Viral Induced Gene Silencing in Crop Species

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Virus induced gene silencing (VIGS) is being used increasingly for reverse genetics because it offers a means for rapid gene knockdown by avoiding stable transformation (Baulcombe 1999). VIGS is an RNA-mediated defense mechanism that directly targets the integrity of the invading viral genome in a sequence-specific manner, and subsequently lowers the titer of the invading virus through an endogenous RNAse-inducible mechanism, which leads to viral RNA degradation (Baulcombe, 1999). By introducing plant cDNA fragments into the viral genome, it is possible to redirect this mechanism to corresponding endogenous host mRNAs, thus providing a means to down-regulate host gene expression.

A PVX vector triggers VIGS of endogenous *pds* in foliar tissues in *Solanum* species. In order to develop VIGS for functional genomic in *Solanum* species, the capacity of a previously described binary-based PVX vector (Jones *et al.*, 1999) was tested for its capacity to infect both wild diploid and cultivated tetraploid *Solanum* species. Cultivars were selected on the basis of their ability to be stably transformed and propagated *in vitro* (*Solanum tuberosum* L. cv Desiree), differential interactions to *Phytophthora infestans* between susceptible and resistant cultivars (*S. tuberosum* L. cvs Bintje and Stirling, respectively; Birch *et al.*, 1999), or their potential as a source of novel resistance genes to *P. infestans* (*S. bulbocastanum*, Song *et al.*, 2003). The efficacy of the PVX vector in silencing was assessed through its ability to silence an endogenous phytoene desaturase (*pds*) gene in these *Solanum* species. Down-regulation of *pds* triggers a characteristic photobleached phenotype (Kumagai *et al.*, 1995). As RNA silencing is homology-dependent, a potato *pds* cDNA fragment was isolated and cloned in antisense orientation into the PVX vector (PVX.PDS$_{AS}$ construct). By 3 weeks post inoculation photobleaching was observed in all infected *Solanum* representing either diploid (*S. bulbocastanum*) or tetraploid (*S. tuberosum* L. cultivars, fig 1). The silenced phenotype correlated with a four- to five-fold decrease in normalized *pds* mRNA levels in all silenced *Solanum* plants. Further HPLC analysis of phytoene levels (the substrate of PDS) showed a five-fold increase increased in the silenced plants (fig.2, left).

Systemic VIGS of pds in potato tubers and *in vitro* generated microtubers. Although VIGS proved effective in potato leaves, much research in potato is directed at improving storage organ quality and resistance to pathogens. Therefore it was important to determine whether gene silencing was observed in tubers. Phytoene levels in tubers harvested from glasshouse grown potatoes challenged with PVX.PDS$_{AS}$ increased by up to five-fold in comparison to control PVX.GFP infected plants (fig. 2, center). This indicates that systemic *pds* silencing does not only extend to foliar tissues but is transmitted through the whole vascular system to tubers. Due to the variability in tuberization time, tuber size and the glasshouse space required for higher-throughput gene function analysis, *in vitro* grown potato could provide an interesting alternative for reverse genetics approaches to study tuber-associated functions, as *in vitro* microtuberization is synchronized and controlled. We therefore evaluated the potential of a VIGS-based approach for *in vitro* propagated potato. *In vitro* grown plants were

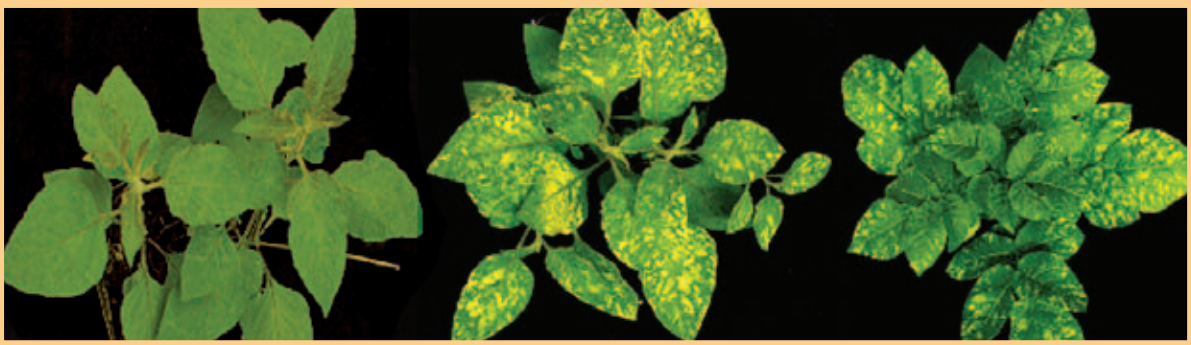

Figure 1 Silencing of the endogenous *pds* gene in *Solanum* species leads to photobleaching of the leaves (from left to right *Solanum bulbocastanum* non-silenced, *S. bulbocastanum* and *S. tuberosum* cv Bintje *pds* VIGSed).

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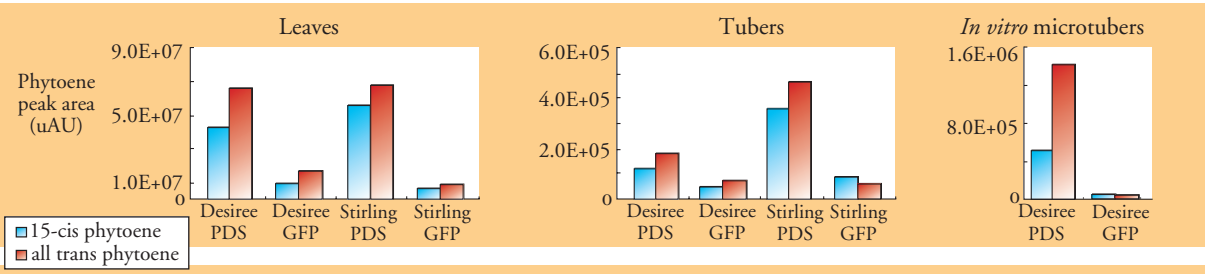

Figure 2 Increased phytoene levels in *pds* VIGSed potato in leaves, tubers and in vitro generated microtubers.

stab-inoculated (Takken *et al.*, 2000) with PVX.PDS$_{AS}$, and photobleaching was observed by 3 weeks post-infection. Micropropagation of VIGSed plants regenerated plants displaying a comparable photobleaching phenotype (sustained up to the fifth micropropagation event). In parallel, *in vitro* microtubers deriving from *in vitro* VIGSed plants were collected to monitor the extent of accumulation of phytoene. In these silenced microtubers, phytoene accumulation increased by 20-fold over than control microtubers infected with PVX.GFP (fig. 2, right), indicating that silencing was triggered efficiently *in vitro*.

Conclusions. We report the first example of an efficient VIGS-mediated manipulation of gene expression in both diploid and tetraploid potato (Faivre-Rampant *et al.,* in press). The microtuberization system, in conjunction with VIGS, has a number of potential benefits compared with analysis of tubers produced conventionally by glasshouse-grown plants. This should enable easier identification of altered tuber phenotype and opens the way for high throughput analysis of gene function enabling screening of genes involved in important traits such as tuber development, metabolism, and pathogen resistance.

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