Single-Cell Complementation of Barley *mlo* Mutants Using a PDS-1000/He Hepta™ System

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Abstract

Transient expression via particle bombardment is widely used as a means of gene transfer to bacteria, yeast, animals, and plants. In this study we describe the use of the Hepta adaptor for the PDS-1000/He biolistic system for transformation and transient expression of *Mlo* in single epidermal cells of detached barley leaves. Barley *Mlo* is known to dampen plant defense and its expression in single epidermal cells of *mlo* resistant mutants restores susceptibility to attack from the powdery mildew fungal pathogen, *Blumeria graminis*. Consistently high transformation efficiencies were obtained upon delivery of a plasmid carrying both Green Fluorescent Protein (GFP) and *Mlo*. After bombardment and fungal spore inoculation, we found leaf sectors with numerous green fluorescent epidermal cells supporting growth of the pathogen. Likewise, we observed a high transformation efficiency of *Arabidopsis* leaf cells upon delivery of a GUS (β-glucuronidase) reporter gene construct. Application of the Hepta adaptor reduces the number of biolistic transfers necessary to obtain sufficient numbers of transformed plant cells for quantitative scoring of single-cell traits.

Introduction

Mutation induced recessive alleles (*mlo*) of the barley *Mlo* gene confer broad spectrum resistance against *Blumeria graminis* f. sp. *hordei*, the causal agent of powdery mildew. Conversely, the presence of wild-type *Mlo* leads to susceptibility upon attack from this obligate biotrophic fungal pathogen. *Mlo* encodes the founder of a novel family of plant-specific integral membrane proteins (Büschgés et al. 1997). The 7-transmembrane-domain protein resides in the plasma membrane and is presumed to act as a negative regulator of a basal defense mechanism (Devoto et al. 1999).

We have previously shown that transient single-cell expression of wild-type *Mlo* in *mlo* resistant leaves restores susceptibility to *Blumeria graminis* (Shirasu et al. 1999). This was based on cobombardment experiments involving 2 plasmids harboring either *Mlo* or GFP (Figure 1).

Here we describe a modification of the transformation procedure by using the PDS-1000/He Hepta adaptor. This device fits into the shocking chamber of the PDS-1000/He unit and splits the helium shock wave over 7 outlets, permitting a more even dispersal of DNA-coated particles and a greater target area.

Methods

Plant and Fungal Material

Leaves of *mlo* resistant barley (*Hordeum vulgare* cv. BC Ingrid *mlo*-5) and *Arabidopsis thaliana* (ecotype Ms-0) were used for this study. Barley plants were grown in a controlled environment at 18°C (16 hr light/8 hr darkness), whereas *Arabidopsis* plants were grown in short-day (8 hr light/16 hr darkness) conditions. First leaves of 8-day-old barley plants or rosette leaves of approximately 5-week-old *Arabidopsis* plants were used for the experiments. Barley leaves (apical 5–7 cm segments) were harvested and kept on 1% agar containing 10% sucrose 4 hr prior to bombardment.

*Blumeria graminis* f. sp. *hordei* K1 was propagated on *H. vulgare* cv. Golden Promise as previously described (Shirasu et al. 1999).

![Fig. 1. Scheme of the transient single-cell expression system. Detached resistant (*mlo*) barley leaves were bombarded with either 2 separate plasmids carrying GFP and *Mlo* coding sequences (Shirasu et al. 1999) or a single plasmid carrying both genes (pUGLUM, this study). After particle bombardment, leaves were inoculated with powdery mildew spores. Single leaf epidermal cells expressing both genes are fluorescent green and support growth of fungal colonies.](image)
Plasmids

Plasmid pUGLUM (Zhou et al. 2001) carrying GFP and Mlo coding sequences under the control of the strong constitutive maize ubiquitin 1 promoter was used for the barley experiments. For transformation of A. thaliana, plant binary vector pPam-GUS (T Rademacher and R Panstruga, unpublished) was used. In this plasmid the β-glucuronidase reporter gene is driven by a double constitutive cauliflower mosaic virus 35S promoter.

Preparation of DNA-Coated Gold Microcarriers

The preparation of DNA-coated gold microcarriers (1 µM particle size) was carried out according to the manufacturer's instructions (Bio-Rad PDS-1000/He manual). Briefly, gold particles were soaked in 70% ethanol (v/v), washed thoroughly with sterile water, and resuspended in 50% glycerol at a concentration of 60 mg/ml. To coat the particles with plasmid DNA, 50 µl (3 mg) of microcarriers were mixed with 5 µl DNA (1 µg/µl), 50 µl 2.5 M CaCl₂ and 20 µl 0.1 M spermidine (free base). After 2 washing steps with ethanol (first 140 µl 70%, second 140 µl 100%), the coated particles were finally resuspended in 48 µl 100% ethanol.

Conditions for Particle Bombardment and Fungal Inoculation

Each of the 7 macrocarriers of the Hepta adaptor was loaded with 6 µl of the coated microcarriers corresponding to 0.62 µg plasmid DNA. Hence, a total of approximately 4.3 µg plasmid DNA was delivered per shot. The target shelf carrying the petri dish with detached leaf segments was placed 6 cm from the Hepta adaptor. Usually 8–10 leaf sections (barley) or 10–20 rosette leaves (A. thaliana) were placed side by side in a single 9 cm petri dish for the biolistic transfer. After evacuating the shocking chamber to 27" Hg, specimens were bombarded with a helium pressure of 900 or 1,100 psi. Immediately after bombardment, the vacuum was released and leaf segments were kept on the plates 24 hr for recovery. The leaves were transferred to fresh plates (1% agar, 0.002 g/L benzimidazole) and inoculated with a high density of conidiospores of B. graminis f. sp. hordei K1. Petri dishes carrying the detached leaf sections were incubated in a growth cabinet under conditions described above.

Staining for β-glucuronidase (GUS) Activity

Arabidopsis rosette leaves were stained for GUS activity 3–4 days after bombardment by vacuum infiltration (1 hr) in 100 mM Na₂HPO₄/NaH₂PO₄, pH 7.0, 0.1% Triton X-100, 2 mM K₃Fe(CN)₆ containing 0.5 mg/ml 5-bromo-4-chloro-3-indoxyl-β-D-glucuronic acid and incubated overnight at 37°C. The stained leaves were cleared thereafter in ethanol (96%) for several hours.
Modification of the experimental setup described here generated a comparable number of cells expressing the GFP reporter gene after a single bombardment. Application of the Hepta adaptor resulted in consistently high numbers of transfected cells in several independent experiments. Generally, we found multiple clusters of transfected epidermal cells in bombarded leaf segments as shown in Figure 2. Thus, the Hepta adaptor is useful for experiments in which several independent constructs need to be tested or in which high numbers of transformed cells are desired.

Next we tested the efficiency of the Hepta system transformation procedure in the dicot plant *A. thaliana* by delivering a plasmid expressing GUS to detached leaf tissue. In this case we used plasmid pPam-GUS driving expression of the reporter gene from the doubled 35S promoter. Experimental conditions were identical as described above for the transformation of barley leaves. We obtained a high density of GUS-stained cells that were clustered in multiple leaf areas (Figure 4) 3–4 days following bombardment. Our findings demonstrate the usefulness of the Hepta particle delivery system in obtaining high transformation efficiencies in leaves of monocot and dicot plant species.

References


Shirasu K et al., Cell-autonomous complementation of mlo resistance using a biolistic transient expression system, Plant J 17, 293–299 (1999)
