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O-32 (KEYNOTE SPEECH)

RNAi strategy, an alternative to conventional fungicides for the control of botrytis and the cucurbit powdery mildew diseases?

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Botrytis cinerea and *Podosphaera xanthii*, the causal agents of the gray mold and the cucurbit powdery mildew diseases, respectively, are one of the main limiting factors of horticultural crops production worldwide, consuming up to 40% of fungicides in its control. However, these fungi have been categorized by the Fungicide Resistance Action Committee as phytopathogens with a high risk for fungicide resistance development, a fact that has been demonstrated in our country. In addition, and according to the "farm to fork" strategy of the recent European Green Deal, the diversity of fungicides available to growers will be reduced by 50% in 2030. For this reason, alternative control tools and molecules with fungicide activity are needed. In our research group, we intend to check if the efficacy of the emerging RNA interference (RNAi) strategy, called "spray-induced gene silencing" (SIGS), could be a valid sustainable solution and an alternative to the use of conventional fungicides for the control of *B. cinerea* and *P. xanthii*. For this purpose, several double-stranded RNA (dsRNAs) have been designed against targets genes involved in the virulence/pathogenicity of both pathogens. To improve the application of these oligonucleotides in the field, their encapsulation to create nanoparticles is being carried out. If we succeed, new molecules with fungicidal action, could be included to obtain a sustainable plant protection control programs in the field.

Key words: Gray mold, *Botrytis cinerea*, cucurbit powdery mildew, *Podosphaera xanthii*, RNAi, SIGS, nanoparticles

O-33-STU

CRISPR based gene-drive strategy for engineering of disease resistance in plants

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Gene-drives are a powerful new gene-editing tool that allows for the management of detrimental organisms, such as the malaria vector *Anopheles gambiae*, by creating heritable mutations or spread of a genetic payload through genetic manipulation of pest populations. The development of site-specific genome-editing tools has improved gene-drives and made them a promising approach for addressing agricultural, ecological, and human health problems. This technology can be used to manage pests and weeds that reduce yield and quality in agricultural production. Additionally, there is potential to generate disease resistant cultivars using CRISPR gene-drives with only routine pollination after a single transformation assay, eliminating the need for repetitive gene-editing experiments or backcrossing. In this study, we designed two CRISPR/Cas9 based gene-drives with constitutive or inducible promoters to disrupt the function of *CsaMLO8*, the major gene associated with powdery mildew susceptibility in *C. sativus*. We used an *in silico* approach to select targets for knock-in and gene-drive designing for pre-experimental guidance and identify edited plants for post-experimental analysis. Additionally, our study aims to investigate the impact of sex bias and promoter type on the efficiency of gene-drives and the type and heredity of induced mutations. Our overall objective is to assess the potential of CRISPR based gene-drives as a tool for plant breeding and other methods to control harmful organisms that reduce agricultural yield or quality. We expect to provide valuable insights into effective strategies for generating disease-resistant cultivars in a shorter time and at a lower cost by using CRISPR-based gene-drives.

Key words: Cucumber, CRISPR, disease resistance, gene-drives, gene-editing, plant resistance, powdery mildew