Development of a non-chemical RNAi-based strategy for *Amaranthus hybridus* L. weed management

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Weeds are one of the major issues in cropping systems, responsible for significant yield losses. Herbicide applications are the most effective strategy to control weeds, but stricter legislation has resulted in a significant reduction in the number of herbicides available on the market. Furthermore, the recent European legislation on the sustainable use of pesticides will require farmers to drastically reduce chemical use over the next ten years while promoting integrated weed management strategies that improve environmental sustainability and lower the risks to animal and human health. In addition, the over-reliance on chemical control has resulted in the evolution of resistant biotypes. As a result, new technologies to effectively manage weeds and weed resistance should be developed. In this regard, the development of a non-chemical weed control strategy based on RNA interference (RNAi) technology could: i) represent a potential non-chemical weed control strategy, ii) provide an emerging GMO-free strategy for managing invasive and resistant weeds, and iii) provide a valid opportunity to go inside the molecular mechanisms of weed biology.

In this study, the acetolactate synthase (*ALS*) gene of *Amaranthus hybridus* L. has been used as the target to assess the effectiveness and applicability of *in-vitro* synthesized double-stranded RNAs (dsRNAs) direct application for endogenous gene silencing and weed control. *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. *ALS* represents an ideal target for the development and future application of dsRNA-mediated gene silencing because it is an intronless, nucleotide-stable, and single-copy gene. We have produced dsRNAs of various lengths (ranging from 218 to 460bp) targeting three distinct *ALS* regions: the 5'- and 3'-ends, and a central region. dsRNAs molecules were transcribed *in-vitro* by T7 RNA polymerase and externally applied to the abaxial leaf surface of *A. hybridus* plants at 4-6 true leaves developmental stage by: i) mechanical inoculation, or ii) high-pressure spraying. Despite the expression of *ALS* gene transcripts was found to be lightly downregulated when synthetic

ALS-dsRNAs were applied, no phenotypic effects were observed. Our current research focuses on the determination of the effectiveness of *ALS*-dsRNAs silencing using agroinfiltration techniques, and on dsRNAs delivery techniques through the use of nanomaterials to maximize the effectiveness of gene silencing by exogenous dsRNAs application. This second approach was preliminary studied by RNA electrophoretic mobility of functionalized nanomaterial and by means of confocal microscopy on *A. hybridus* leaves. In parallel, we are examining the expression patterns of genes thought to be involved in the RNAi pathway in *A. hybridus* to verify if their expression is triggered by dsRNA applications.