Geminivirus Vectors

Gene function analysis in plants by VIGS, now adapted for use in cotton

Virus induced gene silencing (VIGS) offers an attractive and quick alternative for knocking out expression of a gene in plants without the need to genetically transform the plant. VIGS allows targeting of genes of interest for functional analysis in intact plants, thereby by-passing transformation and selection. It is also ideal for testing genes that could be embryo-lethal. Comparison with an empty vector control allows accurate information to be drawn about target gene function.

Geminiviruses are small circular DNA viruses that replicate in plant nuclei. Silencing of an endogenous gene from a geminivirus vector was first reported by Niki Robertson’s lab at NCSU in 1998, and she is still actively working to improve the vectors and extend the range of plant species used for silencing. Because the geminivirus vectors lack a coat protein gene, they are not transmissible by insect vectors, which are required for plant-to-plant spread and thus use of the disarmed vectors does not require a permit.

Viruses from the geminivirus family naturally infect a wide range of crop plants, including maize, cotton, wheat, bean and cassava and are therefore an ideal system of choice for VIGS-based gene function analyses in a broad range of crop plants. Vectors have now been developed for use in cotton, and work is also ongoing for suitable vectors for roses.

Using these new VIGS vectors, recombinant virus carrying a partial sequence of a host gene is used to infect the plant. As the virus spreads systemically, the endogenous gene transcripts, which are homologous to the insert in the viral vector, are degraded by post-transcriptional gene silencing. These virus vectors have been used in a range of studies to silence single or multiple genes, including the meristematic gene, Proliferating Cell Nuclear Antigen (PCNA).

The inventors have developed three geminivirus silencing vectors using Tomato Golden Mosaic Virus (TGMV), Cabbage Leaf Curl Virus (CaLCuV) and Cotton Leaf Crumple virus (CLCrV). All three viruses were engineered to allow insertion of sequences of 100 - 800 bp while maintaining viral replication and spread. Their work has demonstrated that geminiviruses, can provide new information about plant gene function. For example, Gemini-VIGS of the Retinoblastoma gene RBR in Nicotiana benthamiana led to plant cell death in mature leaves and tissue-specific hyperplasia in developing parts of the plant. The RBR gene is gametohytic lethal in Arabidopsis and silencing in mature parts of the plant has never been reported. This illustrates another advantage of VIGS over transformation with a constitutive promoter, that the virus is able to replicate in mature cells and move to the phloem before cell death begins. Therefore, a range of cell types can be analyzed for their response to loss of an essential gene.

References:


