The diversity, activity, and biosynthesis of bioactive polyacetylenes in *Daucus carota*

Polyacetylenic lipids are produced in various Apiaceae and Asteraceae species in response to pathogen attack. It has long been suspected that these compounds are natural pesticides; a potentially valuable resource for creating crops with enhanced pathogen resistance.

The recent release of a high-quality carrot genome has enabled functional genomics approaches to exploring polyacetylene structure, function, and biosynthesis in this species. We began with a detailed analysis of carrot polyacetylene chemical structures and distribution among carrot tissues in the cultivar Danvers. We identified five major (two novel) and seven trace polyacetylenes, with falcarindiol and falcarinol predominating. In this cultivar, total polyacetylene concentrations were around 2 μg/mg. At this concentration, we found that purified falcarinol inhibited the growth rate of mycelia of the necrotrophic fungus *Sclerotinia sclerotiorum* by 25%. Next, an analysis of five carrot cultivars revealed falcarinol levels ranging from ca. 1 to 5 $\mu g/cm^2$ that were positively correlated with resistance to *S. sclerotiorum*. These data provided a rationale and framework for searching for underlying biosynthetic genes.

Previous work had identified that the polyacetylene biosynthesis begins with the conversion of the monounsaturated fatty acid oleate into the polyunsaturated, acetylenic fatty acid dehydrocrepenynate. In other plant species, these steps are catalyzed by members of the fatty acid desaturase (FAD2) family. We found that the carrot FAD2 family is massive, with 24 members. To identify carrot FAD2s associated with polyacetylene production, we correlated polyacetylene abundance with both public RNAseq data from diverse carrot tissues and RNAseq data from carrot cell cultures before and after elicitation with an extract of fungal mycelia. By testing top candidate genes in yeast and/or Arabidopsis seeds, we identified carrot genes capable of generating dehydrocrepenynate. We are now (i) creating knockout and overexpression lines with altered polyacetylene content to test their pathogen resistance and (ii) examining the evolution of polyacetylene biosynthesis and structure in the euasterid clade.

Significance:

A warmer climate is expected to expand the ranges of *Sclerotinia* and *Phytophthora* plant pathogens, including into large portions of Nebraska and Iowa.[1,2] Here, we present data

suggesting that plants can resist fungal pathogens by producing elaborate lipid metabolites called polyacetylenes. Literature indicates that these compounds also make positive contributions to human health.[3] Based on recent reviews of the diversity of polyacetylene chemical structures, it can be concluded that more than 200 different polyacetylenic lipids are produced in specific, later-diverging plant lineages.[4] A major portion of these are biosynthesized by an offshoot of ubiquitous fatty acid biosynthesis: the crepenynate pathway.[5] This large arsenal of likely anti-pathogen and health-promoting compounds is a tempting research target: can engineering the biosynthesis of these compounds in crop species improve plant pathogen resistance and human health? Unfortunately, little is known about polyacetylene biosynthesis and no tools exist for testing the effects of polyacetylene engineering in a crop species. In the present work, we identify genes controlling the biochemical transformations surrounding the divergence of fatty acid biosynthesis and the crepenynate pathway. *Accordingly, the work described here is highly significant because it provides the genetic tools necessary to engineer polyacetylene biosynthesis in a crop species for the first time.* These tools can be expected to advance agriculture by enabling the engineering of pathogen-resistant, polyacetylene-rich crop cultivars that can reduce pesticide use and improve nutritive value by providing prerequisites for the creation of polyacetylene-rich crop lines or polyacetylene-producing synthetic biological systems.

1. Global Change Biol. 23 (2017), 1661–74
2. Fungal Ecol. 5 (2012), 62-72
3. J. Pharm. Biomed. Anal. 41 (2006) 683–693
4. Fitoterapia 106 (2015) 92–109
5. Prog. Lipid Res. 47 (2008) 233–306