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# Virus-induced gene silencing in rose flowers

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**Abstract:** Virus-induced gene silencing (VIGS) is a favorable method to study gene function by post-transcriptional gene silencing in plants. Here we describe a methodology of graft-accelerated VIGS in rose aimed at obtaining post-transcriptional gene silencing in the flower. The resulting phenotype can be observed within five to six weeks post infiltration. By using this method, we successfully silenced the expression of several genes involved in processes such as scent production, petal coloration or flower architecture. We showed that graft-accelerated VIGS was faster, more efficient and more

convenient than conventional methods previously developed in rose such as agroinfiltration of young plantlets, *in vitro* cultured tissues or seeds.

**Key words:** VIGS, Rose, Vacuum infiltration, Graft, Post-transcriptional gene silencing.

## 1 Introduction

Virus-induced gene silencing is a rapid approach to study gene function by knocking down gene expression in plants (1,2). VIGS is based on post-transcriptional gene silencing (PTGS), in which a sequence specific RNA degradation is triggered by double stranded RNA (1). In VIGS, a recombinant virus carrying a partial sequence of the target gene is used to infect the plant. When the virus replicates and spreads throughout the whole plant, the viral gene transcripts are recognized and degraded together with those of the plant target gene by plant endogenous PTGS, and the expression of the target gene is knocked down (3,4).

Nowadays, VIGS has been broadly used for functional characterization of genes in different plant organs, including leaves of eggplant (5), leaves and tubers of potato (6), cornels of *Gladiolus hybridus* (7), roots of *Nicotiana benthamiana*, *Arabidopsis thaliana* and *Lycopersicon esculentum* (8), fruits of tomato (9, 10), as well as flowers of *Petunia hybrida* (11).

Regarding rose, a VIGS approach using young plantlets issued from *in vitro* cultivated rose cuttings was established by Tian et al (2). The plantlets were submerged in an *Agrobacterium tumefaciens* suspension carrying the vectors encoding the

recombinant Tobacco Rattle Virus (TRV). Vacuum infiltration was used to transform the plant material. Using this procedure, the infected plantlets flowered about two months post inoculation and efficiency of the transformation procedure could be evaluated by observing alteration in flower coloration (2), or petal abscission (12). However, the long delay between agro-inoculation and flowering often leads to attenuated efficiency of gene silencing. Furthermore, when the transformed plantlets are issued from *in vitro* culture, rooting may be difficult for some rose varieties (e.g. *R. odorata* var. *gigantea*). In addition, when plantlets are obtained directly from seeds, cross pollination can also lead to trait segregation (13, 14).

Recently, an alternative approach based on recombinant TRV inoculation in detached rose petals, proved to be able to induce gene silencing (15). An observable phenotype and a measurable down expression of the target gene were detected within a week. This method has been applied to study several processes linked to petal physiology, namely dehydration tolerance (15), petal expansion (16), as well as senescence (17). However, this method has limitations. For instance, detached rose petals can obviously not be used to study flower development or architecture, as well as petal abscission.

Some of the limitations of the previously described procedures could be overcome thanks to the recent development and use of a novel method for rapid gene silencing in complete rose flowers, called 'graft-accelerated VIGS' (18). In our hands graft-accelerated VIGS proved to be able to generate silenced flowers in 30-35 days with an improved efficiency : 46% silenced flowers versus 10% to 30% for previously

developed agroinfiltration methods. We previously demonstrated that this procedure could be used for rapid and efficient studies of gene function in rose flowers, such as genes involved in fragrance production, flower architecture, as well as petal coloration (18, 19). The rose genome has recently been uncovered (20, 21), thus enabling to study the function of genes involved in many processes of interest such as fragrance biosynthesis, development *etc...* This brings a real need to develop reliable methods for reverse genetic studies. We propose that graft-accelerated VIGS can be extensively used in reverse genetics of rose flowers.

## 2 Materials

### 2.1 Media and buffers

1. YEB medium: 1 g/L yeast extract, 5 g/L tryptone (see **Note 1**), 5 g/L beef extract, 5 g/L sucrose, 2 g/L MgSO<sub>4</sub>, 10 mM MES, pH 7.0, 20 mM acetosyringone, 50 mg/L kanamycin. Solid YEB medium contained 15 g/L agar.
2. 50 µg/mL kanamycin stock solution in water and 25 µg/mL rifampicin stock solution in DMSO.
3. *Agrobacterium* infiltration buffer: 10 mM MgCl<sub>2</sub>, 10 mM MES, pH 5.6, 150 mM acetosyringone.

### 2.2 Vectors and constructs

1. The binary pTRV vectors, pTRV1 and pTRV2 were used for VIGS experiments (3). The pTRV1 plasmid contains genes for virus replication and spread, while pTRV2

encodes virus coat proteins and carries a fragment of the VIGS-targeted gene of interest (see **Note 2**). Both vectors carry duplicated CaMV 35S promoter ( $2 \times 35S$  promoter) and the selection marker for kanamycin resistance. The gene of interest fragment sequence to be inserted in the pTRV2 vector should be 200-400 bp and specific to the gene to be silenced. The SGN VIGS Tool (<http://vigs.solgenomics.net/>) (22) now includes the genome of *Rosa chinensis* 'Old Blush' and can be used to design the fragment sequence and avoid off-target silencing. The gene fragment can be inserted in the pTRV2-gateway vector through Gateway LR cloning using the attR1 and attR2 integration sites located on the vector (see **Note 3**).

### **2.3 Plant material**

1. *Rosa chinensis* 'Old Blush' plants.
2. Greenhouse: 13h / 11h day/night cycle, 28 / 20 °C day / night temperature, the relative humidity was 75% - 80% (see **Note 4**).

### **2.4 Equipments**

1. Spectrophotometer
2. Bacterial incubator
3. Vacuum pump and vacuum bell jar
4. Centrifuge
5. Polyethylene plastic gloves
6. Parafilm

7. Grafting knife

8. Thermocycler

### **3 Methods**

All procedures are carried out at room temperature (see **Note 5**).

#### **3.1 Preparation of the scions**

1. The grafted organ was taken from a stem with axillary sprouts. It was cut from the plant as a scion. The scion must be sprouting (not dormant), with one axillary vegetative bud located approximately in its middle, and should be about 4 cm long (**Fig.1A**) (see **Notes 6 and 7**).

2. The scions were kept in the water until grafting (**Fig. 1B**).

#### **3.2 Vacuum agro-infiltration**

1. The *Agrobacterium* strain EHA105 (see **Note 8**) was transformed with pTRV1 or with recombinant pTRV2 carrying a fragment of the gene of interest (pTRV2-GOI). The resulting agrobacteria were grown at 28 °C on YEB solid medium containing 50 µg/mL kanamycin for selecting the pTRV1 and pTRV2 plasmids and 25 µg/mL rifampicin for selection of the *Agrobacterium* strain.

2. 5 mL YEBmedium containing 50 µg/mL kanamycin and 25 µg/mL rifampicin was inoculated with colonies issued from the pTRV1 or pTRV2-GOI transformations and grown overnight at 28 °C under continuous agitation (180 rpm).

3. The next day, 100  $\mu$ L of the overnight *Agrobacterium* cultures were inoculated into 50 mL YEB liquid medium containing 50  $\mu$ g/mL kanamycin and 25  $\mu$ g/mL rifampicin, and cultured in a shaker at 28 °C and 180 rpm until OD<sub>600</sub> reaches a value of 0.3 to 0.5.
4. The *Agrobacteria* containing pTRV1 or recombinant pTRV2-GOI were harvested by centrifugation (500 g, 15 min), resuspended in *Agrobacterium* infiltration buffer and mixed in a ratio of 1:1 (v/v).
5. The cellular density was finally adjusted to an OD<sub>600</sub> of 1.0 with infiltration buffer (see **Note 9**). The resulting suspension was kept in the dark at room temperature for 2 h prior to vacuum infiltration for virulence gene activation with acetosyringone.
6. Twenty rose scions per gene/construct were submerged in 80 mL *Agrobacterium* suspension in a 100 mL glass beaker. The beaker was transferred into a vacuum bell jar (**Fig.1C**). A -25 kPa vacuum was applied to the preparation for 60 s before being released slowly. The procedure was repeated a second time in identical conditions.
7. The treated scions were then washed for three seconds in distilled water and grafted onto appropriate rootstocks (see **Note 10**).

### 3.3 Grafting

1. Using a grafting knife, a wedge-shaped cut was performed at the basal extremity of the infiltrated scions to be grafted onto the rootstocks (**Fig.2A**).
2. The plant to be used as rootstock was from the same cultivar as the scion. Branches 0.5 cm to 1 cm in diameter were used as rootstocks. The branch that was chosen to receive the graft was cut near the middle of the stem, between two nodes. Then, buds



and leaves were removed from the stem (**Fig. 2B**) (see **Note 7**). Finally, a grafting knife was used to split the rootstock in two equal parts over a height of about 1.5 cm.

3. The scion has to be inserted immediately after preparing the rootstock. It should be pushed all the way down into the cut, keeping the cambium layers of scion and rootstock in contact (**Fig.2C**). Finally, a stretchy material such as Parafilm laboratory film was wrapped around the junction to hold the scion in place (**Fig.2D**) (see **Notes 11 and 12**).

4. Grafted material was kept in humid conditions for 7-10 days by glove fingers cut in polyethylene plastic gloves (**Fig.2E, 2F**) (see **Note 13**).

5. The grafted organ bloomed 30 to 40 days after grafting (see **Note 14**) (**Fig.2G**). In our hands the percent survival of the graft was 90%.

6. The efficiency of the VIGS procedure was assessed by RT-PCR measurement of down expression of the target gene in flowers and by apparition of a knock down phenotype. As an example, targeting of the *Nudix hydrolase 1* gene (*RhNUDX1*) (GenBank accession number JQ820249) (**24**) involved in synthesis of the scent compound geraniol, yielded significant decrease in both gene expression (**Fig.3**) and geraniol emission (**Table 1**) in silenced plants compared to control. Among the surviving grafted organs, the percent of material, which was actually transformed and presented a typical phenotype was about 46% (**18, 19**).

## Notes

1. Bacteriological tryptone can be replaced by bacteriological peptone.
2. The pTRV1 plasmid can be ordered from the *Arabidopsis* Biological Resource Center

(ABRC): stock no. CD3-1039, vector name YL192.

The Gateway-compatible pTRV2 vector corresponds to ABRC stock no. CD3-1041, vector name YL279.

3. Following cloning the pTRV2 will contain the antisense sequence of the gene of interest. Cloning into pTRV2 can also be performed by conventional restriction digestion based cloning or ligation independent cloning (LIC) (23).

4. Culture conditions of the plants both prior and after grafting should be carefully monitored. The temperature is a key factor for the success of this procedure, especially for the successful development of the grafted organ since callus development is favored in the indicated conditions.

5. All genetically modified organisms should be cultured, grown, transported, or used in proper way according to requirement of local legislation.

6. For grafting, the terminal bud of the scion was removed.

7. Ideally the diameters of the scion and rootstock should be the same (0,5 – 1 cm).

8. Agrobacterium strain GV3101 can be used instead of EHA105.

9. The volume of infiltration mixture to prepare should be about 80 mL for twenty scions.

10. Following infiltration, the scions are kept in water until grafting. It takes about one hour for a trained experimenter to graft 20 scions.

11. Parafilm film can be replaced by cling film.

12. Several scions can be grafted on different branches of the same plant as long as they are transformed by the same construct.

13. The key point is to have a fast growing tissue to favor wound healing between the scion and the rootstock.

14. The blooming time depends on temperature. The optimal day temperature is 25-28 ° C, and night temperature should be around 18-20 ° C. Lowering temperature below 24 °C delayed blooming by more than 10 days.

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### **References**

1. Senthil-Kumar M, Mysore KS (2011) New dimensions for VIGS in plant functional genomics. *Trends Plant Sci* 16: 656-665
2. Tian J, Pei H, Zhang S, Chen J, Chen W, Yang R, Meng Y, You J, Gao J, Ma N (2014) TRV-GFP: a modified Tobacco rattle virus vector for efficient and visualizable analysis of gene function. *J Exp Bot* 65: 311-322
3. Liu Y, Schiff M, Dinesh-Kumar S (2002) Virus-induced gene silencing in tomato. *Plant J* 31: 777-786
4. Burch-Smith TM, Anderson JC, Martin GB, Dinesh-Kumar SP(2004) Applications and advantages of virus-induced gene silencing for gene function studies in plants. *Plant J* 39: 734-746

5. Liu H, Fu D, Zhu B, Yan H, Shen X, Zuo J, Zhu Y, Luo Y (2012) Virus-induced Gene Silencing in Eggplant (*Solanum melongena*). *J Integr Plant Biol* 54: 422–429
6. Faivre-Rampant O, Gilroy EM, Hrubikova K, Hein I, Millam S, Loake GJ, Birch P, Taylor M, Lacomme C (2004) Potato virus X-induced gene silencing in leaves and tubers of potato. *Plant Physiol* 134: 1308-1316
7. Zhong X, Yuan X, Wu Z, Khan M A, Chen J, Li X, Gong B, Zhao Y, Wu J, Wu C (2014) Virus-induced gene silencing for comparative functional studies in *Gladiolus hybridus*. *Plant Cell Rep* 33: 301-312
8. Valentine T, Shaw J, Blok VC, Phillips MS, Oparka KJ, Lacomme C (2004) Efficient virus-induced gene silencing in roots using a modified tobacco rattle virus vector. *Plant Physiol* 136: 3999-4009
9. Fu DQ, Zhu BZ, Zhu HL, Jiang WB, Luo YB (2005) Virus-induced gene silencing in tomato fruit. *Plant J* 43: 299-308
10. Zhu HL, Zhu BZ, Shao Y, Wang XG, Lin XJ, Xie YH, Li YC, Gao HY, Luo YB (2006) Tomato fruit development and ripening are altered by the silencing of *LeEIN2* gene. *J Integr Plant Biol* 48, 1478–1485
11. Chen J, Jiang C, Gookin T, Hunter D, Clark D, Reid M (2004) Chalcone synthase as a reporter in virus-induced gene silencing studies of flower senescence. *Plant Mol Biol* 55: 521-530
12. Gao Y, Liu C, Li X, Xu H, Liang Y, Ma N, Fei Z, Gao J, Jiang CZ, Ma C (2016) Transcriptome profiling of petal abscission zone and functional analysis of an Aux/IAA family gene RhIAA16 involved in petal shedding in rose. *Front Plant Sci* 7: 1375

13. Sassa H, Nishio T, Kowyama Y, Hirano H, Koba T and Ikehashi H (1996)  
Self-incompatibility (S) alleles of the Rosaceae encode members of a distinct class of the  
T 2/S ribonuclease superfamily. *Mol Gen Genet* 250: 547-557
14. Serge G (2001) Rose breeding technologies. *Acta Hort* 547: 23-26
15. Dai F, Zhang C, Jiang X, Kang M, Yin X, Lü P, Zhang X, Zheng Y, Gao J (2012)  
RhNAC2 and RhEXPA4 are involved in the regulation of dehydration tolerance during  
the expansion of rose petals. *Plant Physiol* 160: 2064-2082
16. Luo J, Ma N, Pei H, Chen J, Li J, Gao J (2013) A DELLA gene, *RhGAIL*, is a direct  
target of EIN3 and mediates ethylene-regulated rose petal cell expansion via repressing  
the expression of *RhCesA2*. *J Exp Bot* 64: 5075-5084
17. Lü P, Zhang C, Liu J, Liu X, Jiang G, Jiang X, Khan M A, Wang L, Hong B, Gao J  
(2014) RhHB1 mediates the antagonism of gibberellins to ABA and ethylene during rose  
(*Rosa hybrida*) petal senescence. *Plant J* 78: 578-590
18. Yan HJ, Shi S, Ma N, Cao X, Zhang H, Qiu X, Wang Q, Jian H, Zhou N, Zhang Z,  
Tang KX (2018) Graft-accelerated virus-induced gene silencing facilitates functional  
genomics in rose flowers. *J Integr Plant Biol* 60: 34-44.
19. Yan H, Baudino S, Caissard JC, Nicolè F, Zhang H, Tang K, Li S, Lu S (2018)  
Functionnal characterization of the eugenol synthase gene in rose. *Plant Physiol  
Biochem* 129: 21-26
20. Raymond O, Gouzy J, Just, Badouin H, Verdenaud M, Lemainque A, Vergne P, Moja S,  
Choisne N, Pont C, Carrère S, Caissard JC, Couloux A, Cottret L, Aury JM, Szecsi J,  
Latrasse D, Madoui MA, François L, Fu XP, Yang SH, Dubois A, Piola A, Larrieu A,

- Perez M, Labadie K, Perrier L, Govetto B, Labrousse Y, Villand P, Bardoux C, Boltz V, Lopez-Roques C, Heitzler P, Vernoux T, Vandebussche M, Quesneville H, Boualem A, Bendahmane A, Liu C, Le Bris M, Salse J, Baudino S, Benhamed M, Wincker P, Bendahmane M (2018) The *Rosa* genome provides new insights into the domestication of modern roses. *Nature Genetics* 50: 772-777
21. Hibrand Saint-Oyant L, Ruttink T, Hamama L, Kirov I, Lakhwani D, Zhou NN, Bourke PM, Daccord N, Leus L, Schulz D, Van de Geest H, Hesselink T, Van Laere K, Debray K, Balzergue S, Thouroude T, Chastellier A, Jeuffre J, Voisine L, Gaillard S, Borm TJA, Arens P, Voorrips RE, Maliepaard C, Neu E, Linde M, Le Paslier MC, Bérard A, Bounon R, Clotault J, Choisine N, Quesneville H, Kawamura K, Aubourg S, Sakr S, Smulders MJM, Schijlen E, Bucher E, Debener T, De Riek J, Foucher F (2018) A high-quality genome sequence of *Rosa chinensis* to elucidate ornamental traits. *Nature Plants* 4: 473-484
22. Fernandez-Pozo N, Rosli HG, Martin GB, Mueller LA (2015) The SGN VIGS tool: user-friendly software to design virus-induced gene silencing (VIGS) constructs for functional genomics. *Mol Plant* 8: 486-488
23. Dong Y, Burch-Smith TM, Liu Y, Mamillapalli P, Dinesh-Kumar SP (2007) A ligation-independent cloning tobacco rattle virus vector for high-throughput virus-induced gene silencing identifies roles for NbMADS4-1 and -2 in floral development. *Plant Physiol* 145: 1161-1170
24. Magnard JL, Roccia A, Caissard JC, Vergne P, Sun P, Hecquet R, Dubois A, Hibrand-Saint Oyant L, Jullien F, Nicolè F (2015) Biosynthesis of monoterpene scent

compounds in roses. Science 349: 81-83

**Table 1. Geraniol emission in control and *RhNUDX1* silenced flowers.**

	<b>NT</b>	<b>TRV::00</b>	<b>Line A</b>	<b>Line B</b>	<b>Line C</b>
<b>Relative content (%)</b>	4.19 ± 0.20	3.73 ± 0.35	1.52 ± 0.38	2.16 ± 0.52	1.51 ± 0.27

Rose flowers (*R. chinensis* ‘Old Blush’) were harvested at the fully open stage of development. Floral volatiles were collected by solid-phase micro-extraction (SPME) at 30 °C for 45 min using a 75 µm CAR/PDMS SPME fiber (Supelco, PA, USA) inserted into a 100 mL sample vial containing 1 g of petals. The SPME fiber was then inserted into the front inlet of a gas chromatograph (GC, HP 6890A) connected to a mass spectrometer (MS, HP 5973, GC/MS: Agilent Technologies, USA) and desorbed at 250 °C for 2 min. The temperature of the transfer line and ion trap were 250 °C and 300 °C, respectively. Values correspond to the emission of geraniol as a percentage of total volatiles and are the means ± standard deviations of three independent measurements. Turkey's test indicates significant differences ( $p < 0,01$ ) between silenced lines (A, B or C) and both not transformed or TRV::00 transformed plants. See Figure 3 for sample nomenclature.



## Figure captions

### Figure 1. Preparation of grafting scions and vacuum infiltration.

(A) Sampling of the scion used for vacuum infiltration. (B) The scions in water prior to vacuum infiltration. (C) Vacuum bell jar and pump used for agrobacterium infiltration.

### Figure 2. Grafting procedure

(A) Wedge-shaped section on transformed scions. (B) Rose branch used as a rootstock. (C) Grafting of an infiltrated scion onto a rootstock. (D) Fixation of the graft using Parafilm laboratory film. (E) PE plastic glove fingers. (F) Positioning of PE plastic gloves on grafted material. (G) Blooming of the first flower on a grafted scion. The arrow points to the junction between the scion and rootstock.

### Figure 3. Silencing of *RhNUDX1* in *R. chinensis* 'Old Blush'.

(A) Expression of *RhNUDX1* (24) in non-inoculated flowers (NT), flowers transformed by an empty pTRV2 vector (TRV::00) and by a vector containing a 331bp fragment of the *RhNUDX1* gene (TRV::*RhNUDX1*). Lines A, B and C represent three independent *RhNUDX1* silenced flowers. Semi quantitative RT-PCR was performed with the *RhNUDX1*-specific primers RhNUDX1-F (5'-TGTTGAGAGGCCAAAACGTG-3') and RhNUDX1-R (5'-GTACTGCGATGGTTTGGCTT-3'). Glyceraldehyde phosphate dehydrogenase (*RhGAPDH*) amplified with primers RhGAPDH-F (5'-ATCCATTCATCACCACCGACTACA-3') and RhGAPDH-R (5'-GCATCCTTACTTGGGGCAGAGA-3') was used as a reference gene.







