Expression of human hepatitis C virus core antigen in tobacco plants by tobacco mosaic virus-based vector system

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Abstract Tobacco mosaic virus (TMV) has the potential to highly express foreign gene. A novel TMV-based *in trans* expression system was constructed. A TMV mutant TSHc had its coat protein replaced with hepatitis C virus (HCV) core antigen gene. Anotherr TMV mutant TSBD was replicase-defective. Coinfection of the two mutants could cause systemic infection in tobacco plants by *in trans* complementation of their functions. TSHc could effectively replicate and assemble to viral particles, which were a little longer than that of wild-type TMV. HCV core antigen was expressed in whole tobacco plants. A similar expression level of HCV core antigen was detected on serial passages, which suggested that this viral expression system be stable.

Keywords: TMV vector, complementation, systemic infection, HCV core antigen.

Using plant cell to express foreign genes is a potential field and is gaining much attention. Transgenic plants constructed through T-DNA, chemical or physical methods can set up foreign genes expression system[1]. Compared with transgenic plants, the expression systems using plant viruses as vectors are more attracting. They may have many hosts and need a relatively short time to fabricate expression system. In addition, the expression of target genes by such systems is likely at a higher level[2].

TMV, a rod-shaped plant virus, has been well studied. Its genome is 6.4 kb single-strand RNA with a cap structure. It can produce three kinds of subgenomic mRNAs encoding 4 proteins (126, 183, 30 and 17.5 ku) respectively, which function in replication, transportation and assembly regulated differently in time and quantity[3,4]. Although the expression vectors with TMV have been reported[2,4–8], there has been no report by now about using co-infection of two viral mutants with complementary functions to express a foreign gene. Due to the polar effect of TMV gene expression, this strategy could improve the expression level of foreign genes.

Hepatitis is a serious human disease, particularly the chronic viral hepatitis, which is the main reason for hepatocirrhosis and liver cancer. The prevention and therapy of hepatitis A virus and hepatitis B virus are effective, but a lot of problems remain for HCV[9]. HCV core antigen gene is highly conservative, and it can effectively induce protective response of immune system, especially cytotoxic T lymphocyte (CTL) response, which can keep human from HCV infection and should be one of the aims of vaccine development[10−12]. In this work we used the TMV-based vector system to express HCV core antigen gene in tobacco plant and approached the possibility of producing oral vaccine against HCV.

1 Materials and methods

(i) Construction of expression vector. pTSHc: pTSHc was constructed by replacing the *Xho* I - *Pst* I fragment of pT7TB2[7] (provided by Dr. Sean Chapman) with the fused gene (642 bp, 214 amino acids) of trichosanthin signal peptide gene and HCV core antigen gene, which was from pBluescriptSK-SHc (constructed in our experiment, unpublished).

pTSBD: pTSBD was constructed by deleting the *Sma* I -*Bam*H I fragment of T7-pTMV (provided by Dr. Sean Chapman).

(ii) *In vitro* transcription and infection. Two plasmid clones of TMV mutants were prepared by alkaline lysis, purified by DNA purification Kit (Promega), digested with *Kpn* I, filled-up by Klenow and then transcripted by the following system [13]: A total volume of 50 μL contains 10 μL 5×TSC (200 mmol/L TrisCl (pH 7.9), 30 mmol/L $MgCl_2$, 10 mmol/L spermidine, 50 mmol/L NaCl), 7.5 μL 10 mmol/L (rATP+rCTP+rUTP), 2.5 μL 10 mmol/L $m^7G(5')G$, 1 μL 10 mmol/L rGTP, 50 U RNAsin, 40 U T7 RNA polymerase. It was kept at 37℃ for 20 min, added with 6 μL 10 mmol/L rGTP, then placed at 37℃ for another 40 min. The transcripts was used to infect or co-infect tobacco plant (*N. tobacum* var Samsun-nc and *N. tobacum* var Xanthi) leaves with a mixture of corundum and phosphate buffer (20 mmol/L phosphate buffer (pH 7.4), 5 mmol/L EDTA).

(iii) Northern blotting. Total mRNA of infected tobacco leaves for 12 d was prepared by rapid isolation procedure of plant RNA[14]. Northern blotting was processed according to the method described by Sambrook et al.[15].

(iv) Western blotting and ELISA. After infection for 12 d, the upper tobacco leaves were used to prepare the total soluble protein with 1×PBS (with 0.3% β-mercaptoethanol). The Western blotting analysis followed the method described by Sambrook et al. [15]. 120 μg of protein sample was loaded on each lane. ELISA was performed by Ausubel et al. [16].

2 Results

There were two mutant clones of TMV, pTSHc and pTSBD (fig. 1) in our experiments. The former was derived from T7-pTMV with its coat protein replaced by the fused gene of HCV core antigen and signal peptide genes, and the latter with TMV replicase gene deleted. Active RNAs of these two clones and wild-type TMV RNA were trancripted by T7 RNA polymerase. Inoculation experiments with *N. tobacco* var Samsun-nc proved that the pTSHc transcript had infectivity, but the necrotic spots on test leaves were smaller and produced more slowly compared with that infected with the pTMV transcript (fig. 2). It suggested that the infection ability of mutant TMV

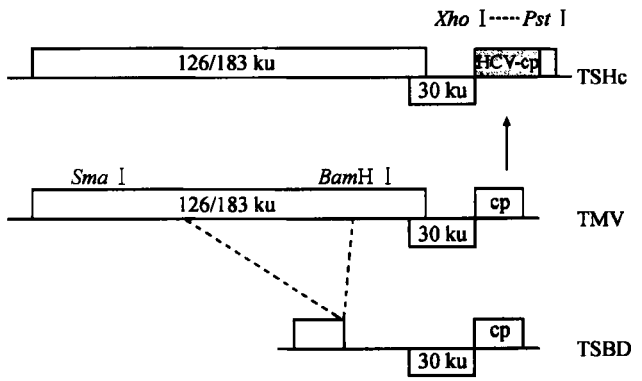

Fig. 1. Genomic structures of wild-type TMV and its two mutants.

with coat protein deleted was declined.

Systemic tobacco plant (*N. Tobacum* var Xanthi) co-infected with TSHc and TSBD trancripts did not show obvious mosaic symptom, even 18 d after inoculation (fig. 2). After infection for 12 d, the

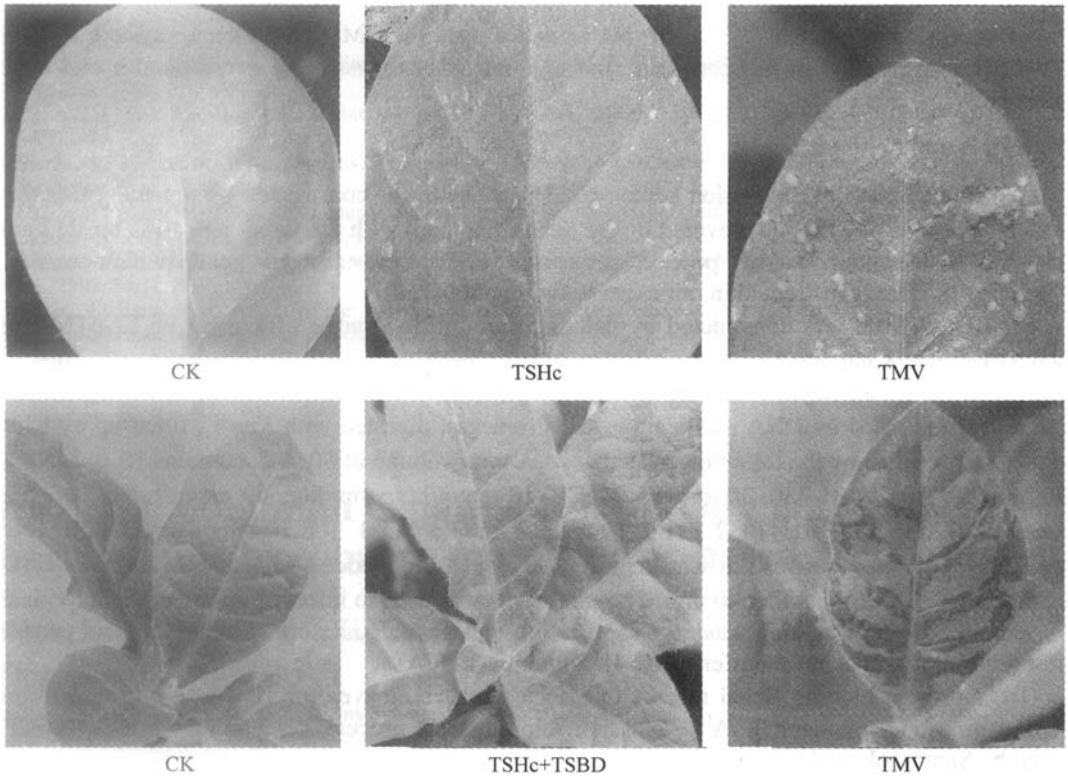

Fig. 2. Symptoms on tobacco plants *N. tobaccum* var Samsun-nc (above) and Xathi (below) after being infected with *in vitro* transcript RNAs of wild-type TMV and its mutants.

upper leaves were analyzed by Northern blotting using HCV core antigen gene fragment as probe. Our results showed that the target gene was expressed in whole tobacco plant (fig. 3(a)). At the same time, using the TMV coat protein gene fragment as probe, we found that only the TSHc and TSBD co-infected tobacco gave positive signal, but the expression level was much lower than that of

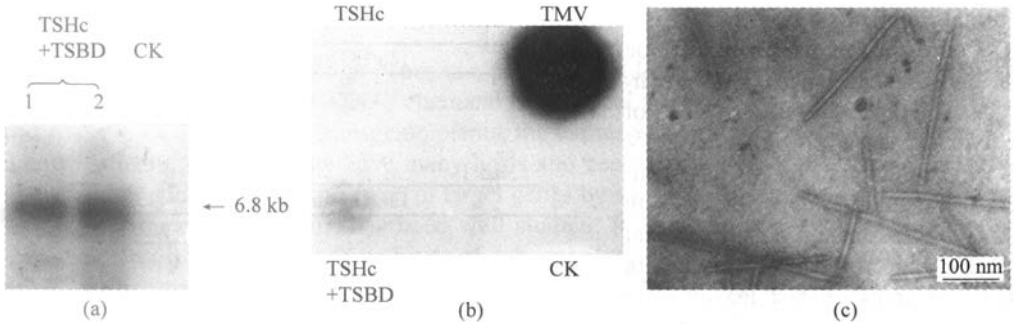

Fig. 3. Northern blotting (a), RNA hybridization (b) and electron microscopy (c) to detect the replication and assemble in co-infected tobacco plant Xanthi with the TMV mutants. (a) HCV core antigen gene was used as probe. 1 and 2 indicate two individuals of co-infected tobacco plants; (b) TMV coat protein gene was used as probe; (c) the juice of upper tobacco leaves was used, stained with 2% uranyl acetate. CK indicates uninfected tobacco leaves in (a) and (b).

wild-type TMV (fig. 3(b)). It is worth noting that there was no hybrid signal in the tobacco leaves infected by TSHc transcript alone (fig. 3(b)). We observed TMV particles of 350 nm in the co-infected tobacco leaves (fig. 3(c)). They were a little longer than that of wild-type TMV (300 nm) under electron microscope. All these results indicated that these two TMV mutants could systemically infect tobacco plants *in trans* complementation of their functions.

Further Western blotting detection revealed that HCV core antigen was expressed in TSHc and TSBD co-infected tobacco plant. Its molecular weight was about 22 ku, similar to native HCV core antigen, which suggested that its N terminal secretory signal peptide might be removed correctly (fig. 4(a)). To prove the stability of this system, we used the juice of co-infected upper leaves to passages and then detected HCV core antigen expression by ELISA. The fact that similar level of core antigen was expressed on three passages gave the evidence of stability of the complementary expression system (fig. 4(b)).

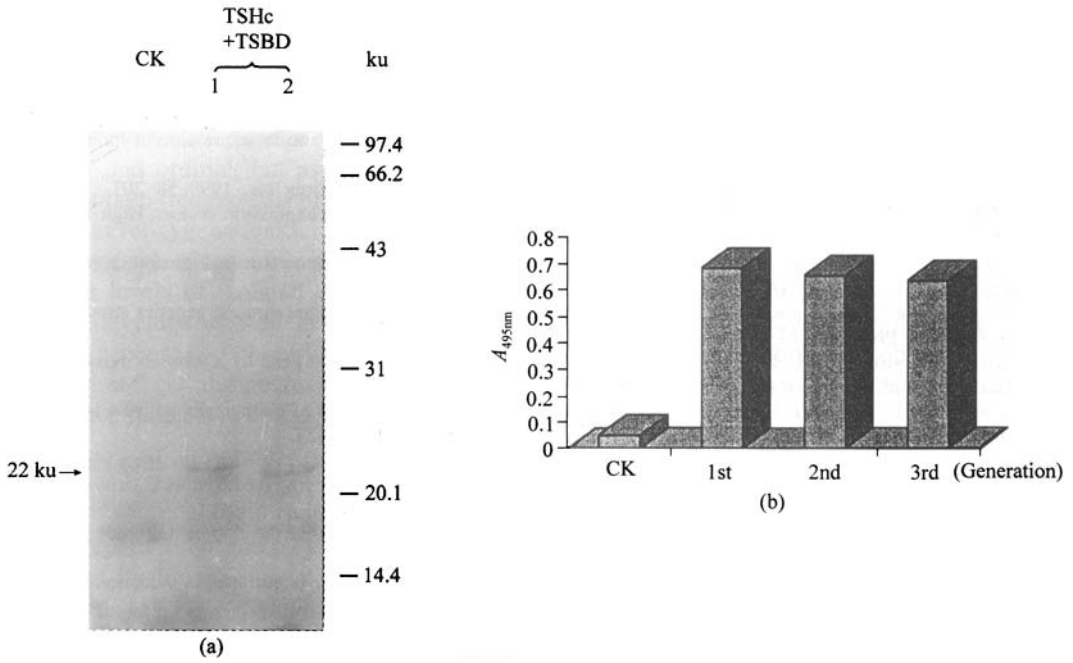

Fig. 4. Expression of HCV core antigen in co-infected tobacco plant Xanthi with TSHc and TSBD detected by Western blotting (a) and ELISA (b). CK indicates uninfected tobacco plant; 1 and 2 indicate two individuals of co-infected tobacco plants. The unit of molecular weight is ku.

3 Discussion

The stable viral genetic complementarity comes from the functional complementation of two or more viral components[17]. The TMV mutant TSHc with coat protein deleted can self-replicate and transport effectively between cells in inoculated leaves, but the efficiency of long-distance movement is very low[18]. So TSHc accumulation in upper uninoculated leaves was undetectable by Northern blotting when tobacco Xanthi was infected with TSHc alone. The TSBD with the *trans*-function of TSHc replicase led to the yield of coat protein subgenome. They could be assembled to viral particles in infected cells and spread to the whole plant. As long as coat protein is expressed in cells, the TMV mutant with coat protein deleted should give rise to systemic infection effectively[19,20]. The replicase deleted mutant replicates along with wild-type TMV, but in this circumstance, the mutant may be continuously recombined, deleted and even lost at last[21]. In our experiments, we even employed wild-type TMV to complementate the function. It was detected that the foreign gene was expressed in primary

TMV cells, but there was no expression detected on its passages.

Ogawa et al. used the two mutants with deletion in replicase and coat protein respectively to co-infect protoplast. Due to their complementation, coat protein was expressed, however, the expression level was only 1/50 of that of wild-type TMV[22]. Similarly in our experiment, TSHc expressed TMV replicase to support TSBD and its own replication and to produce all essential subgenomic RNAs. The complementary mutant TSBD provided coat protein, which improved the stability of mutant TSHc and its accumulation in some degree. They effectively set up tobacco systemic infection and promoted HCV core antigen expression at a higher level. Moreover, the core antigen kept its constant expression level on serial passages, which showed comparative stability of the expression system.

We have successfully used the functional complementation of two mutants to express the HCV core antigen in whole tobacco plants. The strategy used in this work might become an effective and rapid way to express foreign gene in plant after further imporvement.

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