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Rapid Construction and Application of a Vector for Tobacco Ringspot Virus-Induced *McPDS* Silencing in Bitter Gourd

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Abstract: The aim of this study is to facilitate the construction of virus-induced gene silencing vectors and to provide a reference or positive control for gene silencing in bitter gourd. A recombinant TRSV (tobacco ringspot virus) containing two components, pTRSV1 and pTRSV2, was used in this study. The fragment of the *McPDS* target was cloned into pTRSV2 via combined enzymic ligation during digestion. The TRSV components were agro-infiltrated into tobacco leaves to grow virus particles, which were then extracted and mechanically inoculated into the bitter gourd plants. The effect of TRSV-*McPDS*-mediated *McPDS* gene silencing was evaluated by observing the photo-bleaching phenotype, detecting the TRSV virus, and quantifying the downregulation of *MCPDS* gene expression and chlorophyll contents. The results showed that all bitter gourd plants infected with the empty TRSV or TRSV-*McPDS* virus grew and developed normally, with no visible signs of viral disease. However, after seven days of inoculation, only the bitter gourd plants that were inoculated with TRSV-*McPDS* showed obvious photobleaching in the leaves, stems, and buds. The TRSV-specific fragments were tested out in the systemically infected leaves of bitter gourd. The transcription level of the *McPDS* gene in the leaves dropped by 84.7%. The chlorophyll content also dropped significantly. These data suggest that the rapidly constructed VIGS vector TRSV-*McPDS* successfully induced *McPDS* silencing in bitter gourd. Taken together, the results of this study provide a practical method for vector construction in various VIGS applications, as well as a reference and a positive control for TRSV-induced gene silencing in bitter gourd.

Keywords: bitter gourd; virus-induced gene silencing; TRSV; phytoene dehydrogenase

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1. Introduction

Bitter gourd (*Momordica charantia*) is an economically important vegetable crop. It is native to Africa but was first domesticated and widely cultivated in tropical and subtropical Asia [1,2]. As a crop full of nutrients and active ingredients, like polysaccharides, terpenes, saponins, and peptides, bitter gourd is also known as a popular functional vegetable with anti-cancer, anti-virus, and hypoglycemic effects [3]. In 2016, the genome sequence of a monoecious bitter gourd inbred line, OHB3-1, was drafted with 45,859 predicted protein-coding genes; however, these genes were not anchored to chromosomes [4]. In 2021, the first chromosome-level bitter gourd genome was assembled through the whole-genome sequencing of the cultivar Dali-11 and the wild small-fruited line TR. The number of predicted protein-coding genes in Dali-11 and TR was 26,427 and 28,827, respectively [1]. In 2022, the telomere-to-telomere genome of a bitter gourd variety Mca was assembled with

19,895 predicted protein-coding genes [2]. These published genomic resources provide a good basis for understanding bitter gourd's functional genomics and for producing genetic improvements in stress resistance and quality. However, genetic transformation takes a long time and is not very effective [5], thus seriously affecting the identification of functional genes and their applications in the molecular breeding of many crops, including bitter gourd [6,7].

Virus-induced gene silencing (VIGS) technology is valuable for studying gene functions. VIGS is developed by constructing a weakly infectious recombinant viral vector that carries a target fragment of the interested gene-coding sequence and infects plants. The virus's replication, transcription, and transfer in the plant cells produce small interference RNA (SiRNA) specifically matching the target gene. This induces the target mRNA to degrade or change its methylation, which stops the target gene from being translated normally [8]. Using VIGS technology, researchers can quickly figure out the function of a target gene by comparing the number of its downregulated transcripts to changes in the plant's biochemistry and physiology [1–11]. This method does not require stable genetic transformation, and it is easy to use when aiming to study gene functions in a short amount of time and at a low cost [12,13]. VIGS technology is successfully being used in an increasing number of horticultural crops, such as tomato, cabbage, strawberry, banana, and cucurbits, like cucumber, melon, watermelon, and pumpkin [14–21], but not yet in bitter gourd.

For VIGS technology to work, a suitable viral expression vector and a good reporter gene that can indicate the silencing effect must be prepared. Kumagai et al. [22] successfully set up the first implementation of VIGS by using the tobacco mosaic virus (TMV) as a vector and phytoene dehydrogenase (*PDS*) as a reporter gene. *PDS* encodes an enzyme necessary for carotenoid synthesis and plays a photoprotective role in chloroplasts. The tobacco plants showed a clear *PDS*-silencing-associated photo-bleaching phenotype because the carotenoid synthesis and the photoprotection to chloroplasts were affected, leading to chlorophyll degradation and photo-bleaching [22,23]. As their phenotypes are visible and easy to distinguish, *PDS* genes of individual plants are also employed as reporter genes of the gene silencing induced by several different virus vectors in the established VIGS techniques for cucumber, watermelon, melon, and pumpkin [14,24–26].

Viral vector construction is necessary for VIGS to be performed. The process generally includes the following procedures: (1) designing and synthesizing specific primers with 5′-specific restriction enzyme sequences; (2) amplifying the target fragment via PCR; (3) separating the PCR product via agarose electrophoresis before cutting the gel to recover the target fragment; (4) cleaving the purified target fragment and the viral empty vector with a specific restriction endonuclease; (5) separating the cleaved target fragment and linearized viral empty vector via agarose electrophoresis, and then cutting the gel to recover the cleaved target fragment and the cleaved empty vector; (6) mixing the purified cleaved target fragment and the cleaved linearized empty vector in a certain proportion and making a ligation using ligase; (7) transforming the product of ligation reaction into *E.coli* to screen the positive clones; and finally, (8) performing PCR identification and sequencing validation of positive clones. Obviously, these procedures are relatively time-consuming and inefficient, despite being commonly performed to construct various viral vectors [6,9,14,16,18,19,22,23,27,28]. Thus, simplifying the current method for viral vector construction would be meaningful for all VIGS applications.

As discussed above, the *PDS* gene is commonly employed in plant VIGS. So, hopefully, *MCPDS* silencing can quickly identify the visible albino phenotype in bitter gourd, making it easy to judge the silencing effect accurately. Furthermore, we also hope that TRSV-MCPDS can be used as a positive control for future VIGS investigations in bitter gourd. Thus, in this study, we select the *McPDS* gene as a target to perform TRSV-*McPDS* vector construction and TRSV-*McPDS*-induced gene silencing in bitter gourd.

2. Materials and Methods

2.1. Plant Materials and Virus Expression Vector

Bitter gourd (cultivar Cuilv III) and *Nicotiana benthamiana* plants were grown in a controlled growth chamber at 25 °C day/22 °C night with a 12/12 h (light/dark) photoperiod. The TRSV-based gene silencing vector including two parts, pTRSV1 and pTRSV2, was engineered by Fang et al. (2021) and kindly shared by Prof. Geng Chao of Shandong Agricultural University. The genome of TRSV consists of two single-stranded RNA, RNA1 and RNA2. The engineered pTRSV1 and pTRSV2 were generated by cloning TRSV genomic RNA1 and RNA2, respectively, into the binary vector pCB301 downstream of a double cauliflower mosaic virus (CaMV) 35S promoter. RNA1 encodes a polyprotein precursor that can be cleaved into five mature proteins, while RNA2 encodes a polyprotein that can be cleaved into 2a, movement protein (MP), and coat protein (CP). The *Sna* BI restriction enzyme sequences downstream of the CP translational stop codon were introduced into TRSV RNA2 in order to insert a single target fragment to induce gene silencing [5].

2.2. Alignment Analysis of PDS Genes in Cucurbits

The PDS gene-coding sequences (CDS) of cucumber (CsaV3_4G002690/CsaPDS), watermelon (Cla97C07G142100/ClaPDS), melon (MELO3C017772/MELOPDS), pumpkin (CmaCh14G014300/CmaPDS.1, CmaCh06G016940/CmaPDS.2; CmoCh06G016860/CmoPDS.1, CmoCh14G014660/CmoPDS.2), wax gourd (Bhi01G002855/BhiPDS), bottle gourd (HG10002111/HGPDS), and bitter gourd (MC00g0783/McPDS) were downloaded from CuGenDBv2 [29]. With these sequences, the alignment analyses were performed using BioEdit software (v7.2.6.1), and the suitable conserved fragments in McPDS sequences were selected to construct the pTRSV2-McPDS vector.

2.3. Sequence and Primer Design

The following primers were designed using Primer premier 5.0 software (Table 1).

Table 1. The primers used in this study.

Primer ID	Sequence	Description
McPDS-F	TTTACGTATGCACCCATAAATGGGATA	PCR primer for *McPDS* target cloning
McPDS-R	CCTACGTACATCGGGGTTAATGAAGTT	
McActin-F	AATGGGTATGGTCTGCAAGT	qRT-PCR primer for reference gene
McActin-R	GGAGAATGTTTCAAGAGGGTAG	
q McPDS-F	TCAGCCGATTTGATTTCC	qRT-PCR primer for *McPDS*
q McPDS-R	ACATAAGATTGCCCACCA	
pTRSV2-F	ATTTTCTTAGGTTCTTATGCTGG	PCR primer for Specific fragment of TRSV2
pTRSV2-R	TAAGGGCAGGAACCTAAAAC	

2.4. Constructing pTRSV2 Target (McPDS) Vector Using an Improved Method

Extraction of total RNA from bitter gourd leaves and synthesis of cDNA were carried out in accordance with the instructions of the TransZol up plus RNA Kit and the Transcript® One-Step gDNA Removal and cNDA Synthesis Super Mix (Beijing All-Style Gold Biotechnology Co., Ltd., Beijing, China). The specific *McPDS* target fragment was amplified via PCR using the cDNA as a template and the primers *McPDS*-F and *McPDS*-R containing restriction site adaptor, and then recovered. To connect the recovered PCR product of interest into the pTRSV2 vector, a simpler method of combined enzymic ligation during digestion was developed. Each reagent was added to one PCR tube as follows: pTRSV2 vector, 150 ng; recovered PCR product of gene target, 200 ng; T4 DNA ligase (NEB, M0202S), 0.5 μL; rCutSmart buffer (NEB, B6004S), 1 μL; SnaBI (NEB, R0130L), 1 μL; and finally, nuclease-free water to a total volume of 10 μL. The digestion/ligation reaction

solution was incubated in a PCR machine employing the following program: step 1: 37 °C for 5 min; step 2: 16 °C ligation for 5 min; step 3: go back to step 1 for 25 cycles; step 4: storage at 4 °C before transformation into DH-5 *E. coli*. Positive clones are then selected with LB solid medium containing 50 mg L^{-1} kanamycin, identified via PCR, and further confirmed to have the correct insert via sequencing.

2.5. TRSV Cultured in Host and Inoculation

Before being inoculated into bitter gourd, TRSV was cultured in host *N. benthamiana* leaves, as described by Fang et al. [6], with some modifications. GV3101 agrobacteria transformed with pTRSV1, pTRSV2, pTRSV2-McPDS, and P19 (a commonly used silencing suppressor) were cultured in LB liquid medium (containing 50 mg L^{-1} kanamycin, 25 mg L^{-1} rifampicin) at 28 °C, 180 rpm/min. The bacterium of each strain was collected via centrifugation at 4500 rpm/min for 5 min and then resuspended with an appropriate volume of agrobacterium infiltrating buffer (150 μM aceto-butyrone, 10 mM 2-morpholine ethane-sulfonic acid, 10 mM magnesium chloride hexahydrate). The concentration of each resuspension solution was adjusted to OD600 = 1.0, then proportionally mixed with pTRSV1, pTRSV2, and P19 as empty TRSV vectors or pTRSV1, pTRSV2-McPDS, and P19 as the target TRSV-McPDS vectors. Each mixture was incubated at room temperature for 1 h before being infiltrated into the *N. benthamiana* leaves. The injected tobacco plants were placed in the light incubator, where they were incubated at 25 °C for 12 h in the dark, followed by 7 days of normal incubation. The cultured virus particles in the *N. benthamiana* leaves were extracted and mixed with water and quartz sand and then used to inoculate the first true leaf of the bitter gourd plants cultivated in advance. Following inoculation, the plants were placed in a light incubator at 25 °C for 12 h in dark and then 12 h light/12 h dark.

2.6. TRSV Virus Assay, Gene Silencing Efficiency Assay, Chlorophyll Assay

Using the cDNA of the bitter gourd leaves, PCR amplification was used to detect TRSV-specific sequence fragments, and real-time fluorescence quantitative PCR was used to measure the silencing efficiency of target genes in the plants. The qRT-RCR and the chlorophyll assay were conducted using the methods described by Zhang et al. [3] and Yao et al. [30], respectively. Statistical analyses for gene expression and chlorophyll content were performed using independent sample *t* tests in IBM SPSS 25.

3. Results

3.1. Target Selection via Sequence Analysis of Bitter Gourd McPDS

Figure 1A depicts the gene structure of the bitter gourd *McPDS*. The *McPDS* gene is 13.9 kb in size and contains 14 exons, while CDS is 1.7 kb in size. Comparing and analyzing the CDS sequences of bitter gourd *McPDS* and other cucurbits' *PDS*, Figure 1B depicts a relatively conserved region of the cucurbits' *PDS* gene. We designed primers for the conserved region marked by the red line in *McPDS* and constructed the corresponding viral vector pTRSV2-*McPDS*. The sequence marked with a red line is located in the exon 5 to 7 regions of *McPDS*, and the shared sequence identity of the bitter gourd *PDS* fragment with other cucurbits in this region was 95.34% with cucumber, 94.92% with melon, 95.76% with wax gourd, 95.34% with bottle gourd, 95.34% with Indian pumpkin (CmaCh14G014300), 96.61% with CmaCh06G016940, 93.22% with Chinese pumpkin (CmoCh06G016860), 65.68% with CmoCh14G014660, and, finally, a 65.68% sequence identity with watermelon.

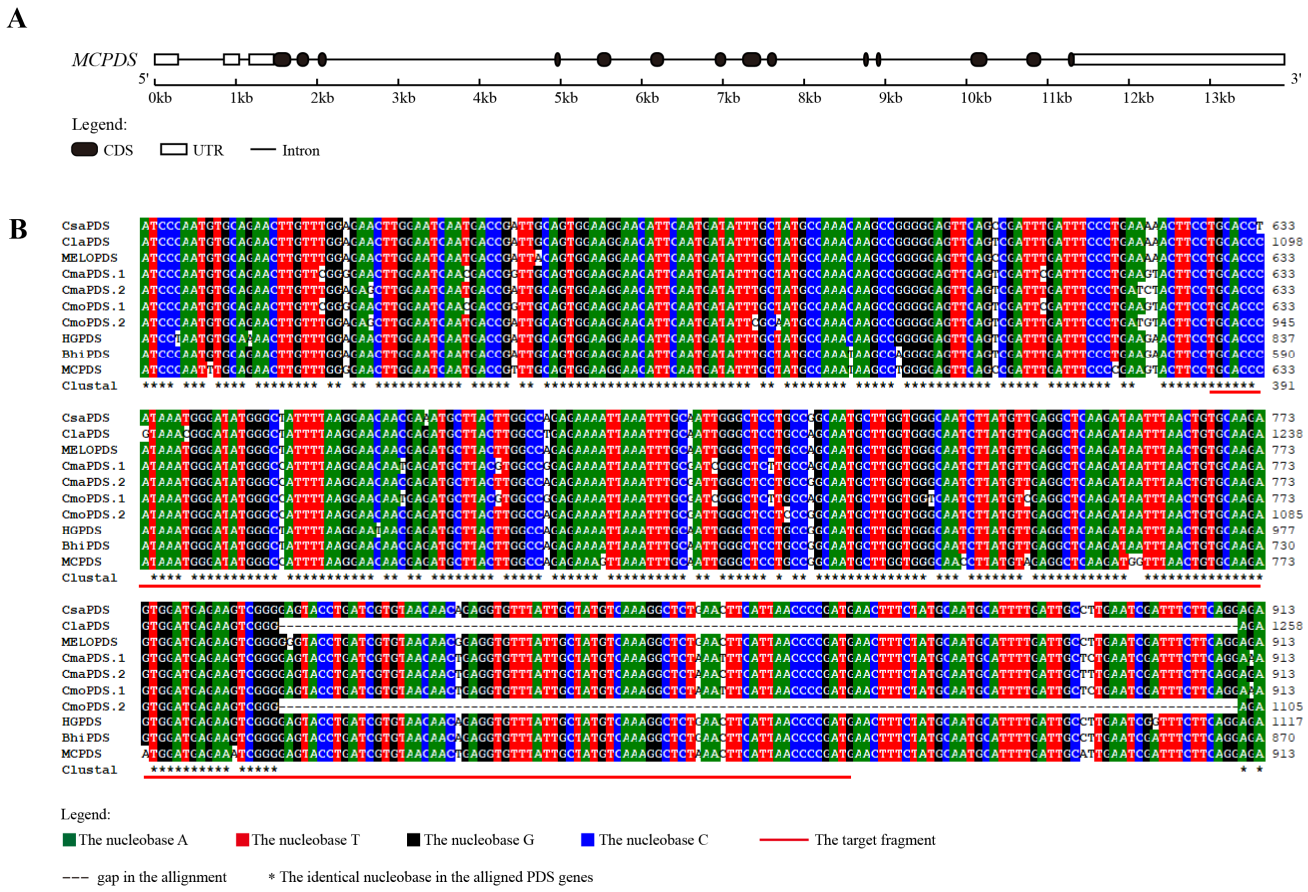

Figure 1. Sequence analysis of bitter gourd *McPDS* gene. (**A**): *McPDS* gene structure map. (**B**): Target sequence selection for *McPDS*. The red-lined sequences in the figure represent the target fragments chosen for vector construction.

3.2. pTRSV2-McPDS Vector Constructed by Enzymic Ligation While Digesting

Figure 2A illustrates the structures of the pTRSV1 and pTRSV2 vectors and the insertion position of the *McPDS* fragment within the pTRSV2 vector. Figure 2B,C show diagrams of viral vector construction using the traditional and optimized methods, respectively. Compared to the traditional method, the optimized method (Figure 2C) is clearly simplified, saving on time, labor, and reagents (Figure 2B); moreover, the pTRSV2-McPDS vector was rapidly constructed using the optimized method. A 252 bp fragment of the target gene with a SnaBI digest site junction was amplified and recovered after electrophoresis (Figure 2D). After the combined ligation/digestion reaction and transformation into *E. coli*, the pTRSV2 vector-specific primers pTRSV2-F/-R were used to identify the positive clones. The PCR band for the pTRSV2 empty vector plasmid or the negative clone was 320 bp, while for the positive clone it was 572 bp (320 bp plus the size of the target fragment), as depicted in Figure 2E. The successfully constructed pTRSV2-McPDS vector plasmid was transformed into *Agrobacterium tumefaciens* GV3101, and the positive clone was identified again using the same vector primers pTRSV2-F/-R, as shown in Figure 2F.

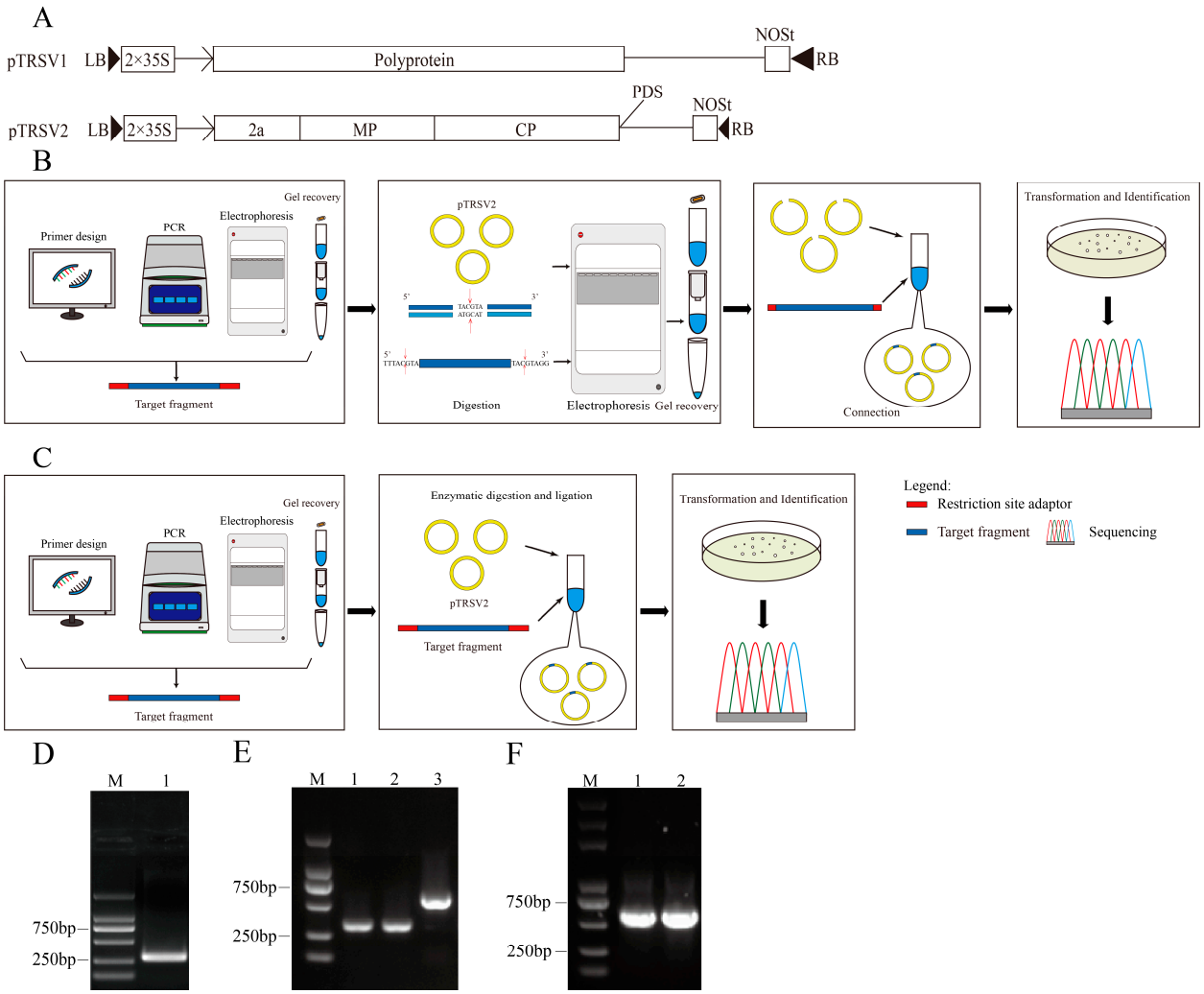

Figure 2. Construction of pTRSV2-*McPDS* vector. (**A**): Vector maps of pTRSV1 and pTRSV2 and cloning sites in pTRSV2. (**B**): Diagram of viral vector construction using the traditional method. (**C**): Diagram of viral vector construction using the optimized method. (**D**–**F**): Agarose electrophoresis of PCR-amplified target fragments. (**D**): Lane 1 is the PCR product of *McPDS* target fragment. (**E**): Lanes 1, 2, and 3 are the PCR products with a template of pTRSV2 empty vector plasmid, a single *E. coli* colony carrying pTRSV2 empty vector plasmid, and a positive *E. coli* colony carrying pTRSV2-*McPDS* vector plasmid, respectively. (**F**): Lanes 1 and 2 represent the single colony amplification products of *Agrobacterium tumefaciens*-positive clones carrying pTRSV2-*McPDS* vector plasmid and the pTRSV2-*McPDS* vector plasmid, respectively.

3.3. TRSV-McPDS Induced Photo-Bleaching in Bitter Gourd

Seven days after the first true leaves of the bitter gourd plants were frictionally inoculated with TRSV (empty vector, negative control) and TRSV-McPDS (carrying the McPDS fragment), the third true leaves and above of the bitter gourd plants inoculated with TRSV-*McPDS* began to show albinism (Figure 3B), and were obviously whitened after 14 days (Figure 3C), while the leaves of the bitter gourd plants inoculated with the negative control still did not show any change. After 35 days, the bitter gourd plants inoculated with TRSV-McPDS entered the flowering stage, where the whitening of leaves was more serious and the stems and flower buds also showed an obvious whitening phenotype (Figure 3D). In contrast, the plants inoculated with the negative control exhibited normal growth and development, no whitening, and no evident virus disease symptoms.

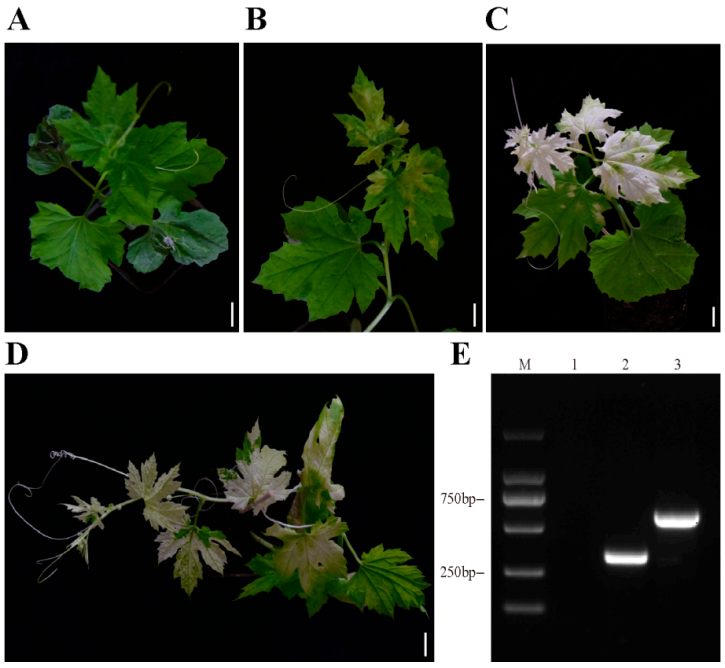

Figure 3. TRSV with/without *McPDS* target works well in bitter gourd. (**A**): The representative plant inoculated with the negative control TRSV after 7 days. (**B**–**D**) represent plants inoculated with TRSV-McPDS after 7 days, 14 days, and 35 days, respectively. (**E**): Lane 1 shows no target band as the PCR template cDNA is from the blank control, lane 2 and lane 3 show different target bands as the PCR template cDNA are from the negative control TRSV (empty vector, 320 bp) and TRSV-MCPDS (320 bp + insert), respectively.

3.4. TRSV Detected in Systemic Leaves of the Bitter Gourd Plants

Before and after the appearance of photo-bleaching in the systemic leaves, the TRSV-specific fragment, as the virus's marker, was detected via PCR. Figure 3E demonstrates that the target bands of 320 bp and 570 bp were amplified in the systemic leaf samples of the bitter gourd inoculated with the negative control TRSV (empty) and TRSV-McPDS, respectively. However, no target band was amplified in the blank control.

3.5. TRSV-McPDS-Induced Downregulation of McPDS Gene Expression

As depicted in Figure 4A, the transcript expression of *McPDS* gene in the bitter gourd leaves inoculated with TRSV-McPDS was significantly lower than that in the bitter gourd leaves inoculated with the negative control TRSV, and its expression was decreased by 84.7%, indicating that the *McPDS* gene was effectively silenced in TRSV-McPDS-infested leaves.

3.6. TRSV-McPDS-Induced Decrease in Chlorophyll Content

Leaf chlorophyll contents were assayed and are illustrated in Figure 4B. The chlorophyll content of the bitter gourd leaves inoculated with TRSV-McPDS was significantly lower than that of leaves inoculated with TRSV negative control leaves. Chlorophyll a content decreased by 88.7%, chlorophyll b content decreased by 75%, and chlorophyll a + b content decreased by 84.3%.

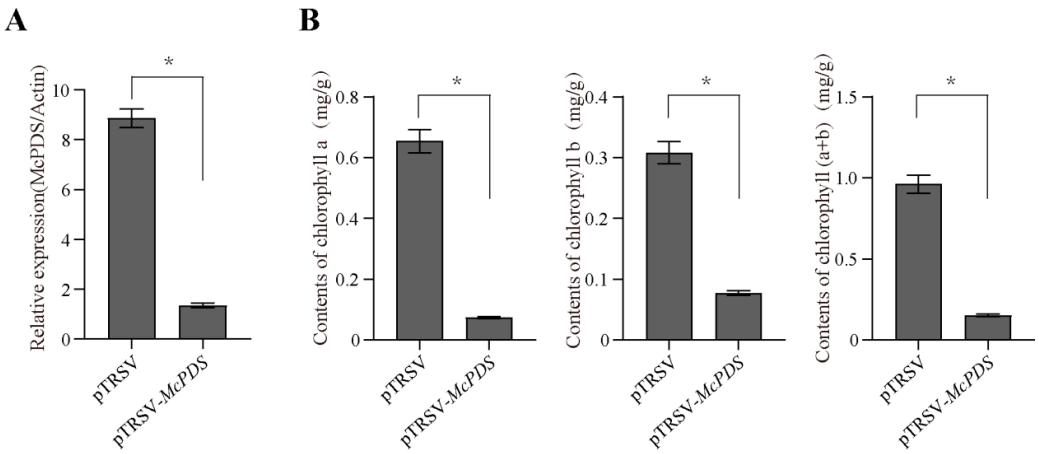

Figure 4. TRSV-McPDS induced decrease in *McPDS* transcripts and chlorophyll content. (**A**): The relative expression of *McPDS* in leaf samples inoculated with empty TRSV and TRSV-*McPDS*. (**B**): The chlorophyll a, b, and a + b contents in leaf samples inoculated with empty TRSV and TRSV-*McPDS*. The values are presented as means of three biological replicates, and * indicates statistically significant differences at $p < 0.05$.

4. Discussion

Compared to the generation of stably transformed transgenic plants, VIGS can be applied to obtain phenotypic information caused by the reduced expression or deletion of target genes relatively quickly. Especially in the case of bitter gourd and other crops whose genetic transformations are time-consuming, laborious, and inefficient, VIGS offers the advantages of simplicity and high efficiency in practice. In this study, we present an improved VIGS approach that enables the target segment to be quickly constructed into the TRSV vector and to effectively silence the *McPDS* gene, which will facilitate vector construction for VIGS and the study of gene functions in bitter gourd.

4.1. Functional TRSV-McPDS (Gene Target) Vector Constructed Rapidly Using an Improved Method

As an enzyme required for the synthesis of carotenoids, PDS is photoprotective of chloroplasts. *PDS* silencing results in a rapid and pronounced bleaching phenotype [21,22]. The McPDS gene was used as the target gene for setting up bitter gourd VIGS in this study. The sequence identity of the fragments carried by the TRSV-McPDS vector in this study was approximately 95% with the PDS fragments of cucumber, melon, wax gourd, bottle gourd, and Indian pumpkin. Since, in many VIGS experiments, a positive control is required to determine the effect of silencing, we speculate that the functional TRSV-McPDS vectors could be used as positive controls for VIGS experiments in bitter gourd.

More importantly, a combined enzymic ligation and digestion technique was developed for constructing pTRSV2-gene target vectors, such as pTRSV2-McPDS. Cloning an interested target fragment into a suitable viral plasmid is necessary for VIGS experiments. However, when using the method traditionally used in the literature, constructing the pTRSV2-McPDS requires at least one more cycle of performing a digestion reaction, conducting agarose electrophoresis of the cleaved target fragment and the empty vector pTRSV2, cutting the gel, and purifying the cleaved target fragment and the cleaved empty vector pTRSV2 [6,9,14,16,18,19,22,24,28,29]. It is therefore hoped that the optimized method for constructing pTRSV2-McPDS will provide a simpler and more efficient reference for the construction of various other VIGS vectors. We speculate that a lot of time, labor, and reagents will be saved in future VIGS applications using the combined enzymic ligation and digestion method.

4.2. The Inoculation Method Optimized for TRSV-Induced McPDS Silencing in Bitter Gourd

Friction inoculation, leaf injection inoculation, juvenile shoot uptake inoculation, and gene gun bombardment inoculation are the primary inoculation methods [14,21,29,31]. Leaf injection inoculation is a good practice for plants with spreading cotyledons and moderate thickness, such as cucumber and melon, but it is not as suitable for plants such as bitter gourd with small, thick, and non-spreading cotyledons. In addition, the first pair of true leaves of bitter gourd and even the later true leaves are generally thin and thus not suitable for injection inoculation. Therefore, we adopted friction inoculation in this study. However, based on our tests, the silencing of *McPDS* was very inefficient when inoculating the cotyledons or directly rubbing the true leaves with the constructed viral vectors instead of the host-cultured TRSV particles. So, the method culturing TRSV in the host and performing inoculation as described in Section 2.5 of this paper was optimized and made applicable for bitter gourd VIGS.

4.3. TRSV-Based Vector Suitable for VIGS in Bitter Gourd

Previous studies have demonstrated that the effect of gene silencing induced by the same viral vector on different plants or by different viral expression vectors on the same plant can vary significantly. Igarashi et al. [24] successfully silenced the *PDS* gene in tobacco, Arabidopsis, soybean, cucumber, and melon using apple latent spherical virus vectors, but not in pumpkin. Recently, Yamagishi and Yoshikawa accomplished effective gene silencing in pumpkin by optimally selecting cotyledon-folding pumpkin seedlings for gene gun bombardment with an apple latent spherical virus expression vector [21,24]. Via the inoculation of CGMMV-constructed VIGS vectors onto watermelon, melon, and cucumber, it was found that the appearance of the albino phenotype was delayed, taking more than 17 days to appear, and the albino phenotype was not obvious during *PDS* gene silencing [26]. However, when the TRSV-based VIGS vectors were inoculated onto watermelon, melon, and cucumber, a faster appearance of a very obvious photo-bleaching phenotype with the silencing of *PDS* was observed [5]. In this study, we present three lines of evidence showing that the TRSV vectors work well in bitter gourd for VIGS. One is the physiological phenotype, including a lack of observed symptoms of viral disease in the bitter gourd plants with/without target TRSV inoculation and an albino phenotype associated with TRSV-*McPDS*-mediated silencing, as shown in Figure 3A–D. The phenotype represents easily visible qualitive evidence. We speculate that, the more severe the leaf albinism, the lower the relative chlorophyll content in the leaves. So, the chlorophyll content was measured as quantitative evidence of an albino phenotype associate with *McPDS* silencing. We then found that the decrease in the relative chlorophyll contents and compositions in the albino bitter gourd leaves were consistent with previous reports that *PDS* gene silencing affects the composition of chloroplast pigments in addition to the photo-bleaching [21,22]. In addition to physiological phenotype, the downregulation of the transcriptional level of *McPDS* in the bitter gourd leaves was also measured as quantitative evidence of *McPDS* silencing. The second line of evidence is therefore the significant decrease in *McPDS* transcripts and chlorophyll content in the leaves, as shown in Figure 4, indicating the good efficiency and persistence of *PDS* gene silencing in bitter gourd. The third line of evidence is the positive detection of the TRSV-specific fragment in systemic leaves, as shown in Figure 3E, indicating that the inoculated *TRSV-McPDS* was successfully replicated, transcribed, and transferred in bitter gourd plants. Thus, these data indicate that the engineered TRSV-based vector is suitable for VIGS in bitter gourd.

5. Conclusions

By constructing the VIGS vector TRSV-McPDS, this study developed a simple method for rapidly constructing the vectors required in future various VIGS applications to save time, labor, and reagents. By performing TRSV-McPDS-induced *McPDS* silencing, this study provides a useful methodological reference and a positive control for TRSV-based VIGS in bitter gourd. However, as more target genes are used for TRS-based VIGS, the

more the effectiveness of this method will be verified. Currently, research on the usage of VIGS systems to silence other genes for gene function analysis in bitter gourd is still lacking. Hopefully, this study will shed light on the functional analysis of those genes required for achieving important agronomic traits in the molecular breeding of bitter gourd.

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Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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