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

Bordignon S.[1], Panozzo S.[1], Farinati S.[2], Milani A.[1], Scarabel L.[1], Scarpin D.[3], Braidot E.[3], Petrussa E.[3], D'Este F.[4], and Varotto S.[2]

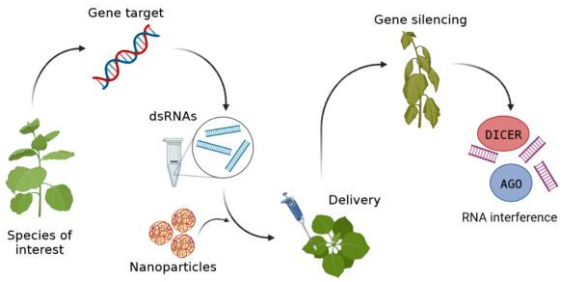
[1] Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Italy
[2] Department of Agronomy Food Natural Resources, Animals and Environment (DAFNAE), University of Padova, Italy
[3] Department of Agriculture, Food, Environment and Animal Sciences (DI4A), University of Udine, Italy
[4] Department of Medicine (DAME), University of Udine, Italy

Introduction

Weeds represent one of the major issues in cropping systems. Nowadays, herbicide applications are the most effective tool for weed control, but repeated use result in the selection of resistant biotypes. The recent European legislation on the sustainable use of pesticides intends to reduce the reliance on herbicides and encourage the development of integrated strategies that are more sustainable for the environment.

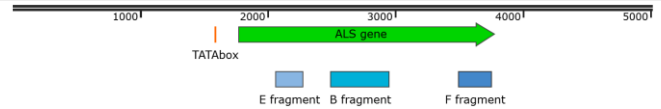
In this study, the acetolactate synthase (*ALS*) gene of *Amaranthus hybridus* L. has been used as the target to assess the effectiveness and applicability of *in-vitro* synthesized double-stranded RNAs (dsRNAs) direct application for endogenous gene silencing and weed control.

Currently, we are focused on the selection of the dsRNAs delivery mechanism, either 1) naked or 2) nano-encapsulated, as well as the evaluation of the effectiveness of gene silencing at both the phenotypic and molecular levels. The two studies are in different stages of development, both ongoing.



Synthesized dsRNAs

Three different dsRNAs of various lengths, ranging from 218 to 460bp, targeting three distinct *ALS* regions were synthesized *in-vitro* by T7 RNA polymerase mediated transcription: the 5'-end (E fragment), the 3'-end (F fragment), and a central region (B fragment).



1. Naked-dsRNA application

At 4-6 true leaf developmental stage, mechanical inoculation or high-pressure spray were used to apply different doses (10- to 30µg) of a mixture of the three dsRNAs to the abaxial leaf surface on *A. hybridus* plants.

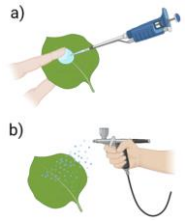


Figure 1 (a) Mechanical inoculation by gently rubbing the inoculation mixture, and (b) high-pressure spray. dsRNAs were resuspended in a specific formulation containing a superwetting surfactant (Silwet™ L-77 0.01%), an osmolyte compound and a buffering agent.

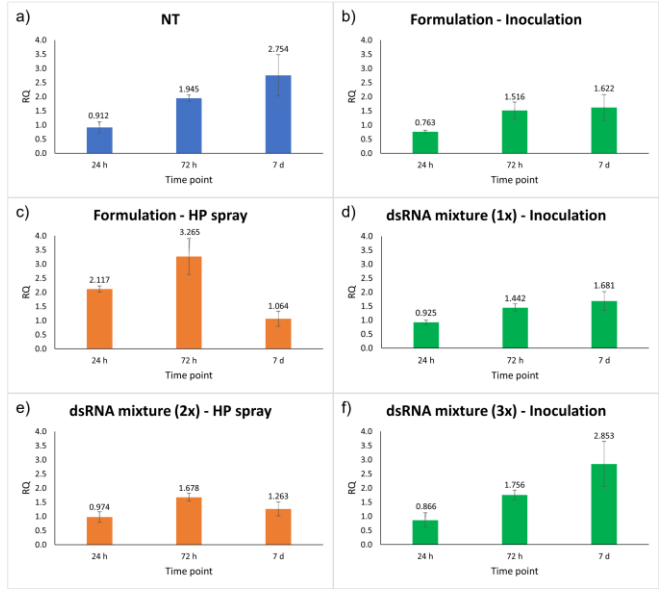


Figure 2 Relative quantification of *A. hybridus ALS* gene expression (through qRT-PCR) normalized to malate dehydrogenase (MDH) transcript quantities.

2. Nanoparticles encapsulation

Chitosan nanoparticles (NPs), used as carriers to maximize the effectiveness of dsRNAs application, were assessed for their dsRNAs retention capacity as well as their ability to be distributed on *A. hybridus* leaf surface.

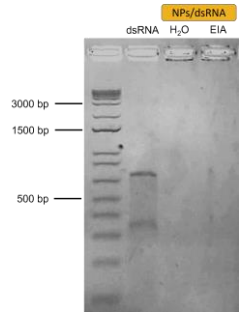


Figure 3 Agarose gel electrophoresis of naked dsRNA and NPs/dsRNA resuspended in either H_2O or 0.1% Ethoxylated Isodecyl Alcohol (EIA) wetting agent (CIFO Srl).

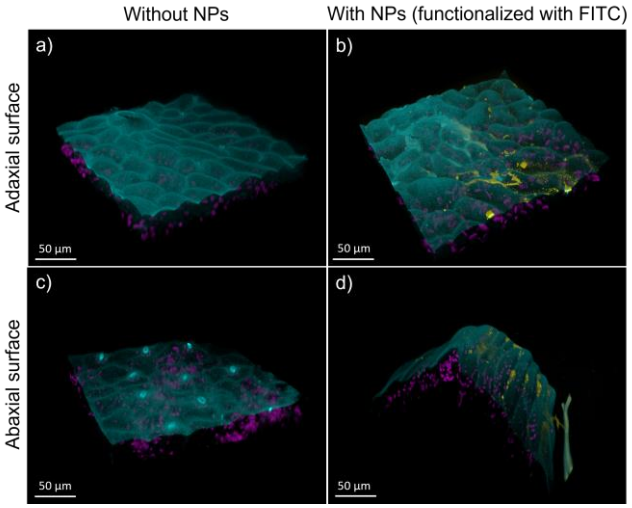


Figure 4 Confocal Microscopy analysis of *A. hybridus* adaxial (a, b) and abaxial (c, d) leaf tegument untreated (a, c) and sprayed with fluorescein isothiocyanate (FITC)-NPs diluted 1:20 in 0.1% EIA (b, d). Yellow, FITC; magenta, chlorophyll autofluorescence; cyan, blue autofluorescence associated with the cuticular layer and guard cells.

First considerations

1. In treated plants, there weren't any evident phenotypic alterations after treatment. Even so, the results of qRT-PCR revealed a slight difference in *ALS* gene transcript level between dsRNA-treated and non-treated plants. Additionally, plants exposed to high-pressure spraying showed *ALS* gene downregulation patterns more consistent with the effect of ALS herbicides, making this approach more promising for endogenous gene silencing.
2. Chitosan NPs were proven to be able to retain dsRNAs and to be distributed on *A. hybridus* leaf surface. On the abaxial page NPs seem to be primarily distributed near leaf veins, while on the adaxial one they are mostly found along the tangential walls of the tegumental cells.