MODIFICATION OF HYPOCOTYL LENGTH AND SEED SIZE IN CAMELINA AND CANOLA BY MANIPULATING THE *AHL* GENE FAMILY

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Project Outcome Oriented Objectives

- To enhance camelina and canola seedling emergence in dryland cropping systems.
- Genetic manipulation of *AHL* gene family to create dominant-negative mutations
  - Taller seedlings
  - Larger embryos
  - Larger seeds

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Project Methods

- 1) Analyze seed size of *AHL* mutations in *Arabidopsis*
- 2) Identify, clone and characterize *AHL* gene family members from camelina
- 3) Generate transgenic *Arabidopsis*, camelina and canola expressing wildtype and mutant forms of *AHL* genes
- 4) Use CRISPR/Cas-9-based gene editing (non-GMO)

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Arabidopsis seedlings

- Dominant-negative AHL mutation
- Blocks DNA binding (*sob3-6* and *sob3-6-like*)
- Taller seedlings!
- Nearly doubles seed size in *Arabidopsis*!
- Larger camelina seeds (~20%)…

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Camelina seedlings

sob3-6 expressing transgenics lead to larger seeds that can be planted deeper in dry soil (Camelina in 8 cm of dry Palouse silt/loam):

Non-transgenic   *sob3-6* transgenics

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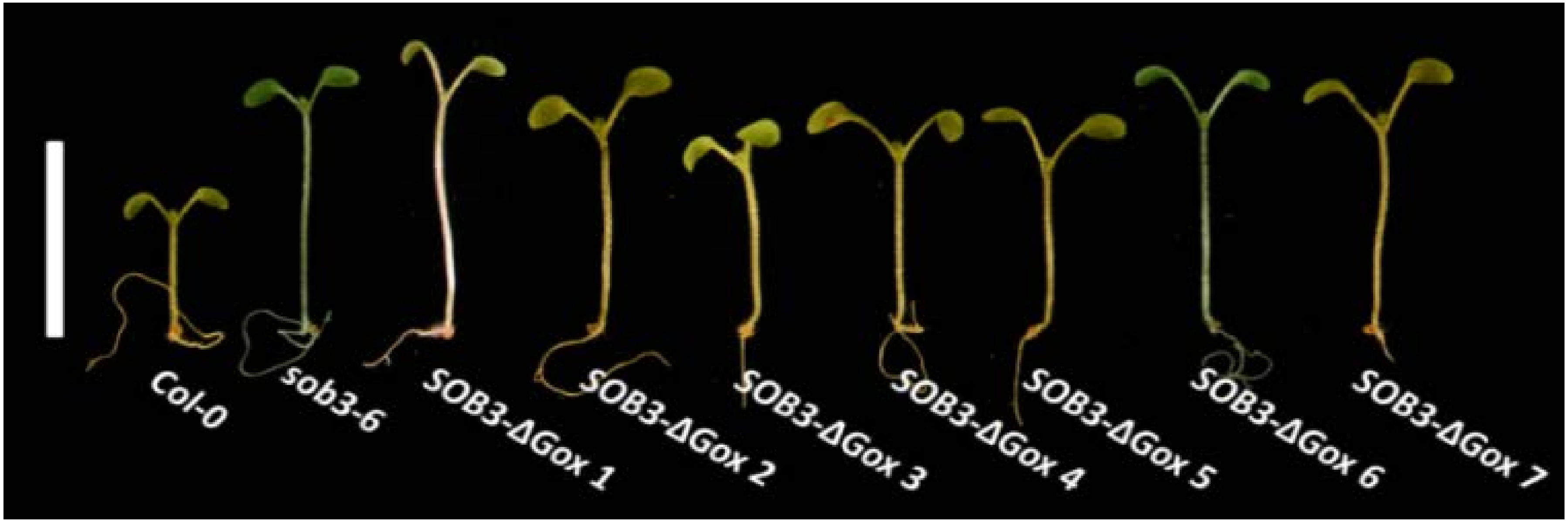
2016 Research Challenges



- Other dominant-negative AHL mutations
- Blocks protein-protein interactions
- Taller seedlings!!!!?
- No increase in seed size…

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2016 Research Challenges

- Camelina genome is complex
- Two distinct families: clade A and clade B
- Transgenic plants expressing AHL genes unstable due to gene silencing…
- Canola transformation requires tissue culture- time consuming…

2016 Research Challenges

- Also compared seedling morphology for canola varieties used in stand establishment field trials
- Too much variability in seedling growth in most varieties…
- Target for breeders?

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2016 Research Highlights

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- *Arabidopsis* AHLs: hormone-mediated development: Auxins (Plant Phys, 2016), Brassinosteroids (Plant J, 2016/2017)
- Camelina draft genome: Identified 81 camelina *AHLs* (most cases 3 copies of each).
- Camelina draft genome: Subfamilies: clades A and B
- Transgenic camelina with *CsAHL29-sob3-6*, clade A (transgene silencing…)
- Transgenic *Arabidopsis*: *AHL6* (clade B- early flowering), *AHL20* (clade A- delayed flowering, same with camelina *AHL20*)

2016 Research Highlights

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- CRISPR/Cas9 gene editing: Target *Arabidopsis* clade B AHLs
- CRISPR/Cas9: Larger *Arabidopsis* plants with 4 genes targeted?
- CRISPR/Cas9: Target camelina clade A AHLs
- *Camelina CsAHL29* (clade A): Longer hypocotyls with a *sob3-6-site* target for CRISPR/Cas9 gene editing?
- Transgenic canola: *sob3-6* but not verified yet (9 putative transgenic plants with roots on soil)

2017 Research Plan

- Finish describing “big seed” phenotypes for existing dominant-negative mutations in *Arabidopsis*, camelina-publish.
- Characterize transgenic canola
- Identify new dominant-negative mutations
- Continue working on CRISPR/Cas-9 genome editing
- Publish flowering phenotypes in clade A and clade B AHLs.

Characterize CRISPR/Cas-9 targeting clade B AHLs and larger plant size. May be the best approach in camelina.

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Cumulative Project Outcomes Towards Basic Knowledge, Furthering Adoption of Oilseed Cropping Systems

- Fundamental knowledge on how to control seed size
- Established a system to hypothesize *AHL* gene function
- Using transgenic plants to test *AHL* gene function
- Extension- talking about GMOs (> 1000 people in 2016)
- Camelina work funded by USDA-NIFA
- Pushpa Koirala- Crops Ph.D. student (supported by project)

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Adoption?- CRISPR/Cas9 to generate a non-GMO plant with altered *AHL* gene function