Evolutionary time machine into chemical defenses: Transforming *Arabidopsis* via *Agrobacterium* Mediated Gene Transfer

Rachel Varnau[1], Julie Herman[1], Kathleen Kay[1]
[1]Department of Ecology and Evolution, UC Santa Cruz

Abstract

Understanding the evolution of plant secondary metabolites is important to everything from crop production to human health. Glucosinolates are well known chemical defenses of the Brassicaceae family that have important roles in defense against herbivory in crop plants as well as possessing anti-cancer and anti-ulcer properties for humans [1]. In order to learn more about the process of evolution of the glucosinolate biosynthetic pathway (GBP), we will study how modern herbivores respond to the difference between various ancestral versus modern plant chemical defenses. However, it is necessary to first perfect a method to develop a transgenic plant with a mutant GBP. This project will provide a preliminary proof of concept that it is possible to alter the GBP in *Arabidopsis thaliana* via *Agrobacterium tumefaciens* mediated gene transfer.

Introduction

Plant biology research is vital to human health because many new drugs and medicines are derived from natural products [2]. Glucosinolates, a major component of the secondary metabolome in Brassicaceae, are used by these plants to deter some herbivores from feeding and stunt the growth of others (Figure 1). Research on glucosinolates is important because of its known anti-cancer and anti-ulcer properties. Plant biology research of chemical defenses is also important for crop health, to invest in natural defenses instead of relying on insecticide pollutants. The Brassicaceae family contains many major crop plants including canola, cabbage, broccoli, and wasabi [3].

Figure 1. Differential Insect Herbivory Between Leaves Making Isothiocyanates (top) and Those Making Nitriles (bottom) from Glucosinolates [1].

Herbivore damage levels are likely controlled by aspects of glucosinolate diversity.. For example, genotypes producing alkenyl glucosinolate phenotypes experience higher damage levels than those with hydroxyalkyl glucosinolates [4]. However, little is known about the processes that have driven the evolution of the diversity of glucosinolates [1]. We would like to study the process of evolution and the phenotypic difference between modern and ancient glucosinolates. To do this, a mutant plant from the Brassicaceae family which exhibits a variety of ancient glucosinolates will be compared to the wild type. Comparisons will be made by recording how modern predators respond to the chemical defenses, as well as analyzing the pattern of released glucosinolates over time. Ancient genotypes can be inferred using phylogenetic methods. However, in order to conduct this experiment, it is necessary to prove that it is possible to have a cost-efficient method to create a plant with a mutant GBP. *Agrobacterium tumefaciens* is a plant pathogen that infects plants' DNA with the bacterium's own DNA. A gene of interest can be transformed into the *Agrobacterium* which could then transfer that DNA to *A. thaliana*, creating a mutant [5]. *A. thaliana* will be used because it is a model organism in the Brassicaceae family with available genomic resources and mutant lines.

References

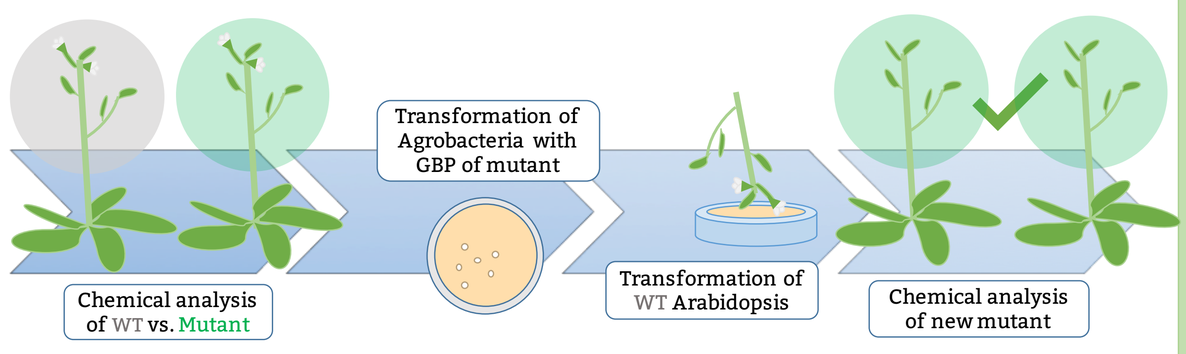
1. Windsor, A.J., Reichelt, M., Figuth, A., Svatoš, A., Kroymann, J., Kliebenstein, D.J., Gershenzon, J. and T. Mitchell-Olds. (2005) Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). *Phytochemistry* 66(11):1321-1333.
2. Katiyar, C., Gupta, A., Kanjilal, S., and S. Katiyar. (2012) Drug discovery from plant sources: An integrated approach. Ayu. 2012;33: 10
3. Kliebenstein, Dan. "Genetic Control of Glucosinolate Hydrolysis." Kliebenstein Lab. UC Davis, n.d. Web. 13 Jan. 2017.
4. Bidart-Bouzat, M.G. and D.J. Kliebenstein. (2008) "Differential levels of insect herbivory in the field associated with genotypic variation in glucosinolates in *Arabidopsis thaliana*". *Journal of Chemical Ecology* 34(8)1026-37.
5. Gelvin, S.B., (2003) "Agrobacterium-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool". *American Society for Microbiology* 10.1128

Engineering Goals

1. Obtain a line of *A. thaliana* with a mutant GBP will produce a chemically different phenotype than the wild type
2. Transform agrobacteria with a specific GBP elements via heat shock
3. Transform *A. thaliana* with *A. fumefaciens* mediated gene transfer so that the wild type now produces the same phenotype of chemical defenses as the mutant

Methods

To test this method, I will take a line of *A. thaliana* that is known to have a genetically different GBP than the wild type and analyze its chemical profile to determine the phenotypic differences. I will then transform the *Agrobacterium* with a vector containing genetically different GBP elements as well as a selective marker with antibiotic resistance via heat shock. Only surviving *Agrobacterium* will have been successfully transformed, which I will use to infect the wild type *A. thaliana.* If the transformation of *A. thaliana* is successful, the chemical profile of the of the two lines will exhibit the same phenotype of chemical defenses after the transformation.



Another method to achieve this desired result is CRISPR; a precise, effective, yet very expensive way to edit genes to create a mutant. This proof of concept is intended to show that it is possible to successfully genetically modify plants' GBP at a reasonable price. If this process is effective, I will use this method create mutants with GBPs of various ancient ancestors of *A. thaliana* to analyze and build a more complete understanding of glucosinolate evolution.

Outlook

With this experiment, we hope to show that using *Agrobacterium fumefaciens* mediated gene transfer will be an efficient, cost-effective method to create a *A. thaliana* with a mutant GBP. The next direction for this project would be to transform plants with various GBPs from different points of evolutionary history and perform chemical analysis to determine phenotype as well as record how modern predators respond to each phenotype. This project will increase our insight into the process of evolution by showing whether defensive phenotypes escalate or oscillate over evolutionary time. Logically, if these chemical defenses give plants an evolutionary advantage, glucosinolate levels would escalate over time (Figure 2.) However, it has been hypothesized that evolutionary constraints of glucosinolates could involve trade-offs among phenotypic traits controlled by the same gene, which could explain a hypothetical oscillating evolutionary pattern [1] (Figure 3.)

Fig. 2 Hypothetical Escallating Evolution

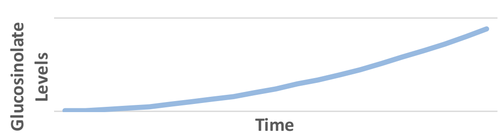
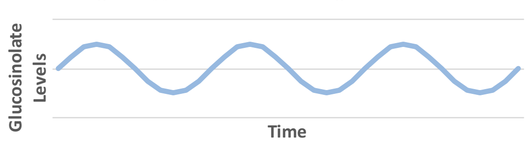


Fig. 3 Hypothetic Oscillating Evolution



Acknowledgements

Special thanks to the Kay lab and my mentor Julie Herman