Estabishment of Agrobacterium-mediated transient expression system in Betula platyphylla

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Abstract

Transient transformation systems have been developed as quick and convenient m ethod and the Agrobacterium-mediated transient expression is a powerful tool for the characterization of gene function in plants. Here, we have established in vivo transient transformation system with *GFP* as a reporter gene by *Agrobacterium*-mediated of ro ots of Betula platyphylla. The results exhibited that the effect of infection on Betula platyphylla roots was the same as that of the whole plant and the expression level of GFP increased obviously. So,we have developed a simple and highly efficient transie nt transformation system that can quickly analyze the gene function in Betula platyph ylla.

Key words：*Agrobacterium* Transformation; Transient Transformation

1. Introduction

Stable genetic transformation and transient transformation are usually used for cha racterization of genes function (Rongli Mo et al., 2015). Furthermore, because of low transformation efficiency and high labor costs, stable transformation is not suitable fo r fast and high-throughput analysis of gene function (Li et al., 2009). In contrast,the t ransient trasnformation offers a number of advantages compare with stable genetic tra nsformation,such as short period, high efficiency, labor and time saving (Wydro et al., 2006). By comparison, on account of some superior characteristics such as simplicity, easy performance and cost saving, the Agrobacterium-mediated transient gene express ion system has been wildly used for characterization of genes function in woody plan ts (Orzaez et al., 2006; Figueiredo et al., 2011).

The *Agrobacterium-mediated* genetic transformation of fruit crops was firstly deve

loped in walnut (McGranahan et al., 1988) and then further applied to apricot, citrus, apple, etc., (Moore et al., 1992; Rugini et al., 1991; James et al., 1989). The persimmon genetic transformation by Agrobacterium-mediated has first been reported by Tao et al. (1994) and was developed and optimized further (Tao et al., 1997; Gao et al., 2000, 2001; Tamura et al., 2004). However, it was due to the higher phenolic content which can easily cause callus browning and the adventitious bud regeneration is genotype-dependent (Tao and Sugiura, 1992; Tetsumura and Yukinaga, 2000; Choi et al., 2001). Plant genome sequencing has resulted in the identification of a large number of uncharacterized genes. To investigate these unknown gene functions, several transient transformation systems have been developed as quick and convenient alternatives to the lengthy transgenic assay. Transient gene expression provides a convenient alternative to stable transformation in analyzing gene function by virtue of its time and labor efficiency.Routine transient assays include biolistic bombardment (ChristouP et al., 1995), protoplast transfection (Sheen J et al., 2001), and *Agrobacterium*-mediated transient purposes (Yang Yet al., 2000), each with advantages and disadvantages depending on the research goals. We present a novel transient assay based on cocultivation of young Betula platyphylla roots with Agrobacterium tumefaciens. Because the leaves of Betula platyphylla are very easy to wilt, it infects the roots.Without damaging the precursors of the leaves. And that, there is a strong need for such a protocol to enhance the study of genes potentially involved in Betula platyphylla.

2. Methods

2.1 Preparation of Agrobacterium cultures for infiltration

A single colony of Agrobacterium strain GV3101 harboring binary vector was inoculated in 1 ml of LB media with appropriate antibiotics. After grown for 1 day at 28°C with agitation it was removed into 50 ml of LB media with appropriate antibiotics. While, an overnight culture of Agrobacterium was harvested at OD 600 of 1.0, centrifuged at 4000 × g for 10 mins, and re-suspended to an optical density (OD) of 0.80 at 600 nm with infiltration medium. Cultures were incubated at room temperature for 3 h before infiltration.

2.2 Infection the roots of Betula platyphylla

Experiments were carried out at ambient temperatures ranging from 22 to 32℃, appropriate light and humidity conditions throughout the experiments. The parameters included the concentration of acetosyringone (50, 100, 150, 200 M) (infiltration medium contained 10 mM MES, 10 mM $MgCl_2$ and final OD 600 0.75), bacteria density (0.5, 0.75, 1.0) at 600 nm (infiltration medium contained 10 mM $MgCl_2$, 10 mM MES and 150 M Acetosyringon) and days after agroinfiltration. All of the parameters were evaluated and optimized based on content of green fluorescent protein. *Agrobacterium tumefaciens* infects Betula platyphylla roots instead of soaking the whole plant.

3. RESULTS

The *GFP* gene was PCR-amplified from pCAMBIA1300 by using a pair of prim ers. The PCR fragment was then purified and cloned into pEASYTM-Blunt Cloning V ector (TransGen Biotech, Beijing, China) resulting in plasmid pEASY-GFP. It was veri fied by sequencing. While, both the plasmid pEASY-GFP and the plant expression ve ctor PBI121 were double digested by using XbaI and SpeI. The binary plasmid pBI12 1-GFP contains NosP-NPTII-Nos terminator expression cassette, GFP reporter gene wit h CaMV 35S promoter, T7 terminator and a spectinomycin bacterial selection marker. The binary plasmid was transferred into *Agrobacterium* strain GV3101. Plasmid integri ty in *Agrobacterium* was confirmed by PCR amplification with specific primer and do uble digestion identification with XmaI and SpeI (data not shown). The constructed v ector map is shown in Figure 1.

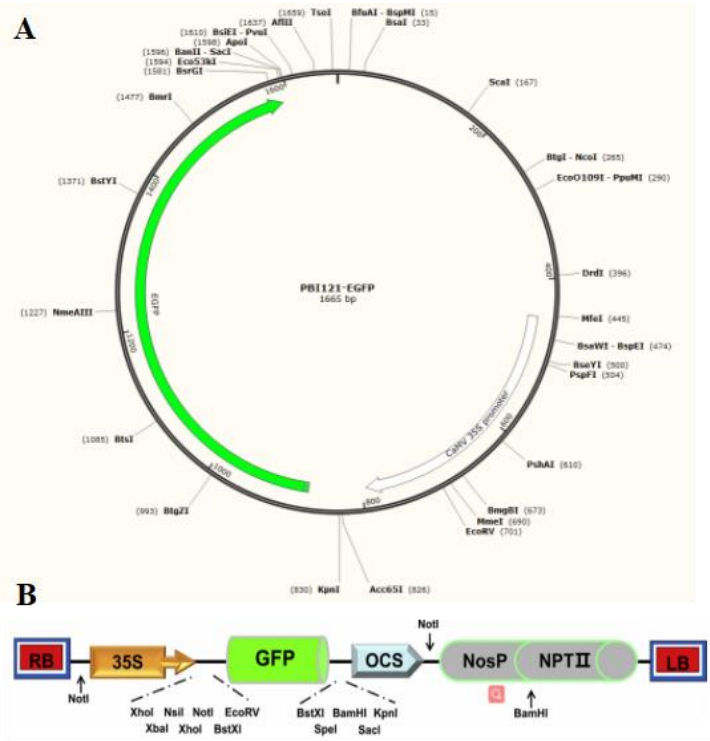

Fig 1. The PBI121-GFP T-DNA region of the binary plant vector. The T-DNA region of PBI121-GFP showing left (LB) and right (RB) border sequences, 35S promotor (35S), GFP coding sequence (GFP), and the coding region f or the neomycin phosphotransferase gene.

3.1 Agrobacterium-mediated infect Betula platyphylla

The leaves of Betula platyphylla are very fragile. If the whole plant is soaked in *Agrobacterium* to infect, the leaves will wilt easily, which will cause great errors in downstream experiments. In this experiment, the root of Betula platyphylla is soaked in *Agrobacterium tumefaciens*, and the leaves are not invaded. During the process of infection, the leaves are sprayed continuously on the surface of the leaves to keep them in normal state (Fig 2).

Fig 2. Transient transformation of Betula platyphylla system by root infection. This method only infects the root to prevent leaf wilting.

3.2 Transient GFP expression in Betula platyphylla

Here, we have constructed a GFP expression vector to investigate the feasibility of using

GFP as a reporter in Betula platyphylla. Suspensions of the A. tumefaciens strain GV3101 carrying plasmid pBI121-GFP were into Betula platyphylla. The whole plant were sampled for GFP fluorescence detection after 72 hours of infection.The results exhibited no any fluorescent signal in non-transformed plants (Fig 3). However, GFP signals were clearly observed in the infection. This result indicates that the GFP gene can be successfully expressed in leaves. Meanwhile, we found that the young leaves were more easily to be transformed than old leaves.

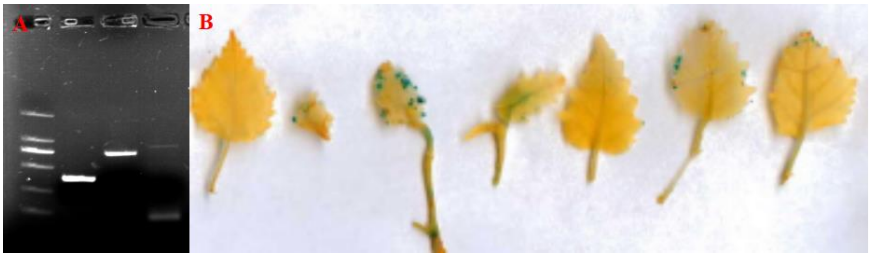

Fig 3 Detection of transient transfection by PCR and GUS staining.Wild-type control without blue spots showed no GFP signal.

We present a novel transient assay based on cocultivation of young Betula platyp hylla seedlings with *Agrobacterium tumefaciens.* This Fast *Agro-mediated* Seedling Tra nsformationwas used successfully to express a wide variety of constructs driven by dif ferent promoters in Betula platyphylla.

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