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Short Communication

An efficient transient assays system using *Agrobacterium*-mediated transformation of onion (*Allium cepa*) epidermal cells

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Abstract

In this study, the *Agrobacterium* infection medium, infection duration, detergent, and cell density were optimized. The sorghum-based infection medium (SbIM), 10-20 min infection time, addition of 0.01% Silwet L-77, and *Agrobacterium* optical density at 600 nm (OD_{600}), improved the competence of onion epidermal cells to support *Agrobacterium* infection at >90% efficiency. Cyclin-dependent kinase D-2 (CDKD-2) and cytochrome c-type biogenesis protein (CYCH), protein-protein interactions were localized. The optimized procedure is a quick and efficient system for examining protein subcellular localization and protein-protein interaction.

Key words: *Agrobacterium*-mediated method, *Allium cepa* (onion), BiFC, Transient gene function assay

Routine transient assays in onion have been conducted using biolistic bombardment (Cheng et al. 2009; Hollender and Liu 2010); however, these assays often exhibit high equipment dependency and sensitive materials. To overcome these disadvantages, the *Agrobacterium*-mediated DNA transfer system has been used and a number of techniques have been optimized (Li et al. 2017; Sun et al. 2007; Xu et al. 2014). To date, less effort has been focused on optimizing the competence of onion epidermal cells in supporting *Agrobacterium* infection for protein-protein studies.

Here, we report an improved method for transient gene function assays using *Agrobacterium*-mediated transformation of onion epidermal cells. Silwet L-77, an effective surfactant that shows relatively low toxicity to plants and often enhances transformation reliability (Chen et al. 2010), the results were obtained within 2 days. The protein-protein interactions were used to verify the robustness of the system.

Fresh red *Allium cepa* "Hongmeigui", purchased from the supermarket was used as explant.

The *Agrobacterium* strain, EHA105, harboring vectors pCAMBIA3300-*UBI-GFP* (p3300-*GFP*, Fig. 1A) and the *Agrobacterium* strain GV3101 harboring vectors pSPYNE-*CYCH*-YCHA/*CDKD;2*-YNEE and pSPYNE-YCHA/YNEE (Fig. 1B) was used for transformation.

Bacterial inoculation and co-cultivation with onion epidermal peels was conducted according to the method of Gurel (2012). The infection culture medium is shown in Table 1.

Under sterile conditions, the onion was cut into half (Fig. 2A a-b) and the epidermis (concave) was cut into several 1 cm^2 blocks and placed in three different types of infection media (Fig. 2A a-d): SbIM for sorghum transformation (Gurel et al. 2012), TnIM for tobacco transformation (Sparkes et al. 2006), and AtIM for *Arabidopsis* transformation (Clough et al. 1998) (Table 1). After infection, the onion epidermal peels were dried on filter paper and then transferred onto a solid co-cultivation medium (SbCOM, Table 2) and incubated in the light at 20 ± 2°C for 24 hours (Fig. 2B a-e). The onion peels were loaded onto microscopic

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Table 1. Media used in the transient expression assay

	SbIM	SbIM+s	SbCOM	TnIM	TnIM+s	AtIM	AtIM+s
MS (l^{-1})	4.43 g	4.43 g	4.43 g	-	-	2.215 g	2.215 g
Glucose (l^{-1})	36 g	36 g	10 g	-	-	-	-
Sucrose (l^{-1})	68.5 g	68.5 g	20 g	-	-	50 g	50 g
MES (l^{-1})	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
$MgCl_2$	-	-	-	10 mM	10 mM	-	-
Silwet L-77	-	0.01		-	0.01	-	0.01
As	100 μM	100 μM	100 μM	100 μM	100 μM	100 μM	100 μM
PHAgar (l^{-1})	5.2-	5.2-	5.88 g	5.2-	5.2-	5.2-	5.2-

Note: +s means the infection medium with Silwet L-77

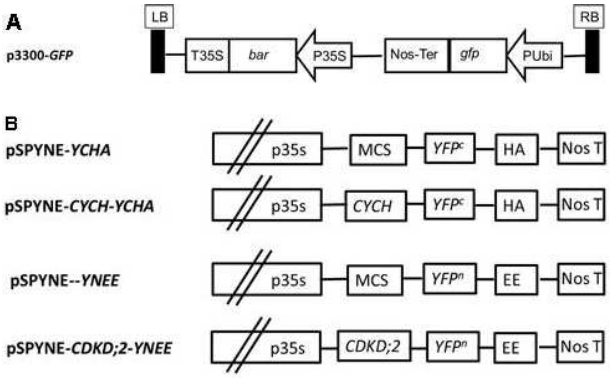

Fig. 1. Schematic representation of constructs used in the experiment

slides and then assayed for GFP activity (Fig. 2A a-f, Fig. 2B).

Competence of onion cell

The inclusion of Silwet L-77 to the SbIM in the presence of *Agrobacterium* at an optical density at 600 nm (OD_{600}) of 0.3 improved the competence of onion epidermal cells to support *Agrobacterium* infection at >90% efficiency (Table 2, Fig. 2B). Under this condition, the infection time (10min, 20min) did not affect the infection efficiency (Table 2).

Agrobacterium OD_{600} at both 0.3 and 0.5 reached higher efficiency (>90%) than that at *Agrobacterium* OD_{600} 0.1 (74%). To avoid a possible high *Agrobacterium* OD_{600} (0.5) that would be toxic to plant cells, we chose a density of 0.3 (OD_{600}) in the assay system.

Protein-protein interaction studies using the developed system

For protein-protein interaction, co-transformed

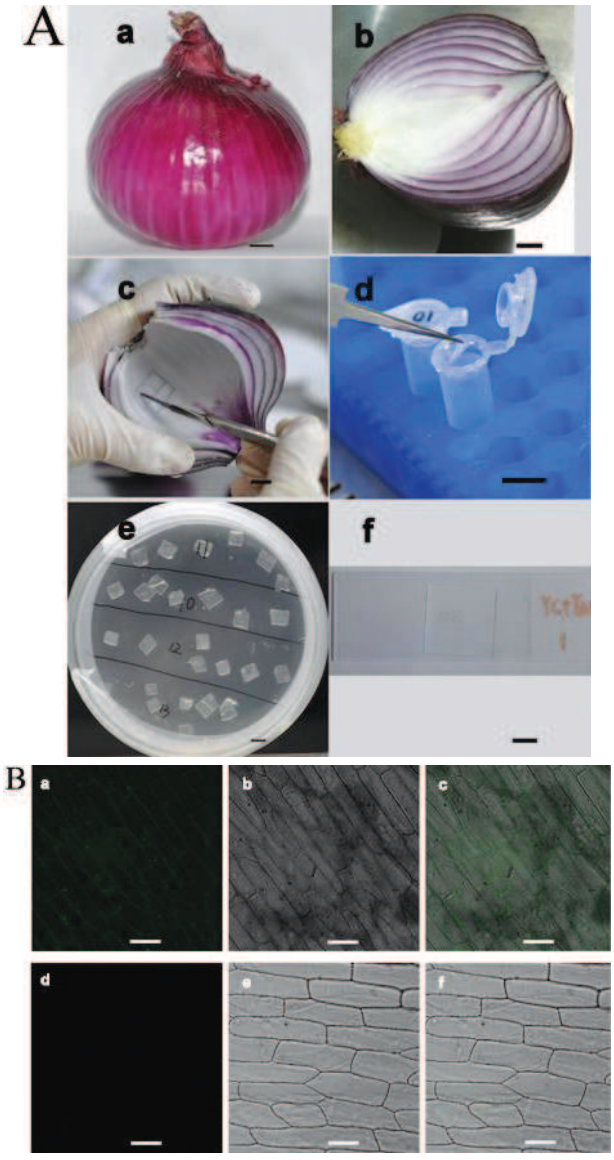

Fig. 2. *Agrobacterium*-mediated transformation of onion epidermal peels and transient expression of p3300-GFP

Table 2. Effects of infection media and infection time on transient transformation efficiencies

Infection media	OD_{600} = 0.3 with different infection times	
	10 min	20 min
SbIM	50.1 ± 2.9 bcB	60.3 ± 6.1 bB
SbIM +s	92.7 ± 0.9 aA	92.9 ± 1.7 aA
TnIM	8.3 ± 8 eCD	4.0± 2.6 eD
TnIM +s	40.1 ± 3.0 cdBC	43.1 ± 2.0 bcdB
AtIM	27.5 ± 13.8 dBCD	29.7 ± 7.3 dBCD
AtIM +s	49.8 ± 1.7 bcB	61.2 ± 9.1 bB

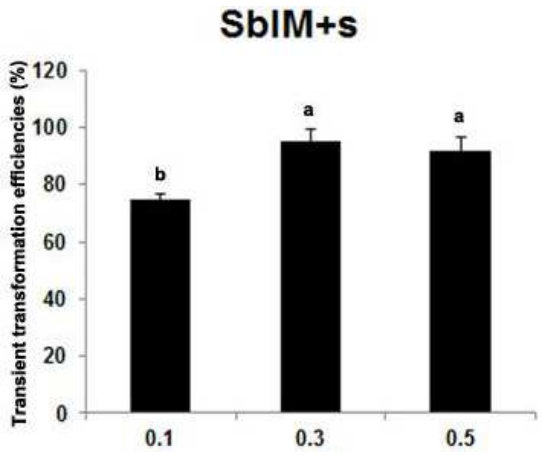

Fig. 3. Effects of *Agrobacterium* density on transient transformation efficiencies

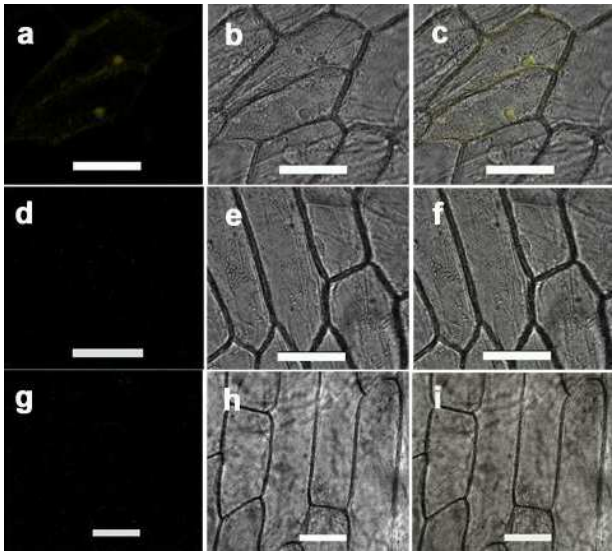

Fig. 4. Detection protein-protein interactions via BiFC using this competence system in onion epidermal cells. a: **pSPYNE-*CYCH-YCHA/CDKD;2-YNEE*,** d g: **negative control,** b e h: **bright field images and** c f i: **merged images**

pSPYNE-*CYCH-YCHA/CDKD;2-YNEE* cells produced strong YFP fluorescence signals preferentially enriched in the cell nuclei, and some were also detected in the cytoplasm (Fig. 4a). The negative control (pSPYNE-*YCHA/YNEE* co-transformed or pSPYNE-*CYCH-YCHA/CDKD;2-YNEE* transformed separately) showed no YFP fluorescence signals (Fig. 4d,g). Thus, functional complementation of the two complementary non-fluorescent fragments of the YFP fluorophore was achieved, and indicated the interaction of corresponding fusion proteins.

Authors' contribution

Conceptualization of research (YMZ); Designing of the experiments (YMZ); Contribution of experimental materials (YMZ, JN); Execution of field/lab experiments and data collection (JN, TW); Analysis of data and interpretation (TW); Preparation of manuscript (YMZ, JW).

Declaration

The authors declare no conflict of interest.

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