Construction of a Plant Binary Expression Vector for the Potato Host Factor Genes StTOM1 and StTOM3

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Abstract: In this article, we designed PCR primers according to the cDNA sequence of potato host factor genes *StTOM1* and *StTOM3* and the restriction enzyme sites of the intermediate vector pUCCRNAi to gain two RNA interference segments, which length were 272bp and 182bp, and then connected to the vector pUCCRNAi from two directions simultaneously. After the validation of sequencing and digestion, the bidirectional RNA interference segment with intron was digested from pUStT1-StT3-dRi(±) and connected to pBI121, thus the plant binary expression vector pBIStT1-StT3-dRi(±) was constructed.

Keywords: Potato, StTOM1, StTOM3, Plant binary expression vector.

1. Introduction

Potato is the world's fourth most-widely-consumed crop, the development of potato industry is severely restricted by viral diseases as its reproduction through tubers facilitates the spreading of viruses in potato seed, and causes the deterioration of potato seed. For this reason, it appears very important to cultivate the anti-viral disease potato.

As intracellular obligatory parasites, viruses relied on DNA or RNA segments to encode, replicate, infect and spread the required genetic information. For its simple structure, a virus needs to complete the cycle of infection with the help of host factor. Host factor refers to the host protein and structure supporting the replication of virus and its transportation inside host (Huang *et al.*, 2010). In recent years, some host factor genes have been discovered gradually and proved to support the replication of viruses. For instance, *AtTOM1* discovered in arabidopsis thaliana was reported to support the replication of *tobacco mosaic virus* (TMV), *cucumber mosaic virus* (CMV) and *tomato mosaic virus* (ToMV) (Hagiwara-Komoda *et al.*, 2008). It was also proved in some studies that when the genes *TOM1* and *TOM3* were knocked out simultaneously in both arabidopsis thaliana and tobacco, the replication of TMV was inhibited (Asano *et al.*, 2005). With all these evidences, it is proved that *TOM1* and *TOM3* are the host factors for replication of viruses in plants. Additionally, some other host factors have been also discovered in plants, including *RTM1* and *RTM2* (Chisholm *et al.*, 2001), *LSM1* and *LSM6* (Noueiry and Ahlquist, 2003), *THH1* (Fujisaki *et al.*, 2006), *TOM2A* (Fujisaki and Ishikawa, 2008), *CUM1* and *CUM2 (Roudet-Tavert et al.*, 2007) and pectin methylesterase (PME) (Micheli, 2001). Now, few studies have focused on the information and acting mechanism of these host factors.

2. Result and Analysis

2.1 PCR Amplification of Interference Segment of Potato Genes StTOM1and StTOM3

PCR amplification was conducted to gain *StTOM1* and *StTOM3* interference segments by taking StTOM1-dRi-F /StTOM1-dRi-R and StTOM3-dRi-F /StTOM3-dRi-R as primer respectively, and the cDNA cloning of *StTOM1* and *StTOM3* as the template. For gene *StTOM1*, the expected length of interference segment was 272bp. For *StTOM3*, it was 182bp. The stripes of gene obtained through amplification were clear, and had expected size (Fig. 1).

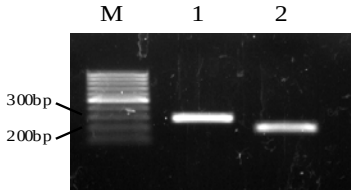

Fig.1 PCR result of target fragment

Note: M: GeneRuler™ 100bp DNA Ladder; 1: PCR amplification of RNA interference fragment of *StTOM1*; 2: PCR amplification of RNA interference fragment of *StTOM3*

2.2 Construction and Identification of Intermediate Vector for Potato Genes StTOM1 and StTOM3

By digestion, *StTOM1* RNAi segment and *StTOM3* RNAi segment were connected to pUCCRNAi plasmid resistant to ampicillin, so as to gain intermediate reverse vector pUStT1-StT3-dRi(-). Subsequently, pUStT1-StT3-dRi(-) was digested with *Pst* I to obtain 660bp segment, which was preliminarily validated as correct clone (Fig. 2A). After validation by sequencing, *StTOM1* and *StTOM3* were connected after being digested with *Spe* I and *Bgl* II, and forward connected to the vector pUStT1-StT3-dRi(-) digested with *Bam*H I and *Xba* I, so as to obtain the duplex connected clone. For validation, 1,100bp stripe was obtained by digestion with *Xho* I and *Sal* I (Fig. 2B). The stripe had the size matching with the target segment, proving that the bidirectional StTOM1-StTOM3-dRNAi segment had been successfully connected to intermediate vector. This successfully constructed intermediate vector was named pUStT1-StT3-dRi(±). The process of construction is detailed in Fig. 3.

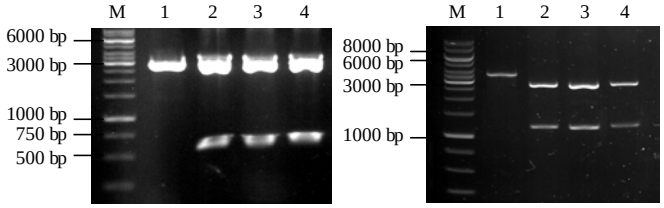

Fig.2 Verification of the intermediate vector pUStT1-StT3-dRi(±) by digestion

Note: A: Identification of the intermediate reverse vector pUStT1-StT3-dRi(－) by digestion; M: GeneRuler™ 1kb DNA Ladder; 1: Plasmid pUCCRNAi digested with *Pst* I (Negative control); 2~4: Vector pUStT1-StT3-dRi(－) digested with *Pst* I; B: Identification of the duplex connected vector pUStT1-StT3-dRi(±) by digestion; M: GeneRuler™ 1kb DNA Ladder; 1: Plasmid pUCCRNAi digested by *Xho* I and *Sal* I (Negative control); 2~4: Vector pUStT1-StT3-dRi(±) digested by *Xho* I and *Sal* I

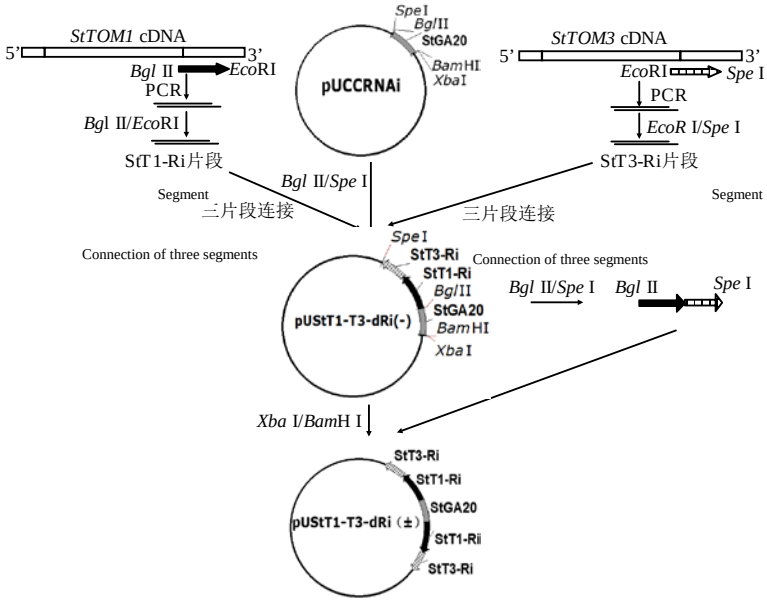

Fig.3 Construction of the intermediate vector pUStT1-StT3-dRi(±)

2.3 Construction and Identification of Plant Binary Expression Vector for Potato Genes StTOM1and StTOM3

Based on the characteristics of restriction enzyme sites on plasmids pUCCRNAi and pBI121, the constructed bidirectional StTOM1- StTOM3-dRNAi segment containing intron was separated from the intermediate vector pUStT1-StT3-dRi(±) by digestion, and connected to the vector pBI121 digested with *Xba* I and *Sma* I, so as to gain the plant binary expression vector pBIStT1-StT3-dRi(±). Through validation by digestion with *Bgl* II, the plasmid pBI121 had two *Bgl* II enzyme sites, so two segments could be taken from the plasmid by digestion with *Bgl* II, i.e. one 8,000bp segment and one 7,000bp segment. Meanwhile, a *Bgl* II enzyme site was transferred when transforming the alien segment into the constructed vector. Hence, one 8,000bp segment and two 4,000bp segments could be gained by digestion with *Bgl* II. The results were presented in Fig. 4A, and had the same size as target segment, proving that the plant binary expression vector pBIStT1-StT3-dRi(±) was successfully constructed. The process of construction was presented in Fig. 5.

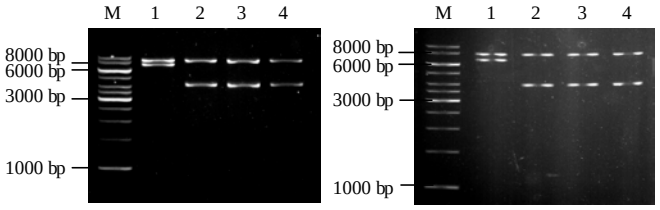

Fig.4 Verification of the plant binary expression vector pBIStT1-StT3-dRi(±) in *E.coli* DH5α and *Agrobacterium* EHA105 by digestion

Note: A: Verification of the vector pBIStT1-StT3-dRi(±) in *E.coli* DH5α; M: GeneRuler^{TM} 1kb DNA Ladder; 1: Plasmid pBI121 digested by *Bgl* II (Negative control); 2~4: Vector pBIStT1-StT3-dRi(±) digested by *Bgl* II; B: Verification of the vector pBIStT1-StT3-dRi(±) in EHA105; M: GeneRuler^{TM} 1kb DNA Ladder; 1: Plasmid pBI121 digested by *Bgl* II (Negative control); 2~4: Vector pBIStT1-StT3-dRi(±) digested by *Bgl* II

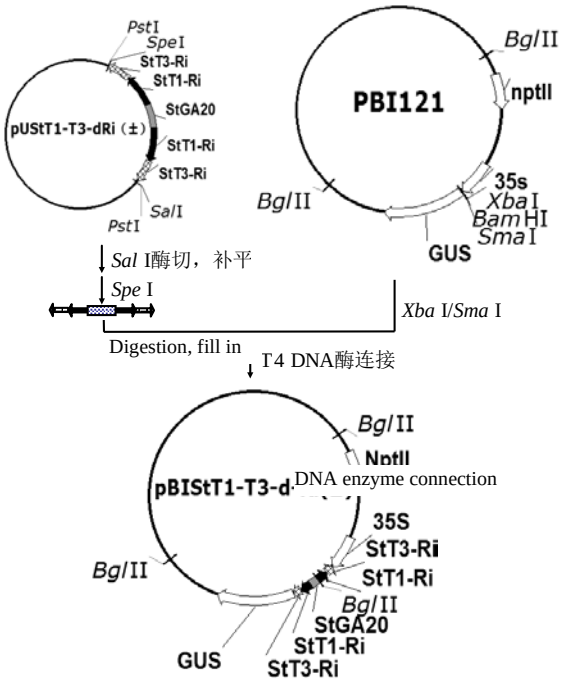

Fig.5 Construction of the plant binary expression vector pBIStT1-StT3-dRi(±)

3. Discussion

Host factors are involved in the infection of viruses, so they can be regulated to prevent the replication and transfer of viruses in host, making it possible to prevent viral diseases. This approach is of great significance to the research, prevention and treatment of viral diseases for humans, animals

and plants, so it is one of the most promising strategy for anti-viral genetic engineering breeding. As proved in some studies, some hosts could effectively control the occurrence of viral diseases after mutation without affecting their normal growth. For instance, when *TOM1* and *TOM3* were knocked out in arabidopsis thaliana and tobacco, the replication of TMV was inhibited, but transgenic plants could grow and develop normally (Asano *et al.*, 2005). After mutation of *LSM1* and *LSM6*, the replication level of *Bean commom mosaic virus* (BMV) was lowered, but mutants still grew and developed as usual (Noueiry and Ahlquist, 2003). It was once reported that *AtTHH1* could also inhibit the replication of TMV without affecting the growth of arabidopsis thaliana (Fujisaki et al., 2006). All these studies reveal that it is feasible to construct new anti-viral germplasms.

4. Material and Method

4.1 Design and Synthesis of Primers

The primer sequences are presented in Table 1. The synthesis of primers was conducted by Sangon Biotech (Shanghai) Co., Ltd.

Table 1 Primers used for PCR

Primer name	Primer sequence (5'-3')
StTOM1-dRi-F	GGAAGATCTGCATCTCTTGACGTCTTGGACC
StTOM1-dRi-R	CGGAATTCGGCCTTTCCACAGAATTATTGC
StTOM3-dRi-F	GGAATTCCCTGTTAGTGGAGATACTGCCTTCT
StTOM3-dRi-R	CGGACTAGTCAAATCACATCCAGGTAAAAGCC
nptII-1F	GGTGGAGAGGCTATTCGGCTATGA
nptII-1R	TGATATTCGGCAAGCAGGCATCG
35S-1F	CTTACGCAGCAGGTCTCATCAAGA
35S-1R	GGCAATGGAATCCGAGGAGGTT

4.2 PCR Amplification of Target Gene

Taking the cDNA clone of genes *StTOM1* and *StTOM3* as a template, amplification was carried out with the primers StTOM1-dRi-F /StTOM1-dRi-R and StTOM3-dRi-F /StTOM3-dRi-R. The PCR reaction system was 25μL: 5×PrimeSTAR^{TM} Buffer 5μL, 2.5mmol/L dNTP 2μL, 1μL each for 10μmol/L primers, 1μL each for 10ng/μL templates, PrimeSTAR^{TM} HS DNA Polymerase(2.5U/μL) 0.2μL, and dd$H_2$0 14.8μL. The reaction conditions were as follows: initial denaturation at 94℃ for 3min, denaturation at 94℃ for 30s, annealing at 61℃ for 30s, extension at 72℃ for 30s, 30 cycles, and extension at 72℃ for 10min. The size and quality of stripes were tested by 1% agarose gel electrophoresis.

4.3 Construction of Intermediate Vector pUStT1-StT3-dRi(±)

After purification of PCR product, *StTOM1* gene segment was digested with *Bgl* II and *Eco*R I, while *StTOM3* gene segment was digested with *Eco*R I and *Spe* I. Meanwhile, the plasmid pUCCRNAi was digested with *Spe* I and *Bgl* II. Through electrophoresis and purification by gel slices, the products from the abovementioned digestions were used to recycle interference segments and vector. Then, *StTOM1* RNAi segment, *StTOM3* RNAi segment and pUCCRNAi vector were connected at 16℃ for 8h under the effect of T4 DNA ligase, and the product from such connection was used to transform the competent cells of *Escherichia coli* DH5α, which were screened on the LA culture medium containing 100mg/L Ampicillin. Through the shaking culture of selected single colonies in the LB culture medium containing 50mg/L Ampicillin, plasmids were extracted and validated by digestion with *Spe* I and *Bgl* II. From plasmids identified to be positive, 3 were selected and sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing.

Based on the results of sequencing, the clone pUStT1-StT3-dRi(-) with the same sequence as the original template was selected, and its plasmids were digested with *Spe* I and *Bgl* II and 454bp connected segments *StTOM1* and *StTOM3* were recycled. Through digestion with *Bam*H I and *Xba* I, the vector pUStT1-StT3-dRi(-) was recycled. The recycled segment and vector were connected by T4 DNA ligase to transform the *Escherichia coli* and select the positive clone resistant to Ampicillin through screening. After selecting the positive clone sway and extracting the plasmids, the bidirectional connected vector pUStT1-StT3-dRi(±) was digested with *Xho* I and *Sal* I to determine whether the size of connected segment was correct.

4.4 Construction of Plant Binary Expression Vector pBIStT1-StT3-dRi(±)

The constructed plasmid pBIStT1-StT3-dRi(±) was digested with *Sal* I , and then filled in by T4 DNA polymerase. After being deactivated and purified, the product was digested with *Spe* I, and then recycled by gel slices to obtain 1,108bp interference segment StTOM1- StTOM3-dRNAi. The plasmid pBI121 was digested with *Xba* I. After purification, it was digested with *Sma* I. The obtained vector was connected to the interference segment StTOM1- StTOM3-dRNAi through T4 DNA ligase at 16℃ for 8h. From the product of such connection, 5μL was taken to transform the competent cells of *Escherichia coli* DH5α, and then placed in screening on the LA culture medium containing 100mg/L Kanamycin. After selecting positive strain sway and extracting plasmids, the interference segment was digested with *Bgl* II to validate whether it was successfully connected.

5. References

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