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Trends in Plant Science | Technology of the Month

Virus-Induced Gene Silencing: Hush Genes to Make Them Talk

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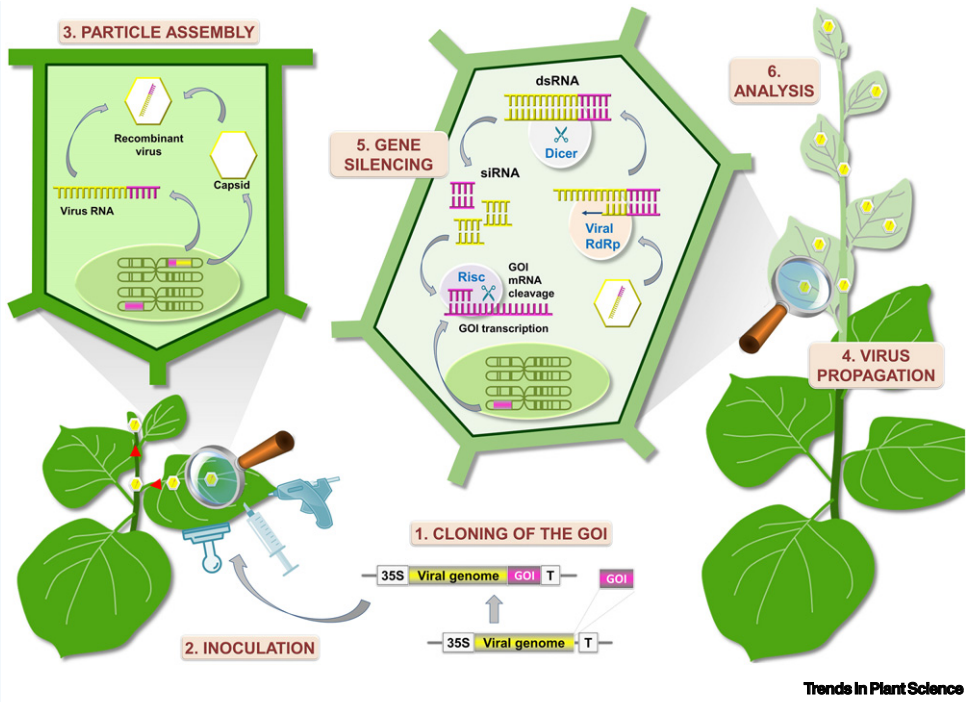
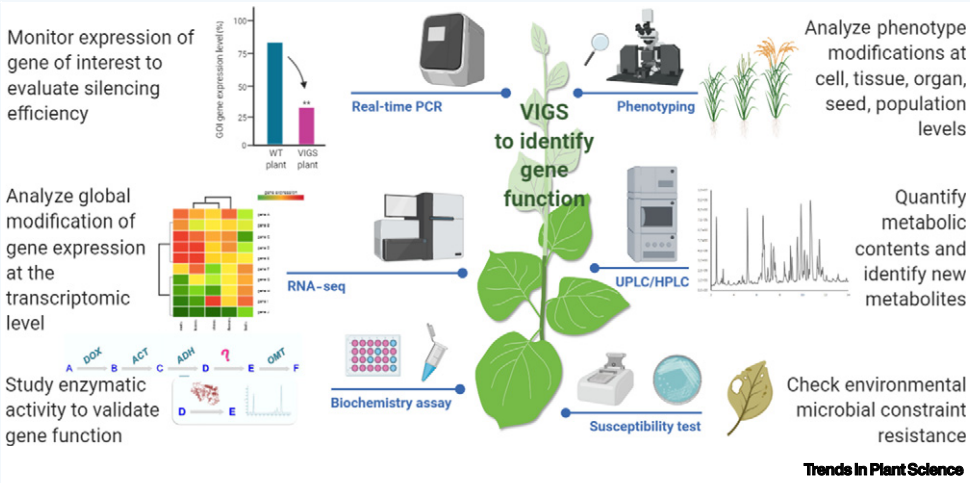


Virus-induced gene silencing (VIGS) silences a gene of interest (GOI) by exploiting plant defense mechanisms against RNA viruses. A GOI fragment is cloned into a construct containing viral genome sequence (1) and then inoculated into plants (2), where viral particles are produced (3). Through cell-to-cell movement using plasmodesmata and systemic progression via the phloem (4), viruses infect newly developing leaves and replicate. This involves the formation of dsRNA that is cleaved by plant DICER to release siRNA. siRNAs are loaded in the multiprotein complex RISC and their antisense strand screen RNA in cells. Hybridization of this complex results in specific RNA degradation (or translation repression) of viral RNA and endogenous GOI transcripts causing its silencing.



After 3–6 weeks, gene silencing efficiency is analyzed by monitoring GOI expression in new aerial parts of silenced plants and plants transformed with empty vector. Gene function can be investigated by comparative studies in similar organs from both types of plant.

ADVANTAGES:

One of the easiest, most cost-effective, and most efficient ways to study gene function *in planta*. Allows reliable silencing of genes (up to 90%) in a short time period (routinely 2–3 weeks post-inoculation).

Wide range of procedures of virus/viral genome inoculation (e.g., agroinfiltration, agrodrench, leaf rubbing, biolistic).

Robust and straightforward protocols reported for model and non-model plants.

Allows study of essential genes that cannot be knocked out.

Large-scale VIGS experiments for EST library screening are possible.

Comparison of gene function between different species with same constructs.

May lead to DNA methylation and heritable transcriptional gene silencing.

CHALLENGES:

Identification of a suitable virus and inoculation method in non-model plants: virus host specificity requires tool development.

Low-level and/or nonuniform gene silencing can result in reduced phenotypic and/or metabolic alterations. Lack of observable phenotype can also result from functional redundancies between gene family members.

Symptoms caused by virus propagation may mask phenotypic variations due to gene silencing.

Gene silencing is mainly transient and does not allow long time-course studies.

Difficulties in specific silencing of individual isoforms encoded by large gene family members.

Metabolic rerouting of biosynthetic intermediates impedes identification of the reaction catalyzed by products of silenced genes.

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