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# Gene Deletions and Silencing via High Pressure Spraying Technique

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## Abstract

Controlling gene expression is critical for improving resistance against biotic and abiotic stress factors. The introduction of expression vectors encoding artificial miRNA or larger hairpin RNA to crops has proven to be effective for higher crop yield and quality by silencing the target gene via the RNA interference (RNAi) mechanism. Similarly, CRISPR/Cas-based genome editing tools have been employed to rewire the transcriptome by deleting genes or regulatory elements for improved crop plants. The expression of RNAi inducers, Cas endonucleases, and guide RNAs all rely on the delivery of expression cassettes via agrobacterium-mediated plant transformation. However, legal regulations, negative public perception, and lengthy and laborious tissue culture requirements restrict the use of agrobacterium-mediated transformation of crop plants. In animal systems ribonucleoproteins (RNP) and RNAs are commonly used to circumvent the use of DNA and tissue culture to induce gene silencing or genome editing *in vivo*. However, cell wall appears as a major barrier preventing the delivery of RNP and RNA molecules into the plant cells. We developed the high-pressure spraying technique (HPST), which successfully delivered sRNAs, mRNAs, and RNP molecules into plant cells *in planta*. In addition, the use of carbon-based nanoparticles, such as carbon dots, improves the uptake efficiency. The delivery of these molecules led to DNA- and tissue culture-free genome editing methods, new methods in epigenetic plant breeding, and substantially faster applications of gene deletions via CRISPR/Cas. In this presentation, the power and the limitations of HPST for RNAi and gene editing will be discussed.

***Key Words:*** *CRISPR/Cas, RNAi, Genome Editing, RNA delivery, Plant Breeding*