Viral-induced gene silencing (VIGS) vector development for functional studies in crop plants

Christophe Lacomme[1], Katarina Hrubikova[1], Eleanor Gilroy[2], Paul Birch[2], Odile Faivre-Rampant[3], Sophie Haupt[3], Jane Shaw[3], Vivian Blok[2], Mark Taylor[3], Tracy Valentine[1], and Karl Oparka[1]

Programmes of Cell-to-Cell Communication[1], Plant-Pathogen Interactions[2] and Quality, Health and Nutrition[3], Scottish Crop Research Institute, Dundee, DD2 5DA UK.

Scottish Crop Research Institute

I: VIGS vector development for *Solanum* species: A potato virus X (PVX) vector trigger VIGS in leaves and tubers of potato

VIGS is increasingly being used to generate transient loss-of-function assays as a more rapid alternative to stable transformation. We demonstrate that a previously described PVX VIGS vector, capable of triggering silencing in the permissive host *N.benthamiana* (1), is also efficient in triggering VIGS in diploid and tetraploid *Solanum* species (2).

A. PVX infects both diploid and tetraploid *Solanae* and trigger systemic VIGS of endogenous phytoene desaturase (*pds*) in foliar tissues.

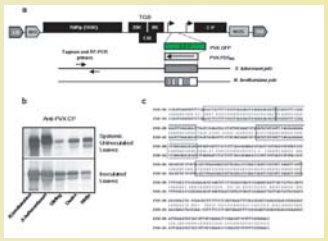
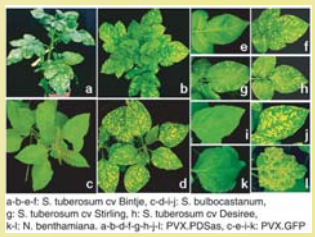


Figure 1: a- Genome organization of PVX (pGR106) VIGS vector. b- Western blot analysis of PVX accumulation in *Solanae*. c- Nucleotide alignment of *pds* cDNA region cloned into PVX from *S. tuberosum* (PDS-St) with *N. benthamiana* (*PDS-Nb*)



a-b-e-f: S. tuberosum cv Bintje, c-d-i-j: S. bulbocastanum, g: S. tuberosum cv Stirling, h: S. tuberosum cv Desiree, k-l: N. benthamiana. a-b-d-f-g-h-j-l: PVX.PDSas, c-e-i-k: PVX.GFP

Figure 2: PVX.PDSas triggers VIGS in diploid and tetraploid Solanum species. Photobleaching phenotypes (as a consequence of *pds* downregulation) observed by 21 dpi.

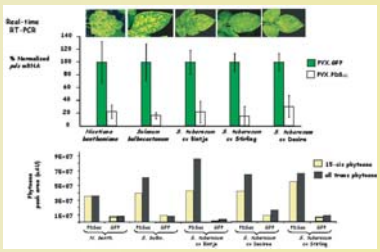


Figure 3: Molecular and biochemical characterization of *pds* VIGS in *Solanae*. Monitoring of normalized *pds* mRNA levels and phytoene (the substrate of PDS) accumulation in silenced and control plants.

B. Development of an *in vitro* silencing assay: systemic VIGS in potato tubers and *in vitro* generated microtubers.

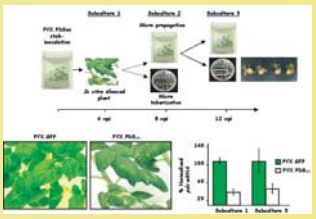


Figure 4: Photobleaching phenotype observed on *in vitro* propagated *S. tuberosum* cv Desiree after 3 subcultures.

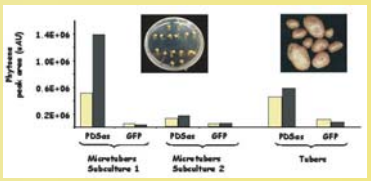


Figure 5: Quantification of phytoene accumulation in tubers and *in vitro* microtubers.

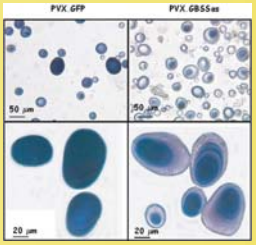


Figure 6: VIGS of granule bound starch synthase (GBSS) in tubers and microtubers. Downregulation of GBSS results in reduction of the production of amylose in starch granules. Microscopical observation of iodine-stained starch granules in control (PVX.GFP: complete blue staining) and silenced (PVX.GBSSas: pale-red with blue core) tubers.

Here we report that VIGS-mediated systemic down-regulation of gene expression can be achieved in both diploid and tetraploid Solanum species (as exemplified here by manipulating carotenoid and starch metabolism). Both foliar and tuber tissues are affected making this approach amenable for high-throughput analysis of gene function associated to important traits, such as tuber metabolism and pathogen resistance.

II: Efficient virus induced gene silencing in roots using a modified tobacco rattle virus vector

Several factors affect the silencing response including host range and viral tropism within the plant. Here, we report that a modified tobacco rattle virus (TRV) vector retaining the helper-protein 2b, required for transmission by a specific vector nematode, not only invades and replicates extensively in whole plants, including meristems, but also triggers a pervasive systemic VIGS response in roots.

A. TRV-2b vectors efficiently invade meristems and trigger a pervasive VIGS response in *N. benthamiana* and *A. thaliana*.

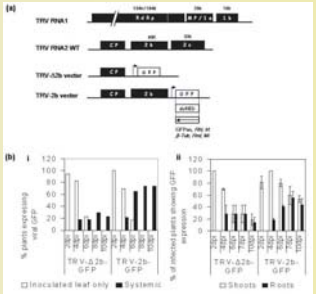


Figure 7: Invasion of roots by TRV. a- Genome organization of TRV. b-Movement of TRV vectors in *A. thaliana* (i, n=17-23) and *N. benthamiana* (ii, n=26-45).

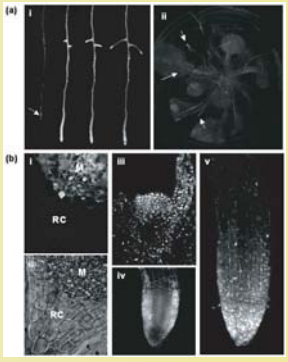


Figure 8: Distribution of TRV-2b constructs in roots. a- Invasion of the root systems by TRV-2b-GFP. b- Shoot and root meristem invasion by TRV-2b constructs. Vibraslice section of *N. benthamiana* infected root meristem (M) (i, and corresponding transmission image ii) and shoot meristem (iii). TRV-2b-dsRED infection of A. thaliana root meristem, optical section (iv) and stacked image (v) showing extensive infection of meristem.

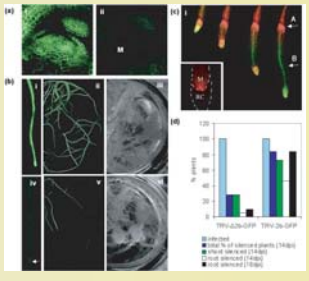


Figure 9: VIGS and recovery of viral replication in *A. thaliana* and *N. benthamiana*. Extensive VIGS of GFP transgene in a- shoot meristems and b- root meristems. c- Viral replication (TRV-2b-dsRED) suppression in newly grown root tissues and recovery in root meristems of *A. thaliana* harbouring a *GFP* transgene. Arrow A indicates the zone of constriction due to TRV invasion, arrow B new root growth exhibiting suppression of viral replication. d- Relative number of plants infected with TRV-Δ2b-GFP$_{VGS}$ (n=17) or TRV-2b-GFP$_{VGS}$ (n=23) vectors.

B. TRV-2b VIGS vectors for silencing in root tissues. The efficacy of the TRV-2b VIGS vector was evaluated by silencing endogenous genes whose functions are associated to root development and resistance to root-pathogen.

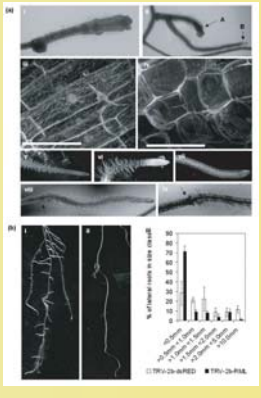


Figure 10: VIGS of a- *beta-tubulin* (change in cell shape and microtubule structure: i, ii, iv), *transparent testa glabra* (ectopic root hair, vi), *root hairless* (no root hair, vii), *iron-regulated metal transporter* (extended root hair, viii-ix) and b- *root meristemless* (reduction of lateral root size, i-ii-iii). Unsilenced control (iii, v).

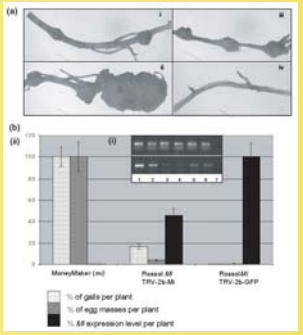


Figure 11: VIGS of nematode resistance gene *Mi* (confers resistance to root-knot nematodes) in tomato. *Mi* resistance–breaking phenotype in tomato (cv Rossol) silenced roots. a- Small and large galls observed on susceptible MoneyMaker (*mi*) plants (i, ii). Galls observed on silenced *Mi* plants (iii). No galls were observed in all control unsilenced *Mi* plants (iv). b- Semi-quantitative (i: lane 1: unsilenced, lane 2-6: independent silenced plants, 7: non-template control; upper panel *pds* PCR product, lower panel *Mi* PCR product) and quantitative Real-time RT-PCR determination of Mi mRNA levels (ii: % Mi expression levels) in control and silenced plants.
The averaged percentage of galls and egg masses per plant from two independent experiment is presented.

These results demonstrate that the TRV-2b vector displays an increased infectivity and meristem invasion, both key requirements for efficient VIGS-based functional characterization of genes in root tissues.
Our data suggest that the TRV helper-protein 2b may have an essential role in the host regulatory mechanisms that control TRV invasion.

Reference/Acknowledgements:

(1) Ruiz et al. Initiation and maintenance of virus-induced gene silencing. *The Plant Cell*, 1998, 10, 937-946. (2) Faivre-Rampant et al. Potato virus-X induced gene silencing in leaves and tubers of potato. *Plant Physiology,* April 2004, Vol.134, pp. 1308-1316.

We gratefully acknowledge Prof. David Baulcombe for the gift of pGR106 vector.

This work was supported by Large Scale Biology Corporation and the Scottish Executive Enviroment and Rural Affairs Department.