Virus induced gene silencing from model to crops: development and applications for the functional characterization of plant genes associated to pathogen resistance

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I / A modified Tobacco rattle virus (TRV) vector displays an increased root tropism and trigger a pervasive VIGS response in roots (Valentine *et al.*, 2004).

We demonstrate that a modified TRV vector retaining the helper-protein 2b, (required for transmission by a specific vector nematode), not only invades and replicates extensively in the whole of the plant (Figure 1) including meristems (Figure 2) but also triggers a pervasive VIGS response in *N. benthamiana* and *A. thaliana* roots. TRV-2b silencing efficacy and sustained VIGS response was demonstrated by silencing genes involved in root development (*root hairless1*, β-*tubulin;* Figure 3 left panel) and the resistance gene *Mi* associated to root-knot nematode resistance in tomato (Figure 3 right panel). The resultant TRV-2b vector displays an increased infectivity and root tropism than TRV 2b-deleted vector (Figure 1), key characteristics for VIGS-based functional characterization of genes in root tissues. This suggests that the TRV helper-protein 2b may have additional role in host regulatory mechanisms controlling TRV invasion.

Figure 1

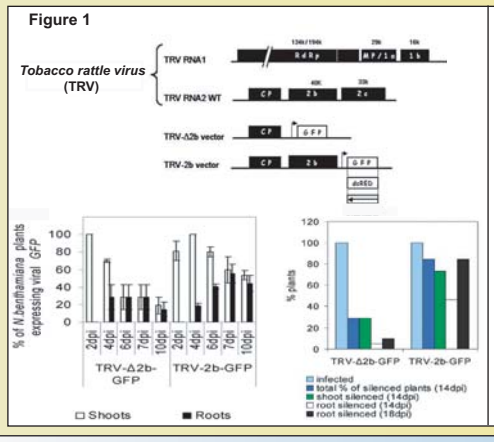


Figure 2

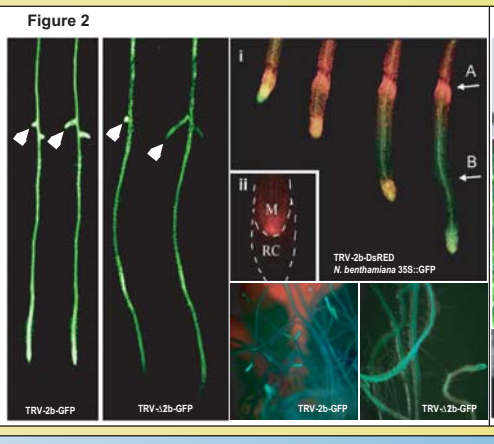
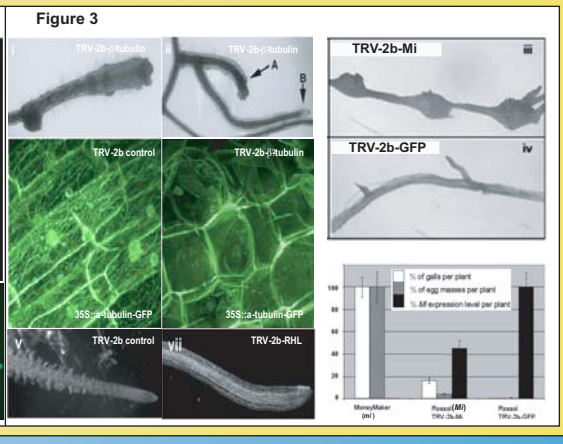


Figure 3



II / Plant virus-derived small interfering RNAs originate predominantly from highly structured single-stranded viral RNAs (Molnar *et al.*, 2005).

Previous work has demonstrated that VIGS can be significantly improved through virus-based expression of small inverted-repeats in comparison to larger antisense cDNA fragments (Lacomme *et al.*, 2003), leading to an increased accumulation of siRNAs (Figure 4) suggesting that such secondary RNA structures folding as short hairpins are efficiently recognised by the silencing machinery. During viral infection by *Potato virus X* (PVX) and *Tobacco mosaic virus* (TMV), a preferential accumulation of positive-strand siRNAs was observed (Figure 5). This was confirmed with *Cymbidium ringspot tombusvirus* (*CymRSV*) where a random sequencing of approximately 230 siRNAs of 20-21 nucleotide long revealed the existence of hot-spots of siRNA originating mostly (80%) from the viral positive strand (Figure 6, the position of each siRNA is represented by a dot). This suggest that during the VIGS-response elicited by positive-strand viruses the majority of siRNA originate predominantly from highly structured single stranded region from the positive strand instead of intermolecular pairing of positive and negative strands (Figure 7). This suggests that virus-derived siRNAs mainly originate by DICER cleavage of positive strand imperfect duplexes, which in turns specifically target the negative-strand viral RNA replicative intermediate for degradation as a part of an RNA-mediated defense pathway (Figure 7).

Figure 4

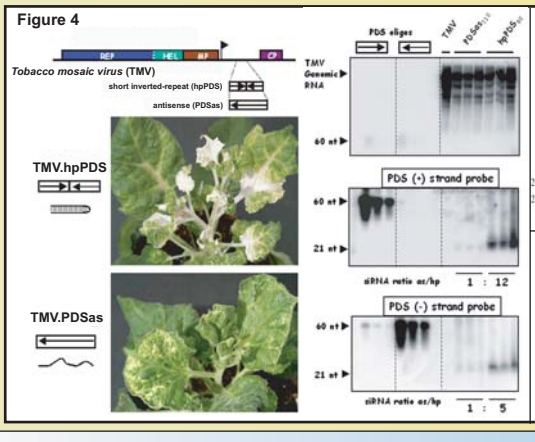


Figure 5

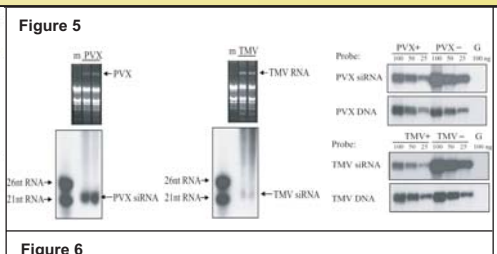


Figure 6

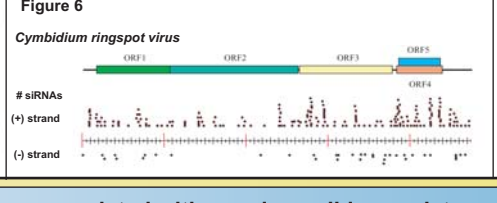
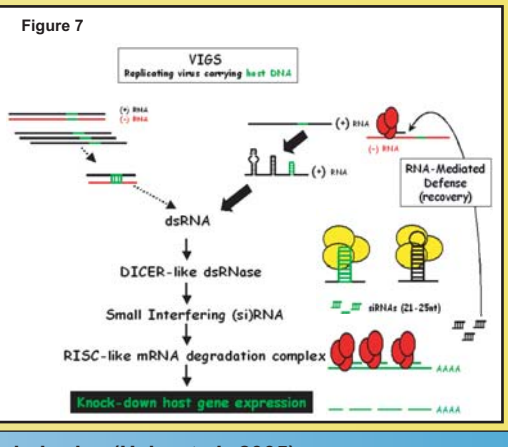


Figure 7



III / VIGS-based functional characterization of genes associated with powdery mildew resistance in barley (Hein *et al.*, 2005).

We successfully implemented the use of *Barley stripe mosaic virus* (BSMV) VIGS vector for the functional characterization of barley genes potentially involved in (*Mla*-based) resistance to the fungal pathogen *Blumeria graminis* (*B. graminis*), the causal agent of barley powdery mildew. BSMV-silenced plants for genes associated to *B. graminis* resistance such as *Sgt1*, *Rar1* and *Hsp90* displays reduced levels of corresponding mRNA and protein by 7dpi to 21 dpi in both main stem and axillary leaves (Figure 8). Silenced leaves from either main stem or tillers for *Sgt1*, *Rar1*, *Hsp90* and *RLK* (a *Receptor-Like Kinase* gene upregulated during the first hours of the incompatible interaction, Hein *et al.*, 2004) were detached and infected by powdery mildew. A significant increase in mycelial growth in plants challenged with either BSMV.SGT1as, BSMV.RAR1as, BSMV.HSP90as or BSMV.RLKas was observed in comparison to control barley *Mla13* plants challenged with control constructs (Figures 9 and 10). These results demonstrate that, in addition to the previously described *Sgt1* and *Rar1* genes, both *Hsp90* and *RLK* are required for the *Mla13*-resistance response to *B. graminis* in barley and that VIGS-based approach are a powerful mean to assign the function of barley genes.

Figure 8

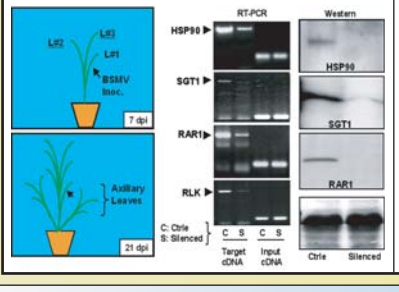


Figure 9

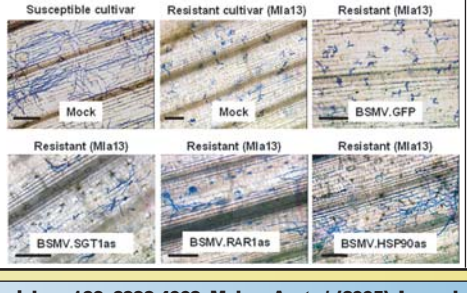
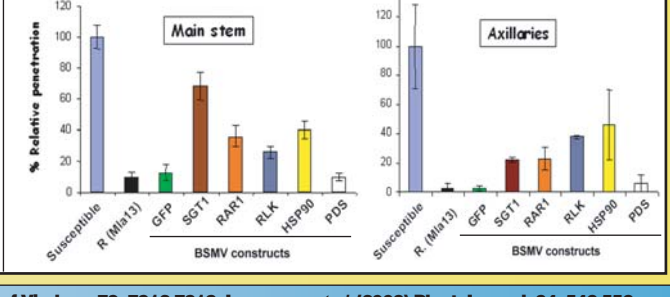


Figure 10



References: Valentine T *et al.* (2004) Plant Physiology 136: 3999-4009. Molnar A. *et al.* (2005) Journal of Virology, 79: 7812-7818. Lacomme *et al.* (2003) Plant Journal, 34: 543-553. Hein *et al.* (2003) Planta 218: 803-813. Hein *et al.* (2005) Plant Physiology, *in press* .