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Rhodopseudomonas palustris PSB-06 Induces Plant Defense and Suppresses the Transmission of Tomato Chlorosis Virus by *Bemisia tabaci* MED

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Abstract: Tomato chlorosis virus (ToCV) is an RNA virus and a member of the Closteroviridae, Crinivirus, that is primarily vectored by *Bemisia tabaci* MED (*B. tabaci* MED). An outbreak of ToCV causes destructive damage to tomato plants and other solanaceous vegetables. Currently, ToCV has not been effectively controlled. *Rhodopseudomonas palustris* PSB-06 is a novel biological agent that is effective at controlling the tobacco mosaic virus (TMV). In this study, we investigated the role of PSB-06 in ToCV-infected tomato plants, and we studied the effects of PSB-06 on plant defense and plant photosynthetic pathways. Furthermore, the effect of PSB-06 on the acquisition and transmission of *B. tabaci* MED was determined. The results showed that compared with water-treated tomato plants, the contents of jasmonic acid increased, and the activities of catalase, peroxidase and superoxide dismutase increased significantly in tomato plants treated with PSB-06. The relative expression of genes involved in chlorophyll development, chlorophyll metabolism and photosynthesis also increased significantly. Simultaneously, treatment with PSB-06 reduced the acquisition and transmission of *B. tabaci* MED. We verified the hypothesis that PSB-06 is effective at controlling ToCV by promoting plant defense responses and reducing the amount of ToCV in tomato plants. We also confirmed the ability of *B. tabaci* MED to transmit ToCV. This study should help to control *B. tabaci* MED and reduce the spread of ToCV.

Keywords: PSB-06; tomato chlorosis virus; *Bemisia tabaci*; plant defense; transmission

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1. Introduction

Photosynthetic bacteria (PSB) are Gram-negative prokaryotes capable of photosynthesizing under anaerobic conditions. Based on whether they produce oxygen during the process of photosynthesis, PSB can be divided into oxygen-producing photosynthetic bacteria, such as cyanobacteria and protogreen bacteria, and non-oxygen-producing photosynthetic bacteria, such as purple bacteria and green bacteria [1,2]. In addition, photosynthetic bacteria can have a variety of metabolic modes, including photoautotrophy, photoheterotrophy, chemoautotrophy, and chemoheterotrophy, and have a strong ability to adapt to their environment [3]. Photosynthetic bacteria grow quickly and are non-toxic and rich in a variety of physiologically active substances, so they are widely used in sewage treatment, livestock and poultry breeding and aquaculture [4–7]. In addition, some studies have found that PSB GJ-22 can induce systemic resistance in plants [8,9]. The application of photosynthetic bacteria can increase the germination rate of seeds, promote plant growth and increase yields [10,11]. Basak et al. (2007) found that the fermentation broth of photosynthetic

bacteria can reduce the amount of ammonia, nitrogen, and oxygen in water, thus improving the quality of aquatic products [12]. Su et al. (2017) found that *Rhodopseudomonas palustris* PSB-06 could effectively control TMV [13].

Tomato chlorosis virus (ToCV) is a Crinivirus (from the Closteroviridae family) with a genome of positive-sense single-stranded RNA [14]. Tomato plants and other economically important crops are affected by ToCV [15]. The virus was first discovered in Florida in the United States and then spread widely around the world [16–20]. ToCV cannot be transmitted by sap friction like other viruses; it can only be transmitted by insect vectors [21]. The main vector, the whitefly *B. tabaci*, transmits ToCV in a semipersistent manner [1,22]. Currently, there is no effective agent to control ToCV, and *B. tabaci* has been found to be resistant to a variety of insecticides [23].

Rhodopseudomonas palustris (*R. palustris*) has been reported to improve plant growth and substantially improve agricultural production [24–26]. A preliminary study found that photosynthetic bacteria-06 (PSB-06), a member of the purple nonsulfur bacteria can prevent and control ToCV in field experiments. Based on the effectiveness of control by PSB-06 in field experiments, we hypothesized that PSB-06 is effective at controlling infection with ToCV by promoting plant defense, reducing the amount of ToCV in tomato plants and reducing the efficiency of *B. tabaci* MED at transmitting ToCV. To verify this hypothesis, we first explored the content of ToCV to choose the optimal time to apply PSB-06. Secondly, we studied the effects of PSB-06 on plant defense pathways. Third, we studied the effects of PSB-06 on plant photosynthetic pathways. Finally, we investigated the indirect effect of PSB-06 on the acquisition and transmission of ToCV by *B. tabaci* MED. The results of this experiment will provide theoretical support and new ideas for the effective and green control of *B. tabaci* MED and ToCV.

2. Materials and Methods

2.1. Plants and Insects

Healthy *B. tabaci* MED and ToCV-infected *B. tabaci* MED colonies were established [27,28]. Healthy *B. tabaci* MED were maintained in whitefly-proof screen cages that contained healthy tomato plants, and infected *B. tabaci* MED were placed in whitefly-proof screen cages that contained ToCV-infected tomato plants. Tomato plants were maintained at a temperature of 26 ± 2 °C and 60 ± 10% humidity. To ensure that the colony remained infectious, biotype identification was performed every other month based on CAPS-PCR technology to amplify the mitochondrial cytochrome oxidase I gene (*mt COI*) [29] (Table 1).

Tomato plants (*Solanum lycopersicum* Mill. Cv. Zuanhongmeina) were cultured in a greenhouse with a temperature of 26 ± 2 °C, relative humidity of 60 ± 10%, and a photoperiod of L, 16 h: d 8 h [30]. The plants were not exposed to *B. tabaci* MED or pesticides and were placed in whitefly-proof screen cages [31]. After 40 days of growth, tomato seedlings were used for inoculation. ToCV-infected *B. tabaci* MED were collected and placed in clip-cages (50/cage), which were attached to healthy tomato plants at the three-true leaf stage. After an inoculation access period (IAP) of 48 h, the clip-cages were removed, and the tomato plants were grown without *B. tabaci* MED [32]. After 40 days, the new leaves of the tomato plants were collected and RT-PCR analysis was conducted using gene-specific primers of the ToCV heat shock protein 70 homolog (HSP70h) to detect whether they were infected with ToCV (Table 1) [33].

R. palustris PSB-06 (CCTCC NO: M2012518) was cultivated at the Hunan Plant Protection Institute (28°12′29.81″ N, 113°5′35.83″ E), Changsha, China [34].

Table 1. Primer pairs.

Gene	Description	Primer Sequence (5′-3′)
mt COI	amplification of *mt COI*	F: CTGAATATCGRCGAGGCATTCC R: TTGATTTTTTGGTCATCCAGAAGT
HSP70h	ToCV RT-PCR	F: GGTTTGGATTTTGGTACTACATTCAGT R: AAACTGCCTGCATGAAAAGTCTC
ToCV-q	ToCV qPCR	F: TTGTTCCTCTTTGGGTTTC R: CGAATCTCCCTGGGTATC
ACT	tomato plant reference gene	F: AGGCAGGATTTGCTGGTGATGATGCT R: ATACGCATCCTTCTGTCCCATTCCGA
UBI	tomato plant reference gene	F: TCGTAAGGAGTGCCCTAATGCTGA R: CAATCGCCTCCAGCCTTGTTGTAA
Actin	*B. tabaci* reference gene	F: CGCTGCCTCCACCTCATT R: ACCGCAAGATTCCATACCC
EF-1α	*B. tabaci* reference gene	F: TAGCCTTGTGCCAATTTCCG R: CCTTCAGCATTACCGTCC
CHLH	qPCR	F: GCTTTGGACCCACAGGCTAT R: CTGTGCCAACGACTCTCCAT
CHLM	qPCR	F: AAGAAGGTGCCATTGTATCAG R: CCATCCAAACTCTCCAAGTC
POR	qPCR	F: GCATCACATTTGCCTCCCTA R: GAGTTCTTGTTCCAGCTCCAGTAC
PAO	qPCR	F: CGAAATTGGCTTAGACGGCAT R: ATCTGTCCATCATCTGGCGTT
PPH	qPCR	F: TGAGGTAACAGAACACCCTGC R: TCATTCGACACCCAGTCAGTG
SGR1	qPCR	F: ACTAGAAGGAAATGCAAGAAGAATCA R: GCAACTTTCCTGGATGCTTTTC
Cab7	qPCR	F: TAGACTTGCTATGTTAGCCGTTATG R: TTCTGCTTCTCACTTGGGACTG
rbcS	qPCR	F: TGCTCAGCGAAATTGAGTACCTAT R: AACTTCCACATGGTCCAGTATCTG
LHCA1	qPCR	F: GATGCCGGTCTACGTTGGAG R: AATCCAAAATCTCCGGGGGC

2.2. Time of Application of PSB-06

The cultured PSB-06 (6.66 mg/L) used to treat the leaves of plants was sprayed every day, for 3 days, 5 days and 7 days. Simultaneously, the control group treated with water was used to treat the plants that had been subjected to the virus for 40 days. After 15 days, three-true leaves of the tomato plant were taken, and the content of ToCV of the tomato plants was detected by RT-qPCR. According to the DNA sequence of the HSP70 gene with ToCV-qF and ToCV-qR as primers (Table 1), target DNA fragments were cloned. When the positive clones were completely correct, plasmids were extracted with the TIANprep Mini Plasmid Kit (Tiangen Biotech Co., Ltd., Beijing, China). After this, their concentrations and OD values were determined with an ultraviolet spectrophotometer. Based on the following formula, the copy number was determined: Copy number (copies/μL) =

$$\frac{\text{Nucleic acid concentration } (\text{g}/\mu\text{L}) \times 6.02 \times 10^{23} (\text{copies/moL})}{660(\text{g/moL}) \times \text{Fragment length(bp)}}$$

Next, the copy number of plasmids was calculated. The plasmids were diluted with DNase/RNase-free water according to 10-times gradient dilution. Then the plasmids were analyzed by RT-qPCR referring to ChamQ Universal SYBR qPCR Master Mix Kit (Vazyme Biology Co., Ltd., Nanjing, China). Finally, the obtained Ct values were used to calculate the standard curve of ToCV. Based on the standard curve of ToCV, the content of ToCV in the tomato plants was calculated.

In addition, the expression of magnesium chelatase H subunit (CHLH) in the chlorophyll development pathway, STAYGREEN1 (SGR1) in the chlorophyll metabolic pathway and chlorophyll-a/b binding7 (Cab7) gene in the photosynthesis pathway were detected. The optimal treatment time was selected based on the relevant expression level, ToCV content and actual cost. The total RNA of the tomato plants was extracted using TRIzol (TransGen Biotech Co., Ltd., Beijing, China). The first chain of cDNA was synthesized using the manufacturer's instructions for Hiscript® II Q RT SuperMix for qPCR (+g DNA wiper) (Vazyme Biology Co., Ltd., Nanjing, China) [35]. ChamQ Universal SYBR qPCR Master Mix using Hiscript® II Q RT SuperMix was used for qPCR (Vazyme Biology Co., Ltd., Nanjing, China) [36]. Each treatment was repeated six times.

2.3. Effect of PSB-06 on Plant Defense Pathways

2.3.1. Effects of PSB-06 on the SA and JA Pathways

After the tomato plants' leaves were sprayed with PSB-06 solution every 5 days, three-true leaves of the tomato plants were collected on days 15, 30, 45 and 60 after treatment and quickly frozen with liquid nitrogen. A grinder (MM 400; Verder Shanghai Instruments and Equipment Co., Ltd., Shanghai, China) was used to grind the plant leaves (0.5 g) at a frequency of 30 Hz for 30 s and they were then transferred to 5 mL microtubules. A total of 3 mL 90% precooled methanol and ddH_2O was added, and then the homogenate was centrifuged at 7500× *g* for 10 min. The supernatant was transferred to a new microtube, and the remaining particles were resuspended in 2 mL 100% methanol and vortexed before centrifugation at 7500× *g* for 10 min. The supernatant was mixed with the supernatant from the first centrifugation, dried under vacuum, and then dissolved in 1.5 mL 5% trichloroacetic acid [37]. After centrifugation, the supernatant was extracted again with the same volume of ethyl acetate and cyclohexane. The extract was dried, resuspended with 3 mL 70% methanol and loaded onto a C18 column for collection. After evaporation, 500 mL of acetonitrile was added and filtered through a 0.22-μm PTFE filter membrane. All samples were analyzed for SA and JA using HPLC (Agilent Technologies Inc., Santa Clara, CA, USA). Tomato plants treated with water were used as the control group. Each process was conducted in triplicate.

2.3.2. Effects of PSB-06 on the Activities of Enzymes Related to Plant Defense

After the ToCV-infected tomato plants' leaves were sprayed with PSB-06 every 5 days, three-true leaves of tomato plants were collected after 45 days of treatment and tested for CAT, POD, and SOD activities (Solarbio Biology Co., Ltd., Beijing, China). Tomato plants treated with water were used as the control group. Each assay was repeated six times.

2.4. Effect of PSB-06 on Plant Photosynthesis-Related Pathways

2.4.1. Effects of PSB-06 on Plant Chlorophyll and Nitrogen Content

After the ToCV-infected tomato plants' leaves were sprayed with PSB-06 every 5 days, the contents of chlorophyll and nitrogen of the three-true leaves of the tomato plants were measured using the OK-Y104 chlorophyll meter (Zhengzhou Okoqi Instrument Manufacturing Co., Ltd., Zhengzhou, China) on days 15, 30, 45, and 60 after treatment. Each treatment was conducted in triplicate.

2.4.2. Effects of PSB-06 on Chlorophyll Development, Chlorophyll Metabolism and the Photosynthetic Gene Expression in Plants

After the ToCV-infected tomato plants' leaves were sprayed with PSB-06 for 5 days, three-true leaves of tomato plants were collected after 45 days. ACT and UBI were used as internal reference genes [37] (Table 1), and ChamQ Universal SYBR qPCR Master (Vazyme Biology Co., Ltd.) was used for fluorescence quantitative PCR. The expression of CHLH, Mg protoporphyrin IX methyltransferase (CHLM), and protoporphyllide reductase (POR) relating to the chlorophyll development pathway was monitored. The expression of pheophorbide a oxygenase (PAO), pheophytin pheophorbide hydrolase (PPH), and SGR1

related to the chlorophyll metabolic pathway and Cab7, light-harvesting chlorophyll-A1 (LHCA1), and the small subunit of RUBISCO (rbcS) related to photosynthesis was measured [38]. Each process was conducted in triplicate.

2.5. The Indirect Effects of PSB-06 on the Acquisition and Transmission of B. tabaci MED

2.5.1. The Effect of PSB-06 on the Acquisition of *B. tabaci MED*

Healthy *B. tabaci* MED were placed in clip-cages (50/cage) and starved for 2 h. The clip-cages were then attached to ToCV-infected tomato plants that had been sprayed with PSB-06 for 45 days. The content of ToCV obtained by *B. tabaci* MED was determined after an acquisition access period (AAP) of 48 h. Simultaneously, healthy *B. tabaci* MED were placed on the ToCV-infected tomato plants and the controls. Each process was repeated six times.

2.5.2. The Effect of PSB-06 on the Transmission of *B. tabaci* MED

After a 48 h AAP, *B. tabaci* MED was collected in clip-cages (50/cage), which were attached to healthy tomato plants (one plant/cage). After the 48 h inoculation access period (IAP), the clip-cages were removed, and the plants were grown normally. At 30, 45 and 60 days, the ToCV that had accumulated in tomato plants was measured. Each treatment was repeated six times.

2.6. Data Analysis

All of the data analyses were performed by SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). The relevant expression level and ToCV content to select the optimal treatment time were analyzed using a one-way ANOVA followed by the tests of Tukey at $p < 0.05$. Independent samples *t*-test was used to compare the effect of PSB-06 on the SA and JA pathways, chlorophyll development, chlorophyll metabolism and photosynthetic gene expression in plants, and the acquisition and transmission of ToCV by *B. tabaci* MED.

3. Results

3.1. Time of Application of PSB-06

The content of ToCV in tomato plants treated with PSB-06 once per day or once every 3 days, 5 days, or 7 days was significantly lower than that in tomato plants treated with water (the control) (Figure 1A; $F_{1,6} = 28.129$, $p < 0.001$). The relative levels of expression of CHLH, SGR1 and Cab7 in tomato plants treated with PSB-06 were significantly higher than those in the control (Figure 1B–D; $F_{1,6} = 15.581$, $p < 0.001$ for *CHLH*, $F_{1,6} = 30.405$, $p < 0.001$ for SGR1 and $F_{1,6} = 56.872$, $p < 0.001$ for Cab7). Simultaneously, the content of ToCV was the lowest in tomato plants treated with PSB-06 each day, and the relative expression of CHLH, SGR1 and Cab7 was the highest. Subsequently, the content of ToCV in tomato plants treated with PSB-06 every 3 days, 5 days, or 7 days increased gradually, while the relative levels of expression of CHLH, SGR1 and Cab7 decreased gradually. There was no significant difference between the tomato plants treated with PSB-06 every 5 days and 3 days. Therefore, to consider the cost and effectiveness, it is more suitable to use PSB-06 to treat the tomato plants every 5 days.

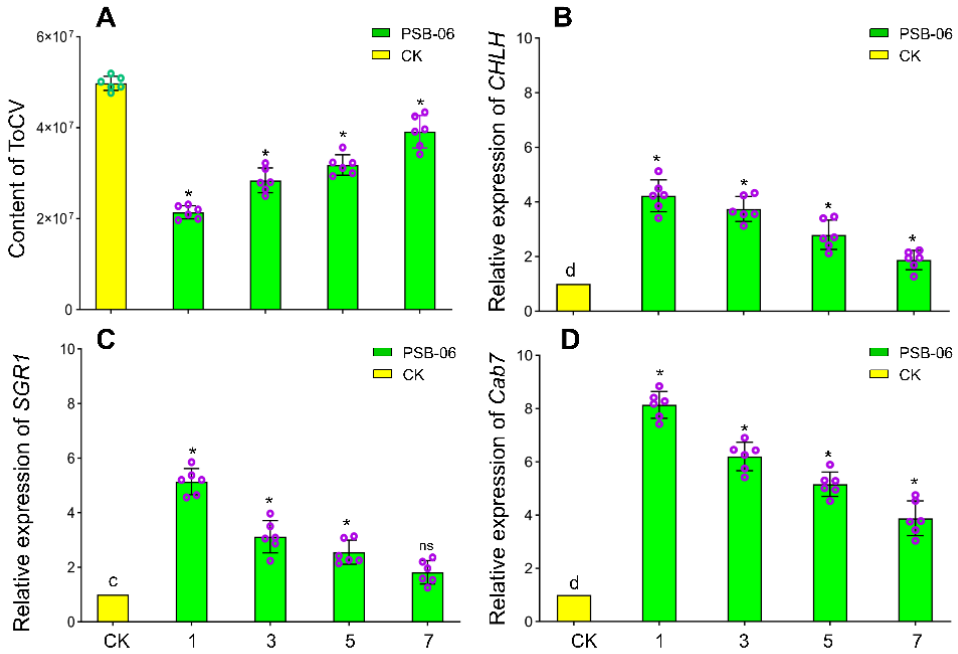

Figure 1. Gene expression in tomato plants after spraying PSB-06 at different times. ToCV accumulation in tomato plants (**A**). The relative expression of CHLH in the pathways of chlorophyll development (**B**). The relative expression of SGR1 in the chlorophyll metabolic pathway (**C**). The relative expression of Cab7 in photosynthesis (**D**). The X-axis indicates the different situations that the tomato plants were treated. CK: The tomato plants treated with water. 1: The tomato plants treated with PSB-06 daily. 3: The tomato plants treated PSB-06 every 3 days. 5: The tomato plants treated PSB-06 every 5 days. 7: The tomato plants treated PSB-06 every 7 days. Data are the means ± SE. Different letters on the bars indicate the significant difference ($p < 0.05$). * $p < 0.05$; ns, not significant. SE, standard error; ToCV, Tomato chlorosis virus.

3.2. Effect of PSB-06 on Plant Defense Pathways

3.2.1. Effects of PSB-06 on the Salicylic Acid and Jasmonic Acid Pathways

After ToCV-infected tomato plants were treated with PSB-06 every 5 days, the content of jasmonic acid (JA) in the tomato plants increased gradually on days 15, 30, 45, and 60 (Figure 2A; $F_{1,3} = 139.981$, $p < 0.001$), while the content of salicylic acid (SA) did not change significantly (Figure 2B; $F_{1,3} = 1.683$, $p = 0.119$). The content of JA in the tomato plants treated with PSB-06 was higher than those of the control. Thus, PSB-06 treatment increased the content of JA in the tomato plants, which promoted the ability of the plants to defend.

3.2.2. Effects of PSB-06 on the Activities of Enzymes Related to Plant Defense

After the tomato plants were treated with PSB-06 every 5 days, the activities of catalase (CAT) (Figure 2C; $F_{1,6} = 1.017$, $p < 0.05$), peroxides (POD) (Figure 2D; $F_{1,6} = 0.004$, $p < 0.01$), and superoxide dismutase (SOD) (Figure 2E; $F_{1,6} = 5.121$, $p < 0.05$) increased significantly in the tomato plants on day 45.

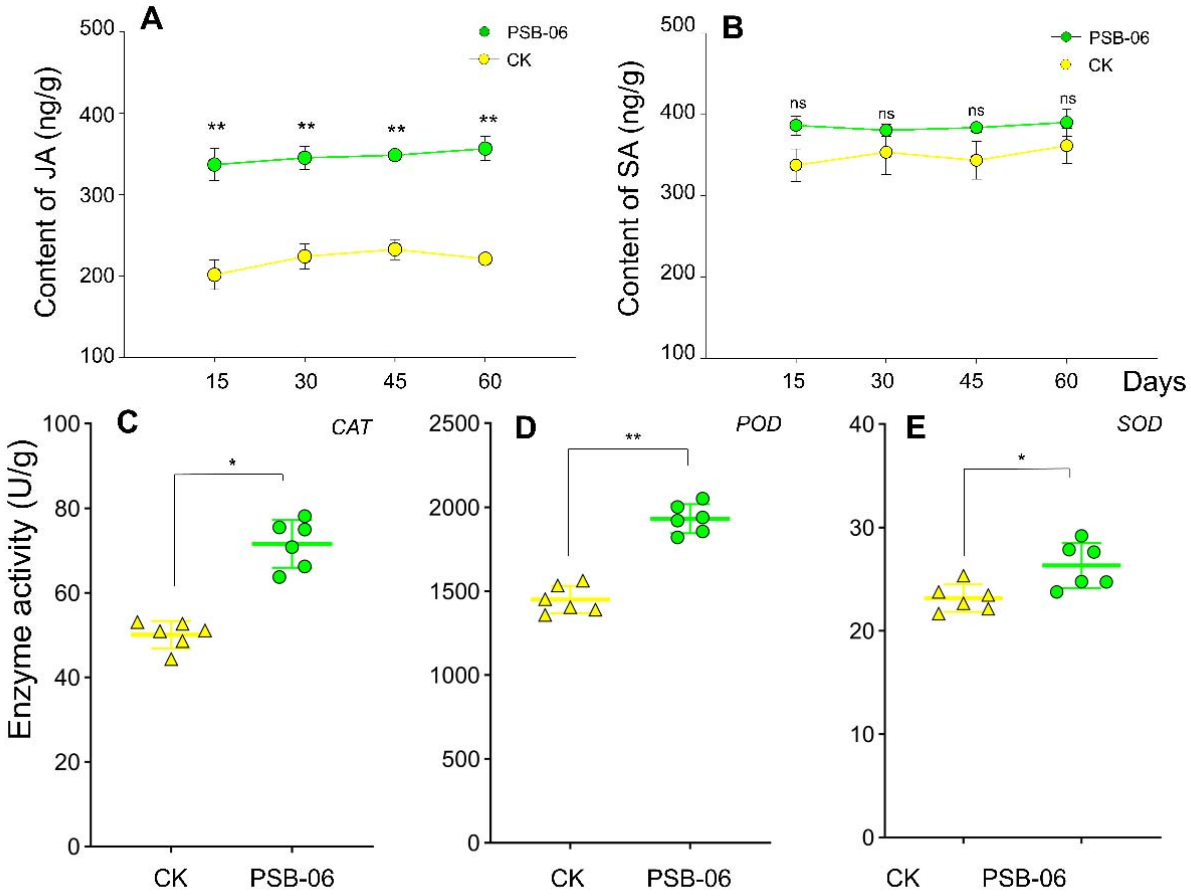

Figure 2. Effect of PSB-06 on plant defense pathways. The content of jasmonic acid (ng/g) in tomato plants at 15, 30, 45, and 60 days (**A**). The content of salicylic acid (ng/g) in tomato plants at 15, 30, 45, and 60 days (**B**). The activity of catalase in tomato plants following treatments of PSB-06 for 45 days (**C**). The activity of peroxidase in tomato plants (**D**). The activity of superoxide dismutase in tomato plants (**E**). CK: The tomato plants treated with water. PSB-06: The tomato plants treated with PSB-06. Data are the mean ± SE; ** $p < 0.01$; * $p < 0.05$; ns: Not Significant. SE, standard error; PSB-06, photosynthetic bacteria-06.

3.3. Effect of PSB-06 on Plant Photosynthetic Pathways

3.3.1. Effects of PSB-06 on the Contents of Chlorophyll and Nitrogen

Compared with the control tomato plants (Figure 3A), treatment with PSB-06 alleviated the chlorotic symptoms of tomato leaves at 45 days (Figure 3B). After the tomato plants were treated with PSB-06 every 5 days, the contents of chlorophyll at days 15, and 45 were higher than that of the control group (Figure 3C; $F_{1,3} = 2.575$, $p < 0.05$), but the content of chlorophyll decreased gradually with time. Simultaneously, the nitrogen content of the tomato plants did not change significantly (Figure 3D; $F_{1,3} = 0.400$, $p = 0.082$).

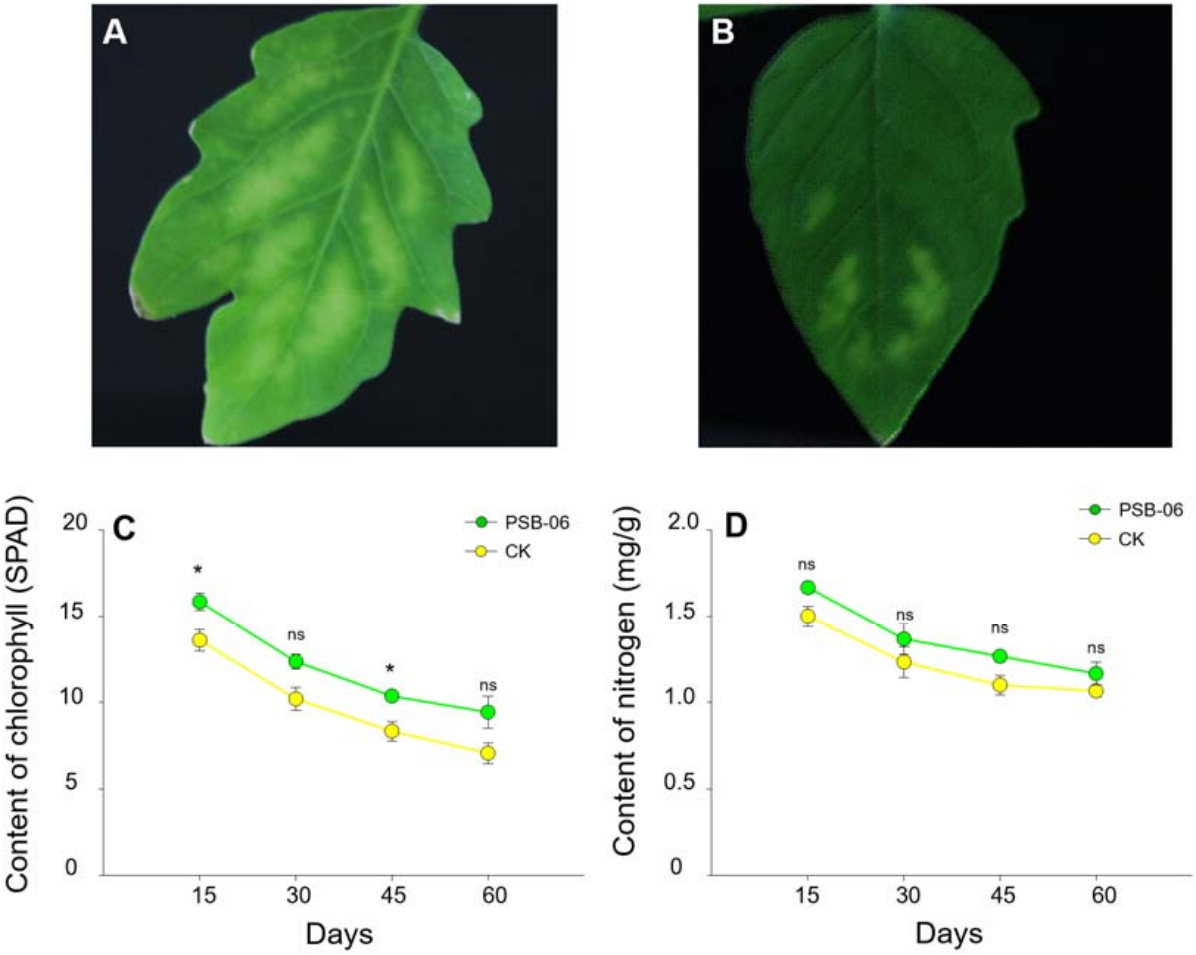

Figure 3. Effect of PSB-06 on plant photosynthesis-related pathways. ToCV-infected tomato plants treated with water after 45 days (**A**). ToCV-infected tomato plants treated with PSB-06 after 45 days (**B**). The content of chlorophyll (SPAD) in the tomato plants (**C**). The content of nitrogen (mg/g) in the tomato plants (**D**). The X-axis indicates the number (15, 30, 45, 60) of days that the tomato plants were treated. Data are the mean ± SE, and different letters on the bars indicate significant differences. * $p < 0.05$; ns, not significant. SE, standard error; ToCV, tomato chlorosis virus.

3.3.2. Effects of PSB-06 on the Development of Chlorophyll, Chlorophyll Metabolism and the Photosynthetic Gene Expression in Plants

Compared with the control tomato plants, the relative expression of CHLH related to the chlorophyll development pathway of tomato plants increased significantly after the treatment with PSB-06 for 45 days (Figure 4A; $F_{1,3} = 3.284$, $p < 0.01$). The relative expression of CHLM did not increase significantly (Figure 4B; $F_{1,3} = 3.731$, $p = 0.087$), and the relative expression of POR increased significantly (Figure 4C; $F_{1,3} = 4.331$, $p < 0.01$). The relative levels of expression of PAO, PPH and SGR1 related to the chlorophyll metabolic pathway increased significantly (Figure 4D–F; $F_{1,3} = 5.206$, $p < 0.01$ for PAO; $F_{1,3} = 4.553$, $p < 0.01$ for PPH; $F_{1,3} = 2.896$, $p < 0.05$ for SGR1); the levels of expression of Cab7, LHCA1 and rbcS related to photosynthesis also increased significantly (Figure 4G–I; $F_{1,3} = 5.057$, $p < 0.01$ for Cab7; $F_{1,3} = 5.600$, $p < 0.01$ for LHCA1; $F_{1,3} = 9.029$, $p < 0.01$ for rbcS).

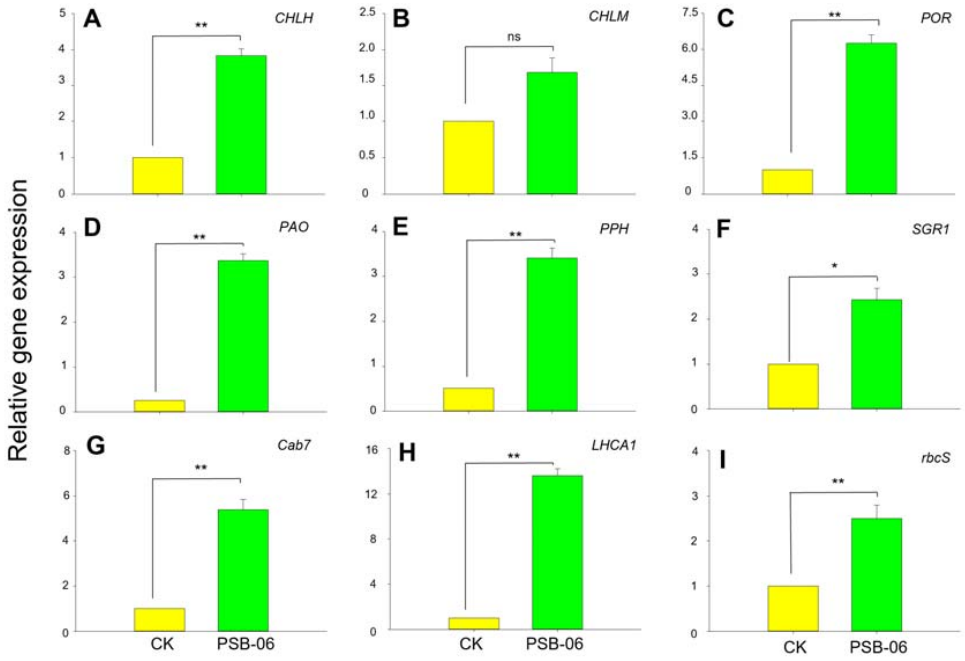

Figure 4. Treatments of PSB-06 affected the development of chlorophyll and the genes relating to chlorophyll metabolism and photosynthesis in tomato plants after 45 days. The relative expression of CHLH, CHLM and POR in the chlorophyll development pathway (**A**–**C**). The relative expression of PAO, PPH and SGR1 in the chlorophyll metabolic pathway (**D**–**F**). The relative expression of Cab7, LHCA1 and rbcS in the photosynthetic pathway (**G**–**I**). CK: The tomato plants treated with water. PSB-06: The tomato plants treated with PSB-06. Data are the mean ± SE, Different letters on the bars indicate significant differences. ** $p < 0.01$; * $p < 0.05$; ns, not significant. SE, standard error.

3.4. The Indirect Effects of PSB-06 on the Acquisition and Transmission of ToCV by B. tabaci MED

3.4.1. The Effect of PSB-06 on the Acquisition of ToCV by *B. tabaci* MED

At 48 h AAP, the ToCV accumulation in the *B. tabaci* MED-fed ToCV-infected tomato plants that had been treated with PSB-06 was 1.66×10^6 copies/μL, and ToCV accumulation in the *B. tabaci* MED-fed ToCV-infected tomato plants that had been treated with water was 1.20×10^6 copies/μL, i.e., 30% lower than the former (Figure 5A; $F_{1,6} = 4.270$, $p < 0.01$).

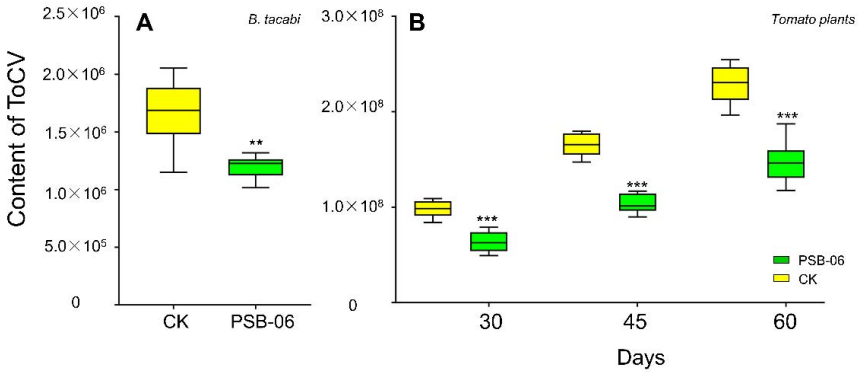

Figure 5. The effect of PSB-06 treatment on the acquisition and transmission of ToCV by *B. tabaci* MED. The acquisition of ToCV by *B. tabaci* MED at 48 AAP (**A**). The content of ToCV in the tomato plants exposed to ToCV-infected *B. tabaci* MED (**B**). In (**B**), X-axis indicates the number (30, 45, 60) of days that the tomato plants had been infested when ToCV accumulation was determined. CK: The tomato plants treated with water. PSB-06: The tomato plants treated with PSB-06. Data are the mean ± SE, Different letters on the bars indicate significant differences. *** $p < 0.001$; ** $p < 0.01$. SE, standard error.

3.4.2. The Effect of PSB-06 on the Transmission of *B. tabaci* MED

The content of ToCV in the tomato plants in the treatment group was lower than that in the control group. At 30 days IAP, the content of ToCV in the tomato plants exposed to ToCV-infected *B. tabaci* MED that had been treated with PSB-06 was 9.82×10^7 copies/μL, and the content of ToCV in the tomato plants exposed to ToCV-infected *B. tabaci* MED that had been treated with water was 6.37×10^7 copies/μL, i.e., 30% lower than the former (Figure 5B; $F_{1,3} = 0.776$, $p < 0.001$). At 60 days IAP, the content of ToCV in the tomato plants of PSB-06 treatment was 2.29×10^8 copies/μL, and the content of ToCV in the tomato plants of water treatment was 1.47×10^8 copies/μL, i.e., 35% lower than the former (Figure 5B; $F_{1,3} = 0.005$, $p < 0.001$). These results showed that PSB-06 treatment could indirectly inhibit the accumulation of ToCV in tomato plants.

4. Discussion

To date, there are no tomato varieties that are resistant to ToCV, and the control of ToCV primarily depends on the control of insect vectors by insecticides and the reduction of old plants and weeds infected with ToCV in and close to the cultivated area [22]. The main measure to control *B. tabaci* is the application of insecticides. Maluta et al. (2020) [39] found that flupyradifurone may decrease the survival of insects to potentially reduce the transmission of ToCV into tomato plants. Insecticides can reduce the spread of plant virus diseases by insect vectors [40,41]. Although insecticides are highly effective at inhibiting the transmission of viral diseases, there will be problems with resistance. Simultaneously, ToCV has a long incubation period of more than 20 days [42]. Thus, insecticides cannot kill viruliferous *B. tabaci* before they have transmitted the virus to plants [43]. In this study, we used PSB-06 to treat ToCV-infected tomato plants every 5 days for nine times, and the incidence of disease in the tomato plants decreased significantly. The accumulation of ToCV in the tomato plants exposed to *B. tabaci* also decreased significantly. The use of photosynthetic bacteria can reduce the insecticide resistance of *B. tabaci* to insecticides and achieve the purpose of a green and sustainable control of plant diseases [44].

Photosynthetic bacteria are widely used to improve water quality in aquaculture [45]. Fewer reports have focused on the use of photosynthetic bacteria to manage the transmission of viruses. In this study, the cultured PSB-06 (6.66 mg/L) were used to treat the plants every day, every 3 days, 5 days and 7 days. We found that there was no significant difference in the tomato plants treated with PSB-06 every 3 days and every 5 days. Therefore, to reduce the cost of treatment yet remain effective, it is more suitable to use PSB-06 to treat the tomato plants every 5 days. This will provide practical applications for the prevention and control of ToCV in the future.

Maurhofer et al. (1998) [46] revealed that *Pseudomonas fluorescens* strain P3 can express SA biosynthetic genes to improve resistance to the tobacco-to-tobacco necrosis virus. Amutharaj et al. (2013) [47] reported that *P. fluorescens*, which produces iron cells, can significantly increase the activity of POD of rice plants after treatment, thereby partially controlling rice blast (*Magnaporthe grisea*). The *Bacillus amyloliquefaciens* strain Bs006 and *P. fluorescens* strain Ps006 promoted growth, stress and defense [48]. Under the conditions of applying PSB-06, the activities of the antioxidant enzymes CAT, POD and SOD involved in the stress responses of tomatoes increased significantly, which indicated that treatment with PSB06 could improve the ability of tomato plants to resist stress. Li et al. (2014) [49] found that the suppression of JA led to the attraction of whiteflies. In this study, the content of JA in the tomato plants increased, inducing the JA pathway and causing the repulsion of whiteflies, which indirectly reduced the transmission of the virus.

Plant growth-promoting rhizobacteria (PGPR) can promote photosynthesis, and bacterial inoculation significantly, enhancing the concentration of chlorophyll [50]. *P. fluorescens* Sasm05 produces indole acetic acid (IAA) to promote plant growth and the concentration of leaf chlorophyll and enhance photosynthesis [51]. *P. fluorescens* N 21.4 increased photosynthesis and activated the expression of pathogenesis-related protein genes to promote plant immunity and improve plant fitness [52]. Meng et al. (2018) [53] reported that protochloro-

phyllide reductase (POR) and magnesium chelatase H subunit (CHLH) are important to the development and synthesis of chloroplasts in tomato fruit. Perveen et al. (2020) [54] found that the overexpression of mEmBP-1 led to an increase in the expression of light-harvesting chlorophyll-A (LHCA) and light-harvesting chlorophyll-B (LHCB) genes in photosynthesis, thereby increasing the photosynthesis and yield of rice fields. After applying PSB-06, the content of chlorophyll in the tomato plants increased, and the content of nitrogen also increased, which improved photosynthesis and significantly increased the expression of chlorophyll synthesis and photosynthetic genes. Under stress conditions, the accumulation of chlorophyll may activate the chlorophyll degradation pathway [38]. In this study, we also found that the chlorophyll synthesis of the tomato plants increased, and PSB-06 increased photosynthesis and activated the expression of pathogenesis to promote plant immunity and improve plant fitness. Thus, the attraction of the whiteflies to the tomato plants was reduced, which indirectly reduced the transmission of virus.

Su et al. (2017) [13] showed that *R. palustris* GJ-22 can promote resistance to plant viruses. After inoculation with GJ-22 in plants, the accumulation of TMV in tobacco decreased, but it is unclear whether it induces the mechanism of resistance to plant viruses in tobacco. The JSC-3b strain of *R. palustris* can inhibit TMV [25]. *Streptomyces thermocarboxydus* isolate BPSAC147 plays a significant role in plant growth to control Fusarium wilt disease and photosynthetic metabolism to enhance productivity [26]. In this study, after tomato plants were inoculated with *R. palustris* PSB-06, plant defense pathways were induced and photosynthesis was enhanced. In addition, the acquisition of ToCV and transmission of *B. tabaci* decreased. This is consistent with the findings of Koh et al. (2007) [24], that plants inoculated with photosynthetic bacteria can enhance photosynthesis, which acts as a biofertilizer to promote plant growth and inhibit pathogenic microorganisms, thus enhancing the resistance to plant disease. However, this study indicated the JA signaling pathways and processes relating to plant defense after using PSB-06 treatment, but the mechanism of action of *R. palustris* requires further analysis.

5. Conclusions

Currently, ToCV is found widely around the world. The management of the disease caused by ToCV has primarily focused on the reduction of whitefly populations using insecticides. However, the control of ToCV has presented some serious problems, such as insecticide resistance and environmental damage. The application of PSB-06 provides an efficient and environmentally friendly way to prevent and control ToCV. In this study, we verified that PSB-06 stimulated the plant defense and photosynthetic pathways of tomato plants, and the acquisition and transmission of *B. tabaci* MED were reduced, thereby alleviating the chlorotic symptoms of ToCV and reducing its transmission. This reduction helps to control *B. tabaci* MED and thus, the spread of ToCV.

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