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Agrobacterium-mediated Transient Expression of Foreign Gene in *Arabidopsis thaliana*

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Abstract Viral vector-*mediated* inoculation is a normal method of transient expression of foreign gene in the plants. In this study, we transiently expressed the foreign gene *GUS* in *Arabidopsis thaliana* by using *Agrobacterium-mediated* leaf-infection and detected the expression of *GUS* gene after ago-inoculation by GUS staining method and semi-quantitative RT-PCR. The results showed that *GUS* was expressed on 7-15 day after inoculation in *Arabidopsis* plants. Moreover， we successfully obtained fast expression of the viral suppressor HCPro in *Arabidopsis* plants, providing a new system for the fast expression of foreign gene and the study of gene function in plants.
Keywords *Arabidopsis thaliana*; Leaf-infection; Foreign gene; *Agrobacterium*

The expression methods of foreign gene include stable expression and transient expression in the plants (Walmsley et al., 2000), the transient expression system has the advantages of simplicity, rapidity and high efficiency (Shivprasad et al., 2000). Viral vector-mediated leaf-infection is a common method for transient expression of foreign gene in the plants. People have transiently expressed a variety of foreign proteins in vitro tobacco leaves by using this inoculation (Kathuria et al., 2002; Wang et al., 2002). Some researchers used viral vector-mediated leaf-infection to expressed high levels green fluorescent protein (GFP) in tobacco (*N. tabacum*) (Jia et al., 2003). Liu et al successfully expressed *human acidic fibroblast growth factor* (ha FGF) with viral vector-mediated leaf-infection in tobacco (*N. tabacum*) (Liu et al., 2007). In 2008, Yang et al transiently expressed GFP in tobacco (*N. tabacum*) by using viral vector 30B that is derived from *Tobacco mosaic virus* (TMV) (Yang et al., 2008). In 2013, we used viral vector-mediated method to transiently express haFGF in pea (*Pisum sativum*) plants (Yang Liping et al., 2013). In 2016, we established and optimized the viral vector-mediated root vacuum inoculation in tobacco (*N. tabacum*), and successfully expressed the foreign gene efficiently with this method (Yang Liping et al., 2016). The studies have shown that these plant transient expression systems and the study of inoculation methods provide a research basis for plant bioreactors application, and also provide an effective platform for the rapid production of medicinal proteins and vaccines.

In this study, we explored an *Agrobacterium-mediated* inoculation for rapid expression of foreign gene and gene function study in the plants. *Agrobacterium-mediated* leaf-infection was used to transiently express the reporter gene *GUS* in *Arabidopsis thaliana* plants. The results of gene expression detection analysis and GUS staining showed that we successfully expressed the *GUS* gene in *Arabidopsis* by using *Agrobacterium-mediated* leaf-infection. Further research results had shown that the viral suppressor HC-Pro was expressed by this method in the plant, which provides a new expression system for the study of its function. *Agrobacterium-mediated* leaf-infection has the advantages of simplicity, rapidity and high expression efficiency, which provides a research basis for the fast expression and the functional identification of foreign gene in the plants.

1 Results and Analysis

1.1 The expression of *GUS* gene by *Agrobacterium-mediated* method

First, we transformed the expression vector pCAMBIA2301-GUS into *Agrobacterium* EHA105, then we used the *Agrobacterium-mediated* leaf-infection to transiently express the reporter gene *GUS* in *Arabidopsis thaliana*, and also observed and photographed for 14 days continuously. GUS staining was used to detect the expression of *GUS* gene in *Arabidopsis*. The results showed that 7 days after agro-inoculation, GUS began to expressed in the wound site of infected leaves (Figure 1A); 10 days after agro-inoculation, *Agrobacterium* was passed around through vascular bundles, and it can be observed that GUS expressed spread out from the wound site of infected leaves (Figure 1B; Figure 1C); 14 days after leaf-infection, GUS expressed heavily in uninfected stems (Figure 1D) and new leaves (Figure 1E).

1.2 The rapid expression of *GUS* gene in *Arabidopsis thaliana*

The plant materials from 7-10 days after inoculation were extracted for gene expression detection. The results showed that the reporter gene *GUS* was successfully expressed in *Arabidopsis thaliana*, indicating the effectiveness of *Agrobacterium-mediated* leaf-infection for fast expression of foreign gene (Figure 2A). In order to further determine whether the foreign gene transiently expressed in the new leaves and new stems, we detected the reporter gene *GUS* by RT-semi-quantitative PCR. The total RNA was extracted form the infected leaves (15 days after inoculation) and the new leaves to reverse transcription, and obtained cDNA was used as template to detect the expression of reporter gene. The result showed that GUS was expressed in both leaves on 15 days after *Agrobacterium* inoculation and new leaves (Figure 2B), that indicated the *Agrobacterium-mediated* leaf-infection can realize the expression of foreign gene in *Arabidopsis*.

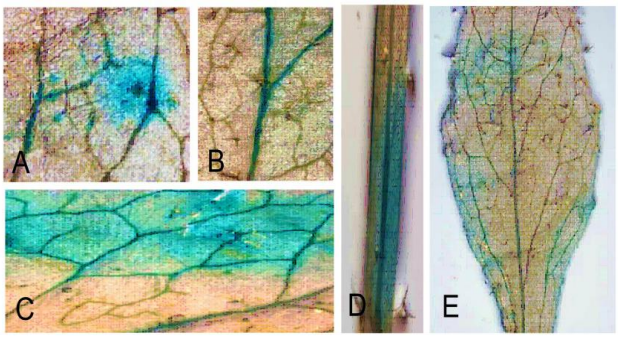

Figure 1 Detection of *GUS* gene expression by GUS staining method

Note: A: The expression of GUS appeared in the infection leaves at 7 days after agro-inoculation; B-C：The expression of GUS appeared in the infection leaves at 10 days after agro-inoculation; D-E：The expression of GUS increased in new stems and leaves at 14 days after agro-inoculation

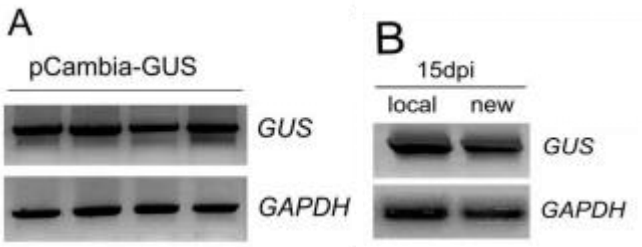

Figure 2 The detection of *GUS* gene expression by RT- sqPCR

Note: A: The expression of GUS was detected in the plants at 10 days after agro-inoculation; B: The expression of GUS was detected in the new and local leaves at 15 days after agro-inoculation

1.3 The expression of HCPro by *Agrobacterium-mediated* method in *Arabidopsis thaliana*

We have successfully constructed the expression vector PBI121-HCPro. In order to further study the function of HCPro, we used *Agrobacterium-mediated* method to express HCPro in *Arabidopsis thaliana*. *Arabidopsis* with stretched leaves were selected as plant materials for leaf-infection inoculation. When *Arabidopsis* infected with *Agrobacterium* carrying empty vector was used as control (Figure 3A), the phenotype of *Arabidopsis* expressing HCPro were obviously changed. 7 days after inoculation, the infected leaves showed mild curled and deformed (Figure 3B); 10 days after inoculation, some infected leaves were obvious curled and deformed, and the new leaves were serrated (Figure 3C), which was very similar to the phenotype of HCPro transgenic *Arabidopsis* obtained previously (Yang et al., 2016), indicating that HCPro began to express in the new leaves. 14-15 days after inoculation, the leaves were more serrated, some leaves were severely curled and deformed, and appeared yellow and wilting symptoms (Figure 3D). The phenotype change of *Arabidopsis* after inoculation suggested that HCPro expression changed the expression of host endogenous gene and the regulation mechanism of gene expression. The total RNA were extracted form infected plants to semi-quantitative RT-PCR detection, and the result confirmed that HCPro expressed in *Arabidopsis* (Figure 3E).

2 Discussion

Viral-derived expression vectors are often used for the transient expression of foreign genes in plants. The main inoculation methods of the plant transient expression system mainly include viral vector-mediated leaf-infection and vacuum infiltration (Kapila et al., 1997). The researchers used viral-mediated method to transiently express *acidic fibroblast growth factor* (aFGF) in tobacco (Liu et al., 2007). This method has the advantages of fast and high expression efficiency and is widely used by people. In this study, we transformed plant expression vectors pCAMBIA2301-GUS and pBI121-HCPro into *Agrobacterium* EHA105, and studied the transient expression of foreign genes in *Arabidopsis* plants by *Agrobacterium-mediated* leaf infection. In this method, the expression vectors and foreign genes carried by *Agrobacterium* are rapidly expressed mainly through the transmission and spread of *Agrobacterium* (Figure 1). Our results indicated that the reporter gene *GUS* (Figure 2) and functional protein HCPro (Figure 3) were successfully expressed with this inoculation method.

In this *Agrobacterium-mediated* leaf-infection, the expression vector carried by *Agrobacterium* may be a plant binary expression vector, *Arabidopsis* or other crops can be selected as the infection material. However, the host is limited by viral-derived expression vectors in viral-mediated transient expression systems. For example, expression vectors derived from *Tobacco mosaic virus* (TMV) are limited to tobacco and other host materials (Liu et al., 2007; Yang et al., 2008). Therefore, this *Agrobacterium-mediated* inoculation method further expands the host range and is suitable for the rapid expression of genes and the study of gene function in plants, providing a new system for plant transient expression systems.

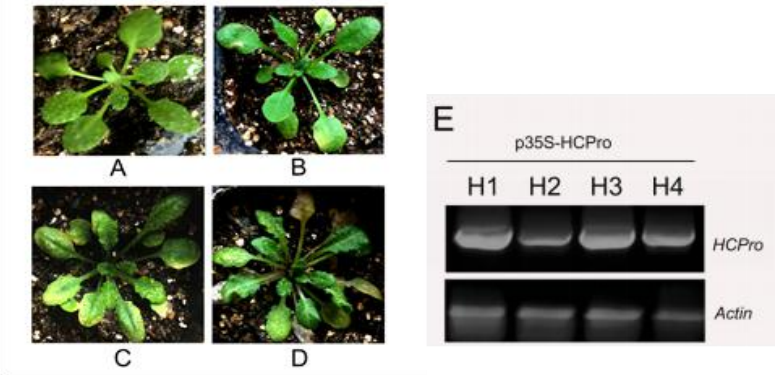

Figure 3 The phenotype of *Arabidopsis thaliana* expressed HCPro and the detection of gene expression

Note：A：mock-infected *Arabidopsis thaliana* served as controls; B：The leaves appeared curled and deformed after agro-inoculation; C-D：The infected *Arabidopsis thaliana* exhibited obvious deformed leaves, new leaves exhibited obvious serration; E：The detection of *HCPro* gene expression in infected plants, *Actin* served as controls

3 Materials and Methods

3.1 Materials and sources

The *Arabidopsis thaliana* seeds and strains are kept by our laboratory; plant expression vector pCambia-GUS is kept by our laboratory.

3.2 Preparation of Agrobacterium tumefaciens resuspension

The EHA105-pBI121-HC-Pro Agrobacterium liquid was activated and cultured in 50 mL LB liquid medium, and 20 μmol AS and 100 μmol MES were added. Centrifuge at 4 000 r/min for 10 min to collect *Agrobacterium*. Formulated solution (100 mL of ddH_2O was added with 1 mmol of $MgCl_2$, 1 mmol of MES and 100 μmol of AS), *Agrobacterium* was resuspended at a concentration of about OD_{600}=1.0, and the *Agrobacterium tumefaciens* resuspension was placed at room temperature for 3 hours for leaf infection.

3.3 Agrobacterium-mediated Arabidopsis inoculation

The stretched leaves of *Arabidopsis thaliana* were selected, and the syringe with the needle removed was used to infect the *Agrobacterium tumefaciens* resuspension into the leaf tissue, and each plant was infected with 2-3 leaves. The plants were cultivated under dark conditions for 24 hours and then transferred to normal conditions. The cultivation temperature of *Arabidopsis thaliana* was 22℃~25℃.

3.4 Semi-quantitative RT-PCR to detect gene expression

We used TRIzol method to extract the total RNA of the plant, semi-quantitative RT-PCR detection, pre-denaturation: take 2.5 μg of RNA, adjusted to 10 μL system with ddH_2O, pre-denatured the RNA first, the pre-denaturation conditions: treatment at 70℃ for 5 min; 4℃, 5 min. The pre-denatured RNA was reverse-transcribed, and the post-transcriptional cDNA was used as the template for PCR detection.

3.5 GUS staining analysis

The GUS staining solution and the GUS staining buffer were configured as the GUS staining working solution according to the volume ratio of 1:50, and placed in darkness at -80℃ for standby use. Put the *Arabidopsis* plant material in a 50mL beaker, added the appropriate amount of GUS staining working solution to completely cover the plant material. Wrapped the beaker with foil paper and used the vacuum infiltration for staining. The vacuum pressure was 0.08 MPa and the time was 5 minutes. We observed that the plant material was immersed in the bottom of the GUS staining solution, and the experiment was repeated twice. The whole beaker was incubated at 37℃ for 24 hours. With the extension of incubation time, GUS expression parts or sites showed blue or blue spots (The whole process is protected from light). Plant materials were fixed with 30 mL Carnoy's fluid at room temperature for 1-3 hours. Then the decolorization process was carried out. The volume fractions of ethanol were set as 25%, 50%, 70%, 85% and anhydrous ethanol, and the decolorization process was conducted at each concentration for 1-3 hours. We observed and took pictures under the microscope. Samples can be stored in 75% ethanol at 4℃ for 1-3 months.

Authors' contributions

Taicheng Jin was the executor of this experimental study, completed the data analysis and paper writing. Liping Yang directed the experimental design and paper modification; Chenjing Lang, Yue Wang, Dawei Meng and Yanju Wu participated in the completion of relevant experiments. All authors read and approved the final manuscript.

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