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### The role of *Fusarium oxysporum* effector protein Avr2 in resistance and pathogenicity

Ma, L.

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## CHAPTER 6

### **The use of Agroinfiltration for transient expression of plant resistance and fungal effector proteins in *Nicotiana benthamiana* leaves**

#### **Abstract**

Agroinfiltration is a versatile, rapid and simple technique that is widely used for transient gene expression in plants. In this chapter we focus on its use in molecular plant pathology, and especially for the expression of plant resistance (*R*) and fungal avirulence (*Avr*) (effector) genes in leaves of *Nicotiana benthamiana*. Co-expression of an *R* gene with the corresponding *Avr* gene triggers host defence responses that often culminate in a hypersensitive response (HR). This HR is visible as a necrotic sector in the infiltrated leaf area. Staining of the infiltrated leaves with trypan blue allows visual scoring of the HR. Furthermore, fusion of a fluorescent tag to the recombinant protein facilitates determination of its sub-cellular localization by confocal microscopy. The matching gene pair *I-2* and *Avr2*, respectively from tomato and the fungal root-pathogen *Fusarium oxysporum* f. sp. *lycopersici*, is presented as a typical example.

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## Introduction

*Agrobacterium*-mediated transient expression (Agroinfiltration) is based on infiltration of *Agrobacterium tumefaciens* cultures into intact plant leaves. The bacterium subsequently transfers a DNA segment, called transfer-DNA or T-DNA, into the plant cells. In nature this T-DNA is part of the bacterial tumour-inducing (Ti) plasmid that, besides the T-DNA, carries the genes required for its transfer. The T-DNA carries effector genes that allow the pathogen to cause crown-gall disease on species in the Rosaceae family. In disarmed laboratory strains the effector genes are deleted and the two essential parts of the T-DNA, its left- and right border, are placed on a separate plasmid. Genes placed between these borders will be transferred to the plant. Since the genes required for T-DNA transfer now reside on a different plasmid, this system is called the “binary vector” system. The binary vectors containing the T-DNA can carry inserts of up to over 100 kbp (Hamilton, 1997). T-DNA transferred to a plant cell will relocate to the nucleus, where its genes can be transcribed and expressed (Kapila *et al.*, 1997). The majority of the plant cells in the infiltrated region express the transgene and the expression typically reaches its highest level 2-3 days after infiltration. At later stages the expression is quenched by RNA silencing (Voinnet *et al.*, 2003). In addition to the regular binary vectors, variants have been developed for specific purposes. One example is the pEAQ series that contain deconstructed viral genes that enhance recombinant protein production by producing a highly stable mRNA that is very efficiently transcribed (Sainsbury *et al.*, 2009). Other types of binary vectors carry viral genomes, such as that of Potato Virus X or Tobacco Mosaic Virus (Scholthof *et al.*, 1996). Use of these binary viral vectors, referred to as agroinfection, result in the production of an autonomous replicating virus that can spread systemically through a plant. A limitation of agroinfection is the relative small size of the insert tolerated (<2 kb) in the viral genome and the formation of a recombinant plant-pathogenic virus that requires elevated containment measures, which hampers broad application (Fischer *et al.*, 2005). We here focus on the use of regular binaries. For details on application of the alternative binary vectors, please refer to Kanneganti *et al.* (Kanneganti *et al.*, 2007).

Unarguably, the main advantage of agroinfiltration over stable transformation procedure is its speed. In addition, compared to other transient expression systems (protoplast transformation (Sheen, 2001), particle bombardment (Schweizer *et al.*, 1999), and microinjection (Bilang *et al.*, 1993)), it has the advantages that it is inexpensive - as it relies on simple technology - and can be exploited in intact plants of which a relative large leaf sector is transformed (Kapila *et al.*, 1997). Agroinfiltration has been exploited in many types of experiments, for instance to study gene function (Wroblewski *et al.*, 2005; Zottini *et al.*, 2008), protein production (Vaquero *et al.*, 1999), host-pathogen interaction (Tang *et al.*, 1996; Van den Ackerveken *et al.*, 1996), protein-protein interaction (Ihara-Ohori *et al.*, 2007) and protein localization (Bhat *et al.*, 2006). *Agrobacterium* is applied either using vacuum

infiltration or by syringe infiltration. Whereas vacuum infiltration has the advantage that whole leaves, and even entire plants, can be infiltrated at once, syringe infiltration is easier to perform and is more frequently utilized for protein production at a small scale (D'Aoust *et al.*, 2008). The main advantage of syringe infiltration is that different genes, either alone or in combination, can be expressed together in a single leaf. Syringe agroinfiltration has been applied successfully in a variety of plant species, including *Nicotiana* spp., tomato, lettuce, Arabidopsis, flax, pea, grapevine, pepper and rose (Abramovitch *et al.*, 2006; Tai *et al.*, 1999; Van den Ackerveken *et al.*, 1996; Van der Hoorn *et al.*, 2000; Wroblewski *et al.*, 2005; Yasmin & Debener; Zottini *et al.*, 2008). Among them, *Nicotiana benthamiana* and *Nicotiana tabacum* (tobacco) are preferably used. Especially *N. benthamiana* as the plant has a short life cycle, carries relatively large and easily infiltratable leaves that produce recombinant proteins at high levels and the leaf does not show necrosis upon infiltration with most *Agrobacterium* strains (D'Aoust *et al.*, 2008). These advantages made it a favourite model to study plant-pathogen interactions (Goodin *et al.*, 2008).

A major challenge in plant pathology is the functional analysis of resistance (*R*) and avirulence (*Avr*) genes. Agroinfiltration in *N. benthamiana* plants has been highly instrumental to tackle this challenge. In our laboratory agroinfiltration has been used for a variety of purposes. Co-expression of the tomato resistance gene *I-2* with candidate *Avr2* genes was used to identify the avirulent allele, as only this candidate triggered the hypersensitive response (Houterman *et al.*, 2009). Moreover, agroinfiltration allowed us to demonstrate physical interactions of *I-2* with plant proteins such as *i*) Heat Shock Protein 90, *ii*) its co-chaperone Protein Phosphatase 5 and *iii*) the small heat shock protein RSI2 (De La Fuente Bentem van *et al.*, 2005; Van Ooijen *et al.*). Also in structure/function analyses this method has shown its merits, as it allowed the rapid testing of a series of *I-2* mutants for either loss-of-function or constitutive activity (Tameling *et al.*, 2006). The ability to co-express constructs encoding different *R* protein sub-domains allowed us to analyse their ability to trans-complement and to examine physical interactions between the domains (van Ooijen *et al.*, 2008a; van Ooijen *et al.*, 2008b). Finally, expression of fluorescent-tagged AVR2 allowed determination of the sub-cellular localization of the recombinant protein in plant cells, providing clues for the endogenous location of protein activity (unpublished data).

The procedures in this chapter describe a generic method for transient production of *R* and AVR proteins in *N. benthamiana* leaves. The method is exemplified by expression of the tomato *R* gene *I-2* and the corresponding *Avr2* gene from the root-invading pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici*. Also detailed protocols for scoring HR and the use of the vital stain (trypan blue) to visualize cell death are provided. The last section describes two methods that can be used to detect the recombinant proteins produced: western blot analysis and confocal microscopy using a GFP-labelled AVR2 protein.

## Materials

### 2.1 Seeds and Plant Growth Materials

1. Seeds of *N. benthamiana*
2. 10 cm square plastic pots
3. Standard germination soil (substrate No.1) and potting soil (No.3) for plant growth

### 2.2 Binary Plasmids and *Agrobacterium* Strains

1. Plasmid constructs that are used as examples in this chapter are listed in table 1 (*see* Note 1).
2. *Agrobacterium tumefaciens* strain GV3101 is used for all infiltrations (*see* Note 2).

### 2.3 Media, buffers and Solutions

1. Luria-Bertani mannitol (LBman) medium: 10 g/L Bacto-tryptone, 5 g/L yeast extract, 2.5 g/L NaCl, 10 g/L mannitol, pH 7.0, autoclave at 120°C
  2. Antibiotics: 10 mg/mL rifampicin, 100 mg/mL spectinomycin and 100 mg/mL kanamycin, filter sterilize the latter two
  3. 0.2 M acetosyringone: dissolve 0.7848 g in 20 mL dimethyl sulfoxide (DMSO), filter sterilize
  4. 1 M MES (2-[N-morpholino]ethanesulfonic acid: dissolve 42.64 g MES in 200 mL ddH<sub>2</sub>O and adjust pH to 5.6 with KOH, filter sterilize
  5. LBmani: 100 mL LBman supplemented with 10 µL 0.2 M acetosyringone and 1 mL 1 M MES
  6. MMAi (made fresh): 5 g/L MS salts (Murashige & Skoog medium without gamborg B5 vitamins, Duchefa, the Netherlands), 20 g/L sucrose, 10 mM MES and 200 µM acetosyringone, non-sterile
  7. Protein extraction buffer: 25 mM Tris-HCl pH 8.0, 1 mM EDTA, 150 mM NaCl, 5 mM DL-Dithiothreitol (DTT), 0.1% NP40, 2% Poly-(Vinyl-Poly-Pyrolidone) (PVPP), 10% glycerol and 1 x Roche Complete protease inhibitor cocktail (Roche Diagnostics, Germany)
  8. Primary antibodies: PAP antibody (Sigma, USA) to detect TAP tag fused to AVR2 and primary antibody from rabbit against I-2 (van Ooijen *et al.*, 2008b)
  9. Secondary antibody: goat-anti-rabbit conjugated with horseradish peroxidase (Rockland, USA)
  10. Trypan blue staining solution: 10 mL lactic acid (DL Sigma L-1250), 10 mL phenol (Buffer-saturated, Invitrogen, USA), 10 mL glycerol, 10 mL ddH<sub>2</sub>O and 10 mg trypan blue (Sigma-Aldrich, USA)
  11. Trypan blue destaining solution: dissolve 250 g chloral hydrate (Sigma-Aldrich, USA) into 100 mL ddH<sub>2</sub>O
  12. 800 mM mannitol: dissolve 14.61 g mannitol into 100 mL ddH<sub>2</sub>O
- ### 2.5 Laboratory Materials and Equipments
1. Spectrophotometer (HITACHI, Japan)
  2. Capped 50 mL polypropylene tubes

3. ROTINA 46 R Centrifuge (Depex, the Netherlands)
4. New Brunswick Incubator (EDISON, NJ, USA)
5. 1 or 2 mL syringes and one syringe needle (BD Plastipak, Madrid, Spain)
6. Liquid nitrogen, mortar and pestle
7. Miracloth™ (EMD Chemicals)
8. Eppendorf 5415 R Centrifuge (Eppendorf, Germany)
9. Perfection 1200 Scanner (EPSON, USA)
10. PVDF membrane
11. LSM 510 confocal microscopy (Zeiss, Germany)

## Methods

### 3.1 *Nicotiana benthamiana* plant growth

1. Germinate 15 seeds in 10 cm square plastic pots containing standard germination soil at 25°C with 70% humidity and 15 h light and 9 h dark photoperiod with 74  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity.
2. Transfer the seedlings 8 days after sowing to a 10 cm plastic pot containing potting soil (one seedling per pot) and grow the plant at 22°C with 60% humidity and 12 h light and 12 h dark photoperiod with 159  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity (*see Note 3*).
3. Three weeks after transplanting the plant has the optimal developmental stage to be used for agroinfiltration (4-5 week old). At this stage the plant has at least five fully developed true leaves and no visible flower buds (Figure 1) (*see Note 4*).

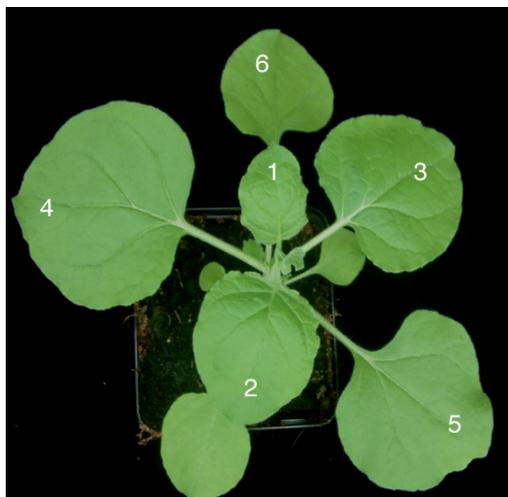
### 3.2 *A. tumefaciens* culturing and preparation of the bacterial suspension

1. Inoculate 4 mL LBman starter cultures containing 25 mg/L rifampicin and 100 mg/L spectinomycin or 50 mg/L kanamycin in a capped 25 mL glass culture tube with the *A. tumefaciens* strain GV3101 harboring the desired plasmid (Table 1) directly from the glycerol stock. Incubate the starter cultures at 28°C under agitation (220 rpm) for 24 h to allow growth (*see Note 5*).
2. Inoculate 10  $\mu\text{L}$  starter cultures into 20 mL LBmani containing 25 mg/L rifampicin and 100 mg/L spectinomycin or 50 mg/L kanamycin in 50 mL polypropylene tube with screw cap and grow approx. 18 h at 28°C with shaking (220 rpm) (*see Note 6*).
3. Measure the OD<sub>600</sub> of the overnight cultures with a spectrophotometer, this should be in the range of 0.6-2.0; an OD<sub>600</sub> of 0.8 is optimal.
4. Pellet the cells by centrifugation for 15 min at 2,800 g using ROTINA 46 R (*see Note 7*) and gently resuspend the cells in MMAi medium to the required final OD<sub>600</sub> value (*see Note 8*) and incubate minimally for 1 h at room temperature.

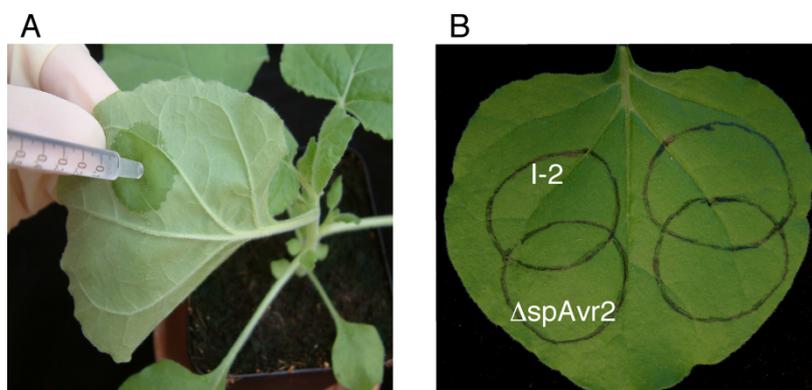
### 3.3 Leaf Selection, Agroinfiltration and Plant Incubation

1. One day prior to infiltration, the 4-5 weeks old *N. benthamiana* plants have to be transferred to a greenhouse compartment (20°C) with low light (*see Note 9*).

2. Use the second, third and fourth leaf for protein expression (Figure 1). The third and fourth leaves are best used for visualization of HR and determination of the *in planta* localization of R or AVR proteins (see Note 10).



**Figure 1.** Optimal growth stage of *N. benthamiana* used for agroinfiltration. Counting from the top of the plant, leaves indicated with number 2, 3 and 4 are best used for agroinfiltration.

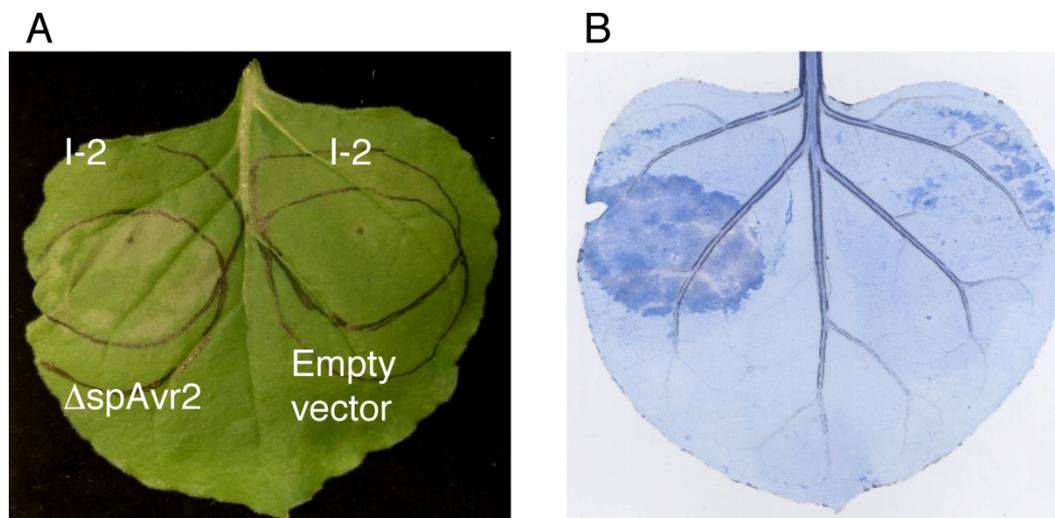


**Figure 2.** **A)** *Agrobacterium* suspension is infiltrated into a *N. benthamiana* leaf. **B)** To visualize specific HR triggered by co-expression of an *R* and *Avr* gene pair they are infiltrated such that both genes are expressed in the overlapping region, which is marked by a black marker.

3. Use a syringe needle to make a small scratch at the lower side of the leaf. Fill a 1 mL needleless syringe with the *Agrobacterium* suspension, and place the syringe tip on the scratch and apply slight counter-pressure to the upper side of the leaf with your gloved finger (Figure 2A).
4. Push the piston slowly down to force entry of the *Agrobacterium* suspension into the leaf. Filling of the apoplastic spaces with the bacterial culture is visible by the formation of a dark-green sector (Figure 2A) (see Note 11). To infiltrate an entire leaf (for protein expression) multiple infiltration points might be necessary.
5. For visualization of HR, infiltrate the *Agrobacterium* suspension carrying either the *R* gene or *Avr* gene. Dry the leaf surface with a tissue and mark the edge of the infiltrated

circle with a black marker. Wait until the infiltration zone is dry (2-3 h) and infiltrate the *Agrobacterium* suspension containing the other gene to form a second overlapping circle, also mark the second circle (Figure 2B) (see Note 12).

6. Keep the plants at 20°C and shield them from direct light, for instance by placing them under a table that is surrounded with black plastic.



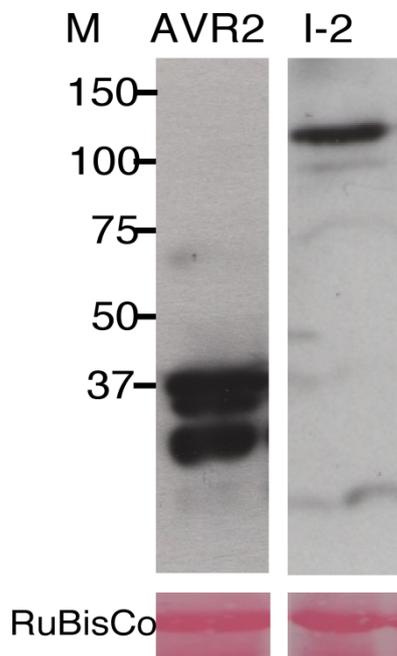
**Figure 3.** Typical example of an *N. benthamiana* leaf showing HR. **A)** HR is induced in the overlapping region that expresses both *Avr2* and *I-2*. **B)** Cell-death representing HR is visualized with a trypan blue staining. There is no HR induction in the negative control (empty vector) or in the regions expressing the genes alone.

7. For protein localization studies (see section 3.5.2), collect the leaves at approximately 36 h post infiltration.
8. For protein expression studies (see section 3.5.1), collect the leaves at approximately 48 h after infiltration and either use them fresh, or transfer them to 50 mL polypropylene tubes and snap-freeze in liquid nitrogen. The frozen leaves can then be stored -80°C (see Note 13).
9. For HR staining studies (see section 3.4), collect the leaves at approximately 60 h after infiltration.
10. HR is normally apparent 3 days-after-infiltration (see Note 14).

### 3.4 Trypan Blue Staining to Visualize Cell Death during Development of the Hypersensitive Response

1. Collect the leaves for trypan blue staining 60 h after agroinfiltration. At this stage HR just becomes visible with the naked eye (Figure 3A) (see Note 15).
2. Mix 50 mL staining solution with 50 mL 96% ethanol (ratio 1:1) in a 1 liter glass beaker.
3. Cover the beaker with a glass plate to avoid evaporation of the ethanol and heat the mixture Bain-marie (by placing the beaker in a boiling water bath) until boiling.
4. Place 1 leaf in the 100 mL solution.

- Boil the leaf for approx. 5 min until its green colour has vanished completely (*see* Note 16).



**Figure 4. Western blot showing production of AVR2 and I-2 extracted from agroinfiltrated *N. benthamiana* leaves.** The Ponceau S staining of RuBisCo (lower panel) is used to confirm equal loading and efficient protein transfer during blotting. The protein size ladder is indicated on the left.

- Transfer max. 2 leaves to a 50 mL polypropylene tube and add 5 mL destaining solution, rotate the tubes overnight at room temperature (*see* Note 17).
- Transfer the leaf when it is fully destained to a petri dish and scan the leaf using Perfection 1200 scanner (Figure 3B) (best results are obtained with light from the top of the scanner).

### 3.5 Detection of Resistance and Effector Protein Expression

#### 3.5.1 Protein extraction for western blot analysis

- Crush the frozen leaves in a 50 mL polypropylene tube with a pestle and weight the tube.
- Place the leaf pieces into a mortar that is pre-cooled with liquid nitrogen and grind the leaves in liquid nitrogen to a fine powder.
- Transfer the powder to a second mortar pre-cooled with ice and add 2 mL ice-cold protein extraction buffer per 1 g of leaf powder (*see* Note 18).
- Keep the mortar on ice and transfer the slurry using a pipet to a 2 mL Eppendorf vial (*see* Note 19).
- Centrifuge for 20 min at 14,000 rpm at 4°C in an Eppendorf centrifuge. Pour supernatant through 4 layers of Miracloth into an Eppendorf tube (*see* Note 20).
- The protein extract can now be used to analyze R and AVR protein expression by western blot analysis (western blot) (*see* Note 21). Typically 30 µL protein extract is used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. After running the gel, the size separated proteins are transferred to PVDF membrane and detection is done according to (van Ooijen *et al.*, 2008a). An example showing the production of AVR2 and I-2 is provided in Figure 4.

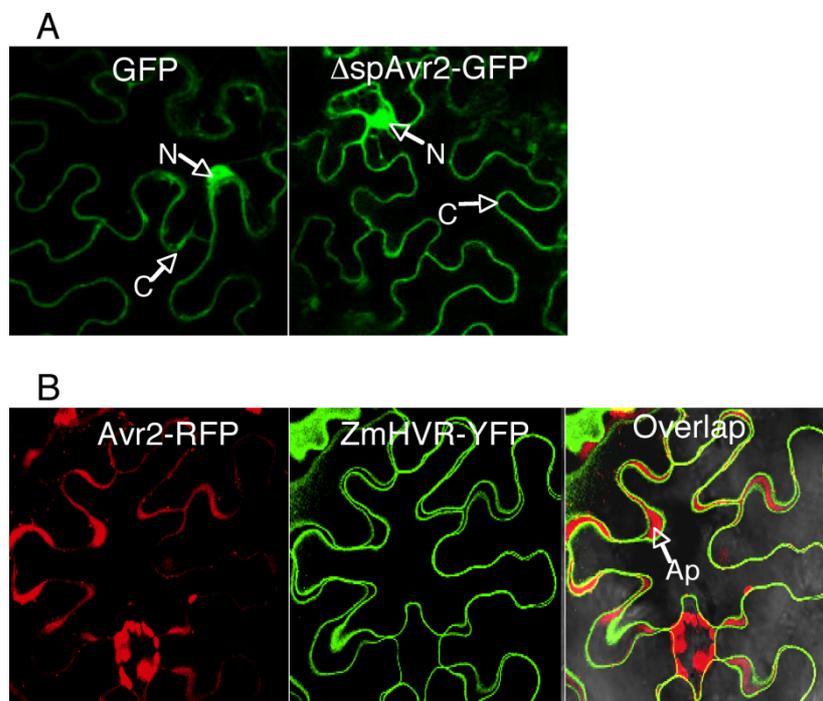
### 3.5.2 *In planta* Localization of Recombinant Proteins

1. Harvest the infiltrated leaves 36 h after infiltration. This example describes the use of *i)* cytoplasmically localized AVR2 labelled with GFP or *ii)* apoplastically localized AVR2 labelled with RFP.
2. Cut the leaf into 3 x 3 mm pieces and place it with its lower side facing up on a microscope slide.
3. Mount the leaf piece with ddH<sub>2</sub>O and cover it with a cover slide.
4. Place a drop of immersion oil on the objective of LSM 510 confocal microscopy and place the sample under the objective.
5. Excite the GFP at 488 nm and capture emission with a 505-530 nm pass filter. The images are typically scanned 8 times (Figure 5A).
6. To detect apoplastic localisation of Avr2-RFP treat the small leaf pieces co-infiltrated with Avr2-RFP and a plasma membrane marker labelled with YFP with 800 mM mannitol for 30 min to induce plasmolysis. Subsequently, mount the pieces in 30% glycerol on the slide (*see* Note 22).
7. For the localization of Avr2-RFP, excite the RFP at 543 nm and the YFP at 514 nm. Capture YFP with a 505-530 nm filter and RFP with 565-615 nm filter. The images are scanned 8 times (Figure 5B).

#### Notes

1. Other binary vectors can also be used for agroinfiltration (Hellens *et al.*, 2005). However, we typically obtain highest expression levels using pPZP200 derived vectors (30, 31).
2. The GV3101 strain is routinely used in our laboratory. Other *Agrobacterium* strains including LBA4404, C58C1, EHA105 and AGL0 have also been used successfully by us and others (D'Aoust *et al.*, 2008; Pruss *et al.*, 2008).
3. Various growth conditions have to be tested to identify optimal conditions for protein accumulation and HR. High humidity and temperature during the growth of the plants are detrimental to both processes.
4. During winter recombinant protein yield and HR development are often compromised. Although the underlying mechanism is not known we suspect this is due to altered physiology of the plants grown during this season.
5. Transformation of *Agrobacterium* is performed as described by Takken *et al.* (Takken *et al.*, 2000) and transformants need to be stored at -80°C in 30% glycerol.
6. When inoculating the starter cultures after 16:00, the OD<sub>600</sub> should range between 0.6 and 2.0 after overnight growth.
7. When the cultures have reached the desired OD<sub>600</sub>, it is not longer necessary to work under sterile conditions.
8. The optimal OD<sub>600</sub> used for infiltration depends on the gene-of-interest. We recommend testing different ODs, but typically for expression of *R* or *Avr* genes we use an OD<sub>600</sub> of

- 1.0. To visualize HR, the OD<sub>600</sub> for the *Agrobacterium* culture carrying the *R* gene lays between 0.2 and 0.5 and for the ones carrying the *Avr* gene at 0.5. An OD<sub>600</sub> that is too high for the *Agrobacterium* containing the *R* gene can trigger AVR-independent HR.
9. Placing the plants under the table to shield them from direct sun and artificial light gives more consistent results.



**Figure 5.** **A)** Transient expression of agroinfiltrated GFP and  $\Delta$ spAvr2-GFP in *N. benthamiana* leaves. GFP and  $\Delta$ spAvr2-GFP are localized in the nucleus and cytoplasm. Position of nucleus and cytoplasm are indicated with N and C, respectively. **B)** Co-transient expression of Avr2-RFP and the plasma membrane marker ZmHVR-YFP in the epidermal cells of *N. benthamiana* after plasmolysis. Avr2-RFP is clearly presented in the apoplastic space (Ap), which is enlarged due to plasmolysis.

10. Although the younger (second) leaves normally shows highest protein accumulation, the third and fourth leaves can also be used for protein expression. For HR visualization, the second leaf often shows aspecific HR development, which is possibly due to overexpression of the *R* gene. Therefore it is best to use the third and fourth leaves for these purposes.
11. Wear glasses and gloves when doing syringe infiltrations.
12. The overlapping method is used to have internal controls on the leaf in which only one of the two genes is expressed. Try to keep the scratches small to confer minimal damage to the leaves.
13. When using autoactive R proteins, which trigger HR in the absence of an AVR protein, it is necessary to collect leaves already at 24 h after infiltration or earlier when the infiltrated sector starts to collapse (van Ooijen *et al.*, 2008b).
14. Onset and timing of HR differs for each *R* and *AVR* gene combination. For *I-2* and *Avr2* the first symptoms are visible two days-after-infiltration and the overlapping region at

the lower side of leaf becomes ‘shiny’. HR developing after day five is considered to be aspecific.

15. For trypan blue staining the leaves have to be harvested before the HR has fully developed. Leaves can be stored overnight at 4°C for staining next day. If the HR has already fully developed staining of the necrotic sector is often poor. Furthermore, a late staining increases background for the sectors only expressing the *R* gene.
16. Fresh ethanol can be added to the staining solution during boiling. The solution should be replaced after boiling five to six leaves.
17. Replacing the destaining solution before overnight incubation reduces background as do longer incubation times (24 h).
18. Since PVPP is not soluble, it is necessary to suspend it in the protein extraction buffer before usage and to use wide-bore pipet tips.
19. The slurry can be transferred to a 2 mL Eppendorf tube using a regular pipet-tip. For efficient protein extraction, the leaves need to be ground quickly and thoroughly while keeping them cold.
20. Instead of Miracloth to clear the protein lysate also a sephadex G-25 column pre-equilibrated with extraction buffer can be used. The latter method results in less background on western blot.
21. Freshly prepared protein extracts give the best results on western blot.
22. The epidermal cells of *N. benthamiana* have big vacuoles making it is difficult to determine the exact localization of proteins residing in either cytoplasm, plasma membrane or apoplast. Plasmolysis can facilitate determination of their localization. However, plasmolysis is inefficient in *N. benthamiana* leaves, but can be increased by incubating an intact leaf in plasmolysis solution (700 mM sucrose) for 5-6 h before analysing it by confocal microscopy (Lim *et al.*, 2009).

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### Supplementary data

**Table 1 List of Plasmid Constructs**

Constructs	Aims
CTAPi (Rohila <i>et al.</i> , 2004)	Empty vector; negative control for HR
CTAPi-I-2	I-2, triggers HR when combined with AVR2
CTAPi- $\Delta$ spAvr2 (Houterman <i>et al.</i> , 2009)	c.p. localised AVR2, triggers HR together with I-2
pGWB451- $\Delta$ spAvr2-GFP (Nakagawa <i>et al.</i> , 2007)	c.p. localised AVR2 labelled with GFP
pB451-GFP	c.p. localised GFP
pGWB454-Avr2-RFP	Apoplastic localised AVR2 labelled with RFP
pBin19-ZmHVR-YFP*	Plasmamembrane marker labelled with YFP

\* Select on 50 mg/L kanamycin, others are 100 mg/L spectinomycine

\*\*c.p. = cytoplasmic

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