Induction of silencing in plants by high-pressure spraying of *in vitro* synthesized small RNAs

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In eukaryotes, double stranded RNA (dsRNA) triggers RNA interference (RNAi), a mechanism that is associated with post- and transcriptional gene regulation (PTGS and TGS). The dsRNA that can derive from exogenous and endogenous sources is processed into small RNAs (sRNAs). In the case of PTGS, sRNAs are loaded into RNAi effector complexes targeting complementary RNA molecules for degradation and/or translational interference. In plants, the initiation of PTGS can involve transitive and systemic silencing. Plant endogenes are less prone to RNA silencing than transgenes. While both can efficiently be targeted by small RNAs for PTGS, generally only transgene PTGS is accompanied by transitivity and systemic silencing.

In plants, RNAi can be induced by the application of exogenous sRNA. We applied various methods to introduce *in vitro* synthesized siRNAs that were designed to target the coding region of a GREEN FLUORESCENT PROTEIN (GFP) transgene onto GFP-expressing transgenic *Nicotiana benthamiana* plants as well as *N. benthamiana* plants containing an intron-containing, endogene-resembling GFP transgene (Nb-GFP^{endo}). In contrast to mere siRNA application, including spraying, syringe injection and siRNA infiltration that all failed to induce RNA silencing, high pressure spraying of siRNAs with sizes of 21, 22 and 24 nucleotides (nt) resulted in efficient local and systemic silencing of the GFP and GFP^{endo} transgene. Systemic silencing of the GFP transgene was basically detected upon spraying of 22 nt siRNAs whereas Nb-GFP^{endo} plants were resistent to systemic silencing.

The failure to trigger silencing of endogenous genes by applying exogenous sRNA molecules complicates the application of this RNAi technology for agricultural purpose in the field. However, high pressure spraying is useful for research purposes, it enables the introduction of clearly defined siRNAs, allowing to investigate their potential to trigger local, transitive and systemic silencing.