AFFILIATED PROJECTS

Title: Modification of hypocotyl length in camelina and canola via manipulation of the AHL gene family

PI: Michael M. Neff Ph.D.

Funding term and duration: 7/1/2009 – 6/30/2010

Graduate students: David Favero and Kim Cotton both worked on this project as rotating MPS graduate students, with David recently joining the Neff Lab. Jianfei Zhao is a MPS graduate student working on this gene family in *Arabidopsis*.

Technical Support: Pushpa Koirala (Technician), Jiwen Qiu (Postdoc).

Background: In low rainfall, dryland-cropping areas of Eastern Washington, such as the regions around Washtucna, Lind and Dusty, stand establishment can have a major impact on yields of camelina and canola. During dry years these seeds need to be planted in deep furrows so that the developing seedling has access to soil moisture. In areas with higher rainfall, canola and camelina are often used in rotations where they are direct seeded into wheat stubble from the previous year. One approach to facilitate stand establishment is to develop varieties with larger seeds and longer hypocotyls as seedlings while maintaining normal stature as adults. Unfortunately, few mechanisms have been identified that uncouple adult stature from seedling height. The Neff lab has identified a group of plant-specific genes that, when mutated in a particular way, increase seed size and seedling height without adversely affecting adult stature. These genes encode AHL (AT-Hook Containing, Nuclear Localized) proteins. When these proteins are over-expressed, the result is seedlings with shorter hypocotyls. When the activity of multiple genes is disrupted the result is seedlings with taller hypocotyls, demonstrating that these genes control seedling height in a redundant manner (Street et al., 2008). In the Brassica *Arabidopsis thaliana*, we have identified a unique mutation (*sob3-6*) in one of these genes, *SOB3/AHL29*, that expresses a protein with a disrupted DNA-binding domain and a normal protein/protein interaction domain. In *Arabidopsis*, this mutation is capable of generating normal adult plants that produce larger seeds and seedlings with hypocotyl stems that are up to twice as long as the wild type.

Objectives: The goal of this project is to enhance camelina and canola seedling emergence when planted deeply in low-rainfall dryland cropping regions (generally less than 12”/year) or in wheat stubble. This can be achieved by manipulating AHL gene family members to develop varieties that have long hypocotyls as seedlings while maintaining normal growth characteristics as adults.

Methods: This project includes three major sub-aims:

1) Continue characterizing the activity of *sob3-6-like* mutations in other *Arabidopsis AHL* genes.
2) Generate transgenic camelina plants expressing wild-type and mutant forms of *Arabidopsis AHL* genes.
3) Identify, clone and characterize *AHL* gene family members from camelina.

Results and Discussion: We have shown that a similar DNA-binding mutation (*esc-11*) in another *AHL* family member in *Arabidopsis*, *ESC/AHL27*, confers similar phenotypes as *sob3-6*. We have also shown that expressing this *Arabidopsis* mutation in the Brassica *Camelina sativa* leads to taller seedlings with no negative impact on adult size. By analyzing seed weight, we have shown that these taller seedlings are, in part, caused by an increase in seed size. However, the increase in height using the *Arabidopsis* mutant allele in camelina is only 30% and not the 100% realized by using the *Arabidopsis* mutant allele in *Arabidopsis*. Even with this 30% increase in hypocotyl length in camelina, we have shown that these
larger seeds and taller seedlings can dramatically enhance emergence from deep planting (2.5 inches) in dry soil.

**Impact/Potential Outcomes:** To date, our work has demonstrated that expressing mutant forms of *AHL* genes encoding proteins with a disrupted DNA-binding domain leads to larger seeds and taller seedlings in both *Arabidopsis* and the oilseed crop camelina. Based on preliminary *Arabidopsis* research, we may be able to double the size of camelina seeds by generating transgenic plants expressing mutant forms of these genes cloned from the camelina genome. Even if the total harvestable oil per plant is unchanged, this may lead to an increase in seed yield per acre by enhancing stand establishment and reducing harvest loss due to blowing out of the combine. Based on these preliminary results, a provisional patent application was filed by WSU during the funding period covered by the progress report. During the current funding period, this provisional patent application was converted to a full patent application.

**Affiliated projects and funding:** The characterization of the *AHL* gene family in *Arabidopsis* was supported by a grant from the Department of Energy and is the subject of a renewal currently in review. The characterization of the *AHL* gene family in wheat was recently supported by grants from the Washington Grain Commission and the Orville A. Vogel Wheat Research Fund. A large-scale transcriptome analysis of this gene family in camelina is part of a large multi-state NIFA AFRI grant proposal to develop camelina as an oilseed crop for generating drop-in Jet A fuel (Farm to Fly). I also applied for support from the Howard Hughes Medical Institute and the Gordon and Betty Moore Foundation to perform transcriptome analysis and develop a non-transgenic TILLING platform for camelina.

**Publications:** At least three publications are being written and/or pending based on this research: 1) A phylogenetic analysis of *AHL* gene evolution in plants. 2) A manuscript describing the mode-of-action for *AHL* family members in *Arabidopsis*. 3) A manuscript describing the manipulation of *AHL* genes to increase seed size and seedling height in *Arabidopsis* and camelina. All publications will be submitted to high-impact peer-reviewed journals. Results from these experiments have been included in eight invited lectures.

**Proposed Future Research/Extension:** We are currently cloning and characterizing the corresponding *AHL* gene members in camelina, creating the same type of mutant allele as was found in *Arabidopsis* and generating transgenic plants expressing these mutant alleles. Seed size, seedling height and stand establishment will be characterized in transgenic plants expressing these mutant alleles. For the second (and current) year of funding, our budget was increased from $10,000 to $15,000 (with 10% being held back in anticipation of state budget cuts that may impact this project). This funding is sufficient to begin characterizing the *AHL* genes in camelina. However, to have the biggest impact on oilseed production, at least ten times this amount of funding is needed to develop the tools that will allow us to efficiently translate this technology into non-transgenic camelina plants via a TILLING platform. We will continue to use these funds to develop proof-of-principal results that may lead to larger funds from either the private or public sector.

**References:**


Provisional patent applied for October 2009: “Genetic manipulation of the AT-hook domain in plant *AHL* genes to modulate cell growth” Inventor: Michael M. Neff. Owner: Washington State University

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