomato plants of 10 days old, and then leaves were harvested following agroinfiltration for four weeks. (A) Disease symptoms of typical leaf samples collected based on plants inoculated with TRV-SlSKIP and those inoculated with TRV-GUS. (B) Leaf lesion size in plants inoculated with TRV-SlSKIP and those inoculated with TRV-GUS. Data presented are the means ± SD from three independent experiments with biological distinct samples and * above the columns indicate significant differences at $p < 0.05$ level.
B. cinerea fungal growth in planta of plants inoculated with TRV-SlSKIP. According to Figure 5A, plants infiltrated with TRV-GUS showed mild disease symptom compared with those inoculated with TRV-SlSKIP1b, while those inoculated with TRV-SlSKIP1a displayed no difference from those inoculated with TRV-GUS at 5 dpi. At 24 and 48 hpi, the B. cinerea growth in planta, which was expressed as B. cinerea BcActinA gene transcript level, notably elevated by threefold in leaves of plants inoculated with TRV-SlSKIP1b compared with those inoculated with TRV-GUS (Figure 5B). While B. cinerea growth for plants inoculated with TRV-SlSKIP1a did not show any significant difference compared with plants inoculated with TRV-GUS (Figure 5B). Collectively, the above findings suggested that SlSKIP1b silencing reduced B. cinerea tolerance in tomato plants, with excessive B. cinerea growth of TRV-SlSKIP1b-infected plants.

To gain insights into the probable mechanism by which SlSKIP1b silencing led to weakened B. cinerea tolerance, this study analyzed the ROS accumulation together with the expression levels of DRGs. Before B. cinerea infection, no obvious \( \text{H}_2\text{O}_2 \) accumulation was observed in plants inoculated with TRV-SlSKIP1b or those inoculated with TRV-GUS, but \( \text{H}_2\text{O}_2 \) accumulation significantly increased at 24 h following B. cinerea inoculation (Figure 6A). The \( \text{H}_2\text{O}_2 \) concentration was further measured. The results showed that the \( \text{H}_2\text{O}_2 \) concentration in plants inoculated with TRV-SlSKIP1b was much higher than that of the ones inoculated with TRV-GUS after B. cinerea infection, but there was no significant difference before B. cinerea infection (Figure 6B). Similarly, SlPRP2 and SlPR1b (the DRGs responding to the SA signaling) and SlLapA and SlPIN2 (DRGs responding to the JA/ET signaling) were almost the same in plants inoculated with TRV-SlSKIP1b as those inoculated with TRV-GUS prior to B. cinerea inoculation (Figure 6C). B. cinerea infection was the primary cause inducing the expression of the above four DRGs, relative to those of uninfected controls. However, at 24 hpi, SlPR1b and SlPRP2 expression slightly decreased, whereas SlPIN2 and SlLapA expression notably reduced in plants inoculated with TRV-SlSKIP1b, relative to those TRV-GUS-infected counterparts (Figure 6C). Collectively, the above results suggested that SlSKIP1b silencing resulted in reduced accumulation of ROS,
FIGURE 6 | Silencing of SISKIP1b elevated H$_2$O$_2$ accumulation but downregulated the JA/ET signaling-responsive DRG expression following B. cinerea inoculation. At 4 weeks following VIGS inoculation, 2 × 10$^5$ spores/mL pore suspension was used to spray on the leaf surface through whole-plant disease assays, then leaves were harvested at 24 h following infection. (A) H$_2$O$_2$ accumulation in plants inoculated with TRV-SISKIP1b and those inoculated with TRV-GUS revealed through DAB staining following B. cinerea inoculation. (B) The H$_2$O$_2$ concentration in plants inoculated with TRV-SISKIP1b and those inoculated with TRV-GUS before and after B. cinerea inoculation. The H$_2$O$_2$ concentration was measured using an H$_2$O$_2$ kit. (C) Specific DRG expression levels in plants inoculated with TRV-SISKIP1b and those inoculated with TRV-GUS following B. cinerea inoculation. Data presented are the means ± SD from three independent experiments with biological distinct samples and * above the columns indicate significant differences at p < 0.05 level.

as well as decreased levels of DRGs responding to the JA/ET signaling after B. cinerea inoculation.

**SISKIP1b Silencing Led to Weakened Pst DC3000 Tolerance**

The potential SISKIP1b functions in the resistance against Pst DC3000 was further studied. The disease phenotype and in planta bacterial quantity of plants inoculated with TRV-SISKIPs were compared with those of plants inoculated with TRV-GUS. Differences in disease symptom and bacterial growth at 3 dpi were not significant between plants inoculated with TRV-SISKIP1a and those inoculated with TRV-GUS (Figures 7A,B), indicating that SISKIP1a did not possibly participate in the Pst DC3000 tolerance. Plants inoculated with TRV-SISKIP1b showed serious disease symptom relative to those inoculated with TRV-GUS (Figure 7A), and the bacterial population at 4 dpi was about 20 times higher than that in control (Figure 7B). The above findings demonstrated the effect of SISKIP1b silencing on reducing Pst DC3000 tolerance of tomato.

For exploring the potential mechanism of action by which SISKIP1b silencing affected Pst DC3000 tolerance, the ROS accumulation together with DRGs expression was examined subsequently. Prior to Pst DC3000 infection, there was no distinct H$_2$O$_2$ accumulation observed in leaves from plants inoculated with TRV-SISKIP1b and TRV-GUS (Figure 8A). Compared with controls, plants inoculated with TRV-SISKIP1b showed obvious H$_2$O$_2$ accumulation at 3 dpi (Figure 8A). We also measured the H$_2$O$_2$ concentration. As shown in Figure 8B, the H$_2$O$_2$ concentration in TRV-SISKIP1b seedlings was much higher than that of TRV-GUS after pathogen infection. There was no
significant difference in SlPRP2, SlPR1b, SlPIN2, or SlLapA expression in plants inoculated with TRV-SlSKIP1b relative to those inoculated with TRV-GUS prior to Pst DC3000 inoculation (Figure 8C). Besides, relative to plants inoculated with TRV-GUS at 2 dpi, those inoculated with TRV-SlSKIP1b showed decreased SlPR1b together with SlPRP2 expression (Figure 8C). However, SlLapA or SlPIN2 expression of plants inoculated with TRV-SlSKIP1b showed no prominent change relative to TRV-GUS-infiltrated counterparts at 2 days following Pst DC3000 infection (Figure 8C). The above results suggested the effect of SlSKIP1b silencing on reducing the SA signaling-responsive DRGs levels by Pst DC3000 inoculation.

DISCUSSION

In this research, two SlSKIP genes were identified in tomato, while only one SKIP gene was discovered in Arabidopsis. Although it is already known that SKIP is involved in transcription regulation and RNA splicing, thus leading to the regulation of several signaling pathways. There is no direct genetic proof for SKIP functions in the disease resistance of plants.

In our experiment, the SlSKIP target gene silencing efficiency was predicted as around 65% (Figure 3A), close to that obtained in our prior works (Li et al., 2014, 2015; Liu et al., 2014; Zhang Y. et al., 2014; Zhang et al., 2016). It is previously reported that pathogen infection may induce SKIP expression...
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FIGURE 8 | Silencing of SlSKIP1b affected H$_2$O$_2$ accumulation and SA signaling-responsive DRG expression following Pst DC3000 inoculation. The Pst DC3000 suspension (OD$_{600}$ = 0.0002) was used to infect plants at negative pressure in the whole-plant disease assays 4 weeks following VIGS inoculation. Leaves were harvested to analyze the H$_2$O$_2$ accumulation together with DRG expression following infection. (A) H$_2$O$_2$ accumulation in plants inoculated with TRV-SlSKIP1b and those inoculated with TRV-GUS following Pst D3000 inoculation measured through DAB staining. (B) The H$_2$O$_2$ concentration in plants inoculated with TRV-SlSKIP1b and those inoculated with TRV-GUS before and after Pst DC3000 inoculation. The H$_2$O$_2$ concentration was measured using an H$_2$O$_2$ kit. (C) Specific DRG expression in plants inoculated with TRV-SlSKIP1b and those inoculated with TRV-GUS following Pst DC3000 inoculation. Data presented are the means ± SD from three independent experiments with biological distinct samples and * above the columns indicate significant differences at $p < 0.05$ level.

(Liu, 2015). As found in the present work, both B. cinerea and Pst DC3000 triggered SlSKIP1b expression (Figure 1). Also, SlSKIP expression was triggered in response to signal molecules associated with defense (Figure 2), which was consistent with previous study reporting that OsSKIPa expression was triggered upon a variety of phytohormone treatments as well as abiotic stress conditions (Hou et al., 2009). The SlSKIP genes showed different responses to B. cinerea or Pst DC3000 inoculation, together with the signal molecules associated with defense, which suggested the potential functions in the B. cinerea as well as Pst DC3000 tolerance.

The VIGS-based method was adopted to analyze the SlSKIP functions in terms of disease resistance. As a result, the silencing of SlSKIP1b led to weakened B. cinerea (Figures 4, 5) along with Pst DC3000 (Figure 7) tolerance. Typically, plants silenced by SlSKIP1b displayed the serious disease symptom, together with excessive pathogen growth, which confirmed their reduced B. cinerea tolerance (Figures 4, 5). At the same time, the SlSKIP1b-silenced plants displayed more severe disease symptoms, along with more bacterial growth, which confirmed the reduced Pst DC3000 tolerance (Figure 7). These results were consistent with a previous report that SKIP did have a certain function in the resistance to biotic stress. Moreover, GhSKIP35 has certain functions in the resistance to verticillium wilt in G. hirsutum (Liu, 2015).

In this study, the alterations of ROS accumulation and certain specific DRG expression were analyzed to explore the cause of the decreased B. cinerea and Pst DC3000 tolerance of SlSKIP1b-silenced plants. In this experiment, plants silenced by SlSKIP1b showed more H$_2$O$_2$ accumulation following B. cinerea and Pst DC3000 inoculation (Figures 6, 8). It is known that late-stage ROS accumulation facilitates disease development resulting from the necrotrophic pathogens (like B. cinerea) and (hemi) biotrophic pathogens (like Pst DC3000) (Govrin and Levine, 2000; Govrin et al., 2006; Temme and Tudzynski, 2009; Ishiga et al., 2012; Mengiste, 2012). Therefore, the increased ROS content resulting from SlSKIP1b silencing was possibly related to the weakened tolerance to B. cinerea as well as Pst DC3000 of plants silenced by SlSKIP1b.
Besides, plants silenced by SISKIP1b had decreased levels of SIRP1b (SA-related gene), SLlapA (JA-related gene), and SIPPIN2 (JA-related gene) following B. cinerea inoculation and increased levels of SIRP1b and SRPP2 (SA-related gene) following Pst DC3000 inoculation (Figures 6C, 8C). It is known that SA-regulated defense responses are good for the infection of necrotrophic pathogen B. cinerea, while JA-regulated defense responses are involved in restricting the disease. The inverse model is proposed for (hem) biotrophic pathogen Pst DC3000 (Glazebrook, 2005; Pieterse et al., 2009). We speculated that SKIP1b may not mediate in the antagonistic effects between SA- and JA-signaling pathways. Instead, SKIP1b may involve in these two pathways. So the expression levels of SA-dependent genes and lower JA-dependent genes changed in TRV-SISKIP1b inoculated plants.

The reduced resistance of the SISKIP1b-silenced plants might be induced by the increased ROS accumulation together with changed DRGs levels. However, further physiological and biochemical experiments are required to find out the mechanisms responsible for the altered disease resistance observed in the SISKIP1b-silenced plants.

Our results showed that the silencing of SKIP1b led to increased susceptibility to both Botrytis and Pst DC3000. It consists with previous reports that the silencing of genes will reduce or increase resistance to both Botrytis and Pseudomonas (Li et al., 2015; Wang et al., 2018, 2020).

It is suggested that SKIP plays a role in abiotic stress tolerance. SKIP is involved in the ABA signaling and renders the osmotic resistance in the case of salt stress through regulating AS genes of Arabidopsis (Lim et al., 2010; Feng et al., 2015). OsSKIPu positively modulates the stress tolerance of rice by regulating different genes associated with stress in rice at the transcription level (Hou et al., 2009). The interaction of OsSKIP with OsCYP18-2 is essential for regulating genes associated with stress at both transcription and post-transcription levels and for enhancing drought resistance (Lee et al., 2015). The ZmSKIP overexpression plants with increased ABA contents exhibit significantly enhanced resistance to drought compared with controls, which suggested that ZmSKIP was involved in the regulation of drought resistance by regulating specific gene levels (Wei et al., 2015). Nonetheless, no experiment was carried out to examine the abiotic stress tolerance in this study, and no difference was found between plants inoculated with TRV-SISKIP1b and those inoculated with TRV-GUS during the vegetative growth process. This might be consistent with previous report that SKIP participated in regulating certain reproductive stage gene expression at the post-transcription level in Arabidopsis thaliana (Wang et al., 2012; Cao et al., 2015; Cui et al., 2017). Unfortunately, this study did not conduct an experiment on plants that had entered the reproductive phase. In our future studies, experiments should be performed to examine the resistance to abiotic stress and plants of the reproductive stag.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HZ, LY, MJ, and FS carried out most of the experiments. MJ and HZ designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the Taizhou Municipal Science and Technology Project (20ny18), Science Foundation for Distinguished Young Scholars of Taizhou University (2019JQ001), and Natural Science Foundation of Zhejiang Province (LY19C150004).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.593267/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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