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NEW INSIGHTS INTO AN EXOGENOUS RNAI-BASED APPROACH FOR ENDOGENOUS GENES SILENCING IN PLANTS

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RNA interference (RNAi) is a natural gene silencing phenomenon triggered by double-stranded RNAs (dsRNAs) which are processed by plant RNAi machinery into 21-24nt small interfering RNAs (siRNAs). siRNAs guide sequence-specific degradation or translational repression of homologous mRNA targets in the cytoplasm. Nowadays, RNAi is being extensively investigated and applied for gene functional studies in the lab and in agriculture for biotechnological applications both in disease control and crop management. So far, conventional RNAi applications in agriculture are largely based on the use of transgenes or viral vectors. Plant transformation enables the production of dsRNA molecules to silence specific genes that control target traits. However, transgenic plants fall under the regulation of genetically modified organisms (GMOs) and their use has always been surrounded by public and political concerns. Thus, the need for new widely accepted, GMO-free, and effective agricultural solutions, has led to the development of exogenous RNAi-based approaches via direct application of dsRNA or siRNA.
In this study, we have developed a model system to test the effectiveness of *in vitro* synthesized dsRNAs direct application for endogenous gene silencing and weed control. A few works have indeed reported that the exogenous application of dsRNAs can down-regulate the expression of endogenous plant genes. In our model system, we are using the acetolactate synthase (*ALS*) gene of *Amaranthus hybridus* L. as the target of silencing. *A.hybridus* is a monoecious and self-pollinated weed that has evolved multiple resistance to herbicides with different sites of action including ALS inhibitors, which are the most used herbicides in soybean. This makes *A.hybridus ALS* gene an excellent bench test for the development and future application of dsRNA-induced RNAi mechanism for weed control. We initially focused on the protocol optimization for the *in vitro* synthesis of dsRNAs of various lengths (ranging from 218 to 460nt) targeting the acetolactate synthase (*ALS*). *ALS* represents an optimum target for RNAi mechanism mediated by dsRNAs because it is an intronless, nucleotide-stable, and single-copy gene. For the synthesis of dsRNAs, we have firstly selected three *ALS* regions: the 5'- and 3'-end and a central region, 218, 263, and 460bp long, respectively. Secondly, dsRNA molecules have been *in vitro*-synthesized through enzymatic T7 RNA polymerase transcription by starting from two different templates: i) PCR products with the T7 promoter upstream of the target sequence, ii) linearized L4440 plasmid DNA containing two converging T7 promoters flanking the target sequence. Both strategies allowed to produce complementary ssRNAs, which were then annealed at nearly equimolar concentration to obtain the three final dsRNAs. Data revealed that linearized L4440 plasmids ensure a higher dsRNAs yield compared to the PCR products. Direct application of dsRNAs to *A.hybridus* leaves is ongoing.