MODIFICATION OF HYPOCOTYL LENGTH AND SEED SIZE IN CAMELINA AND CANOLA VIA MANIPULATION OF 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
  - Larger embryos
  - Larger seeds
  - Taller seedlings

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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 camelina and canola expressing wildtype and mutant forms of *AHL* genes
- 4) Use CRISPR/Cas-9-based genome editing (non-GMO)
- 5) Characterize seedling morphology in existing varieties of canola that have been previously analyzed for stand establishment.

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Arabidopsis seedlings

- Dominant-negative AHL mutations (altered DNA binding):

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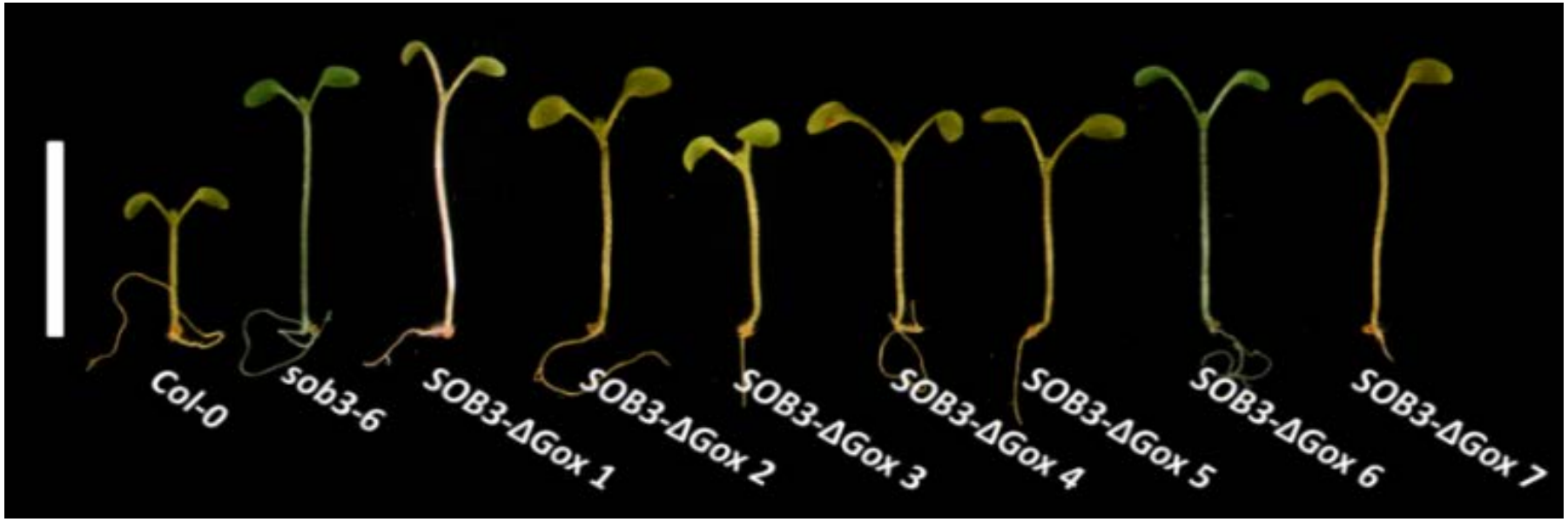
Arabidopsis seedlings

- Dominant-negative AHL mutations (altered protein interactions):



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Camelina seedlings

Mutations lead to larger seeds/seedlings that can be planted deeper in dry soil (Camelina in 8 cm of dry Palouse silt/loam):

Non-transgenic ahl mutant

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2015 Research Challenges

- Camelina genome is complex
- Transgenic plants expressing AHL genes unstable due to gene silencing
- Canola transformation requires tissue culture- time consuming

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2015 Research Highlights

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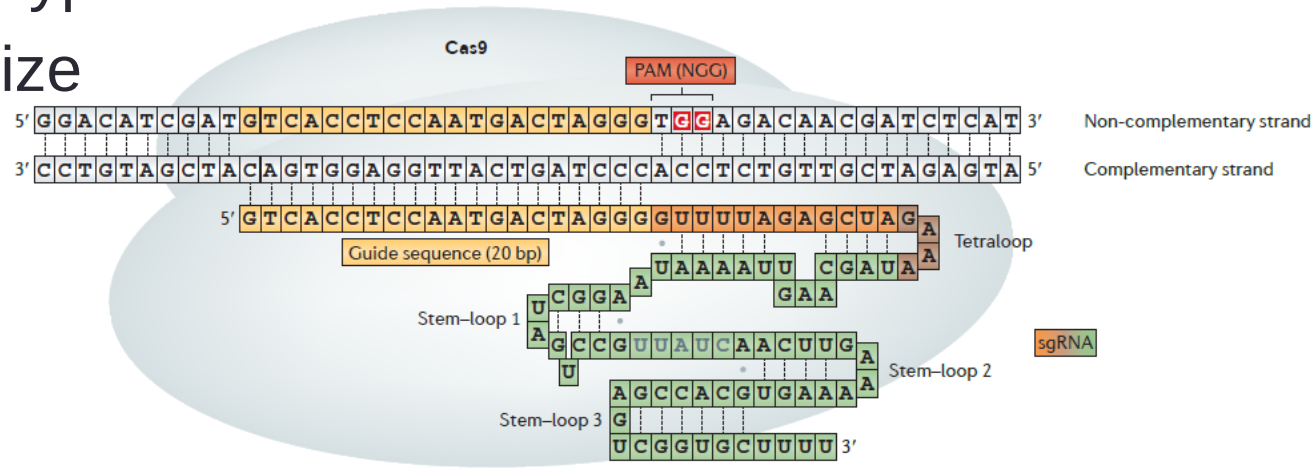
- *Arabidopsis* AHLs- hormone-mediated development: auxin, BRs
- Camelina draft genome- Identified 81 camelina *AHLs* (most cases 3 copies of each)
- Cloned- *CsAHL6, CsAHL14, CsAHL19, CsAHL20* (2 variants), *CsAHL22, CsAHL27, CsAHL29*
- Transformed camelina with *CsAHL29-sob3-6-like*
- Transgenic *Arabidopsis: CsAHL6, CsAHL20* (delayed flowering)
- Transgenic *Arabidopsis:* canola *AHL27* (similar to Arabidopsis but died)
- Transgenic canola: *sob3-6* but not verified yet (8 transgenic plants)

2015 CRIPSR-Cas9 genome editing

- Uses transgenics to edit genome
- Remove transgene- non-GMO product with targeted mutation
- Neff lab- successful using *Arabidopsis*
- Targeting *CsAHL29* AT-hook
- Camelina T2 populations growing (from 75 T1 events)
- From 55 lines harvested- 35 look like T-DNA silencing
- Some adult phenotypes
- Need to characterize



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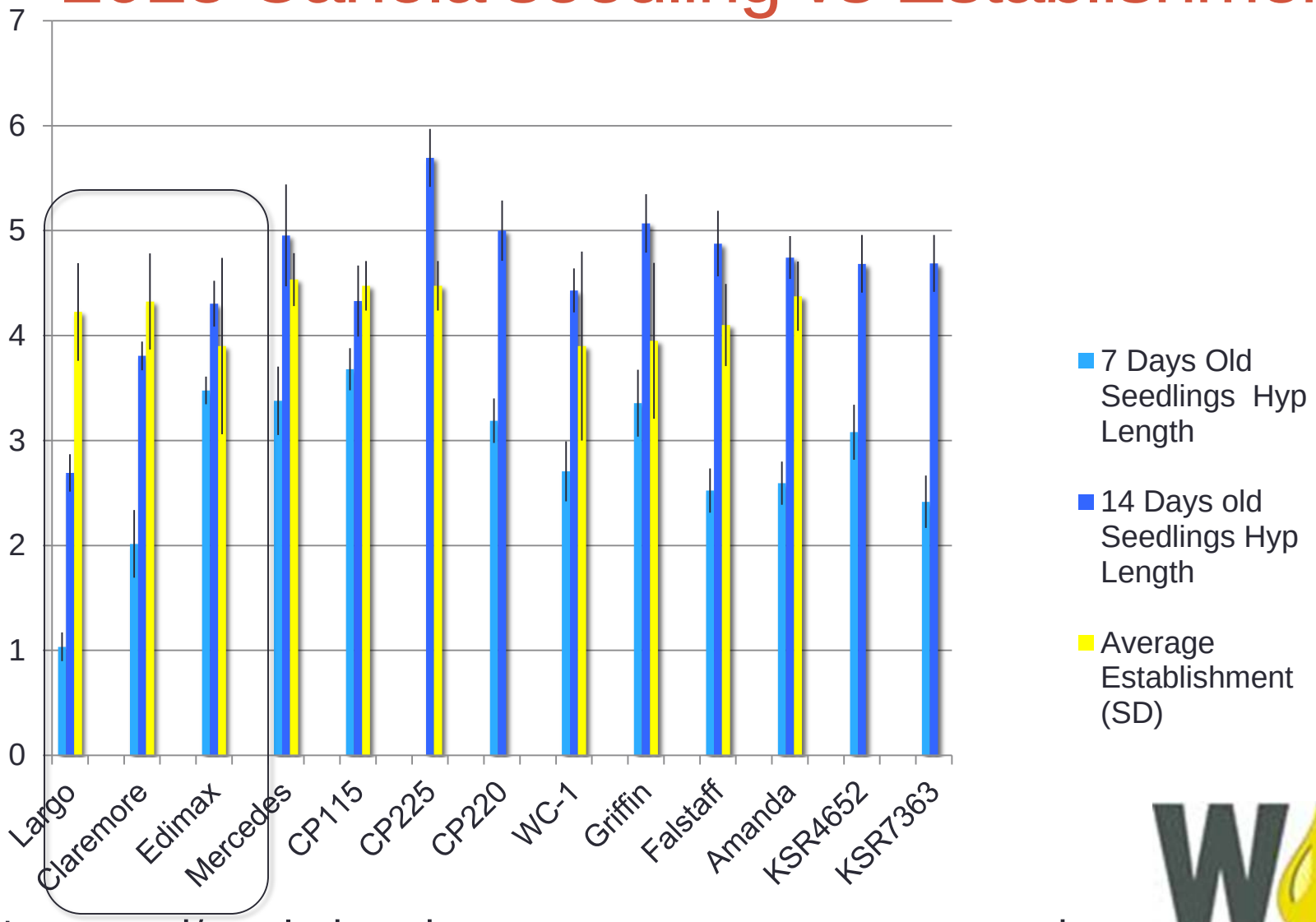
2015 Canola seedling vs Establishment



Next: seed/cotyledon size, response to temperature and hormones, cell number, root growth

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2016 Research Plan

- Finish describing “big seed” phenotypes for existing dominant-negative mutations in *Arabidopsis*, camelina- publish.
- Perform phylogenetic analysis of AHL gene family in camelina (auto- vs. allo-hexaploid?)
- Characterize transgenic canola
- Identify new dominant-negative mutations (Holly Lane in Spain)
- Continue working on CRISPR/Cas-9 genome editing
- Continue working on canola variety characterization

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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 (2000 people in 2015)
- Camelina work funded by USDA-NIFA
- Pushpa Koirala- Crops Ph.D. student

Adoption?- Developing DNA editing to generate a non-GMO plant with altered *AHL* gene function

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