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

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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 Solanaceae and trigger systemic VIGS of endogenous phytoene desaturase (pds) in foliar tissues.

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

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.

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-knot nematode in tomato.

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/acknowledgments:

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