Manipulation of AHL genes in *Arabidopsis* and *Camelina* to increase seed size, seedling height and stand establishment in dry soils

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Michael M. Neff; Jianfei Zhao; Pushpa Koirala; Jiwen Qiu; David Favero

Department of Crop and Soil Sciences, Washington State University, Pullman, WA

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Abstract

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 ground water. In areas with higher rainfall, canola and Camelina are often used in rotations where they are planted in wheat stubble left over to reduce erosion and increase soil quality. 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. In the Brassica *Arabidopsis thaliana,* we have identified a unique mutation in one of these genes, *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. We have shown that a similar mutation in another *AHL* family member confers similar phenotypes. 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. 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 taller seedlings can dramatically enhance emergence from deep planting (2.5 in) in dry soil. 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. The possibility of a non-transgenic TILLING approach in breeding applications may also be explored.

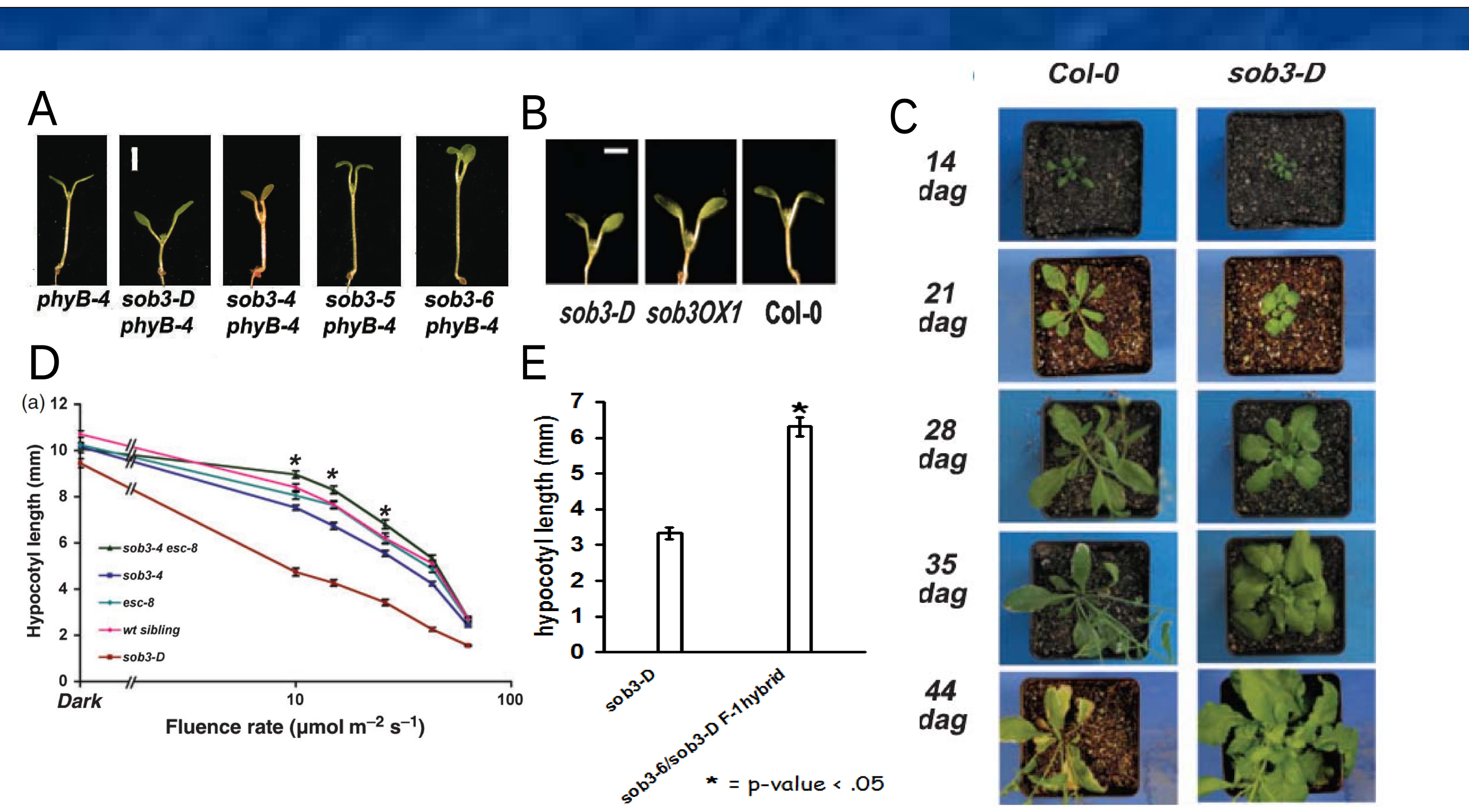

Figure 1 ***SOB3/AHL29*** **and** ***ESC/AHL27*** **are negative modulators of hypocotyl growth in seedlings. (A)** 5-day-old Arabidopsis seedlings grown under continuous light. *sob3-D phyB-4* seedlings shows a suppressed hypocotyl growth phenotype. Three intragenic suppressor seedling plants, *sob3-4*, *sob3-5* and *sob3-6*, have longer hypocotyl than *sob3-D phyB-4* plants. Scale bar=2mm. **(B)** Recapitulation of the suppressed hypocotyl growth phenotype of sob3 over-expression. Scale bar=2mm. **(C)** Wild-type and *SOB3-D* adult plants over a 44-day period under long-day (16h light:8h dark) condition. *SOB3-D* plant shows an adult phenotype with enlarged leaves, delayed growth and flowering time. **(D)** Fluence-rate response assay of 5-day-old seedlings grown in continuous light. *sob3-4 esc-8* double-null seedlings have longer hypocotyls than wild-type seedlings, while, *sob3-D* gain-of-function seedlings have shorter hypocotyls than wild-type seedlings. Asterisk represents a p-value<0.05 by student's unpaired t-test. **(E)** *sob3-6* suppresses the shorter hypocotyl phenotype conveyed by the *SOB3-D* allele suggesting *sob3-6* functions as a dominant-negative allele. Street *et al* (2008), *Plant J*, 54(1):1-14

Figure 2 ***sob3-6*** **is a dominant-negative allele resulting in an elongated hypocotyl.(A)** Recapitulation of the *sob3-6* phenotype in hemizygous primary transformed lines. 6-day-old Arabidopsis seedlings growing in white light. Multiple T1 generation seedlings are shown. **(B)** Over-expression of *esc-11*, which bears a similar mutation as *sob3-6* in the AT-hook motif, generates the same elongated hypocotyl phenotype. This indicates that the dominant-negative feature of the *sob3-6* allele may be common for other *AHL* members.

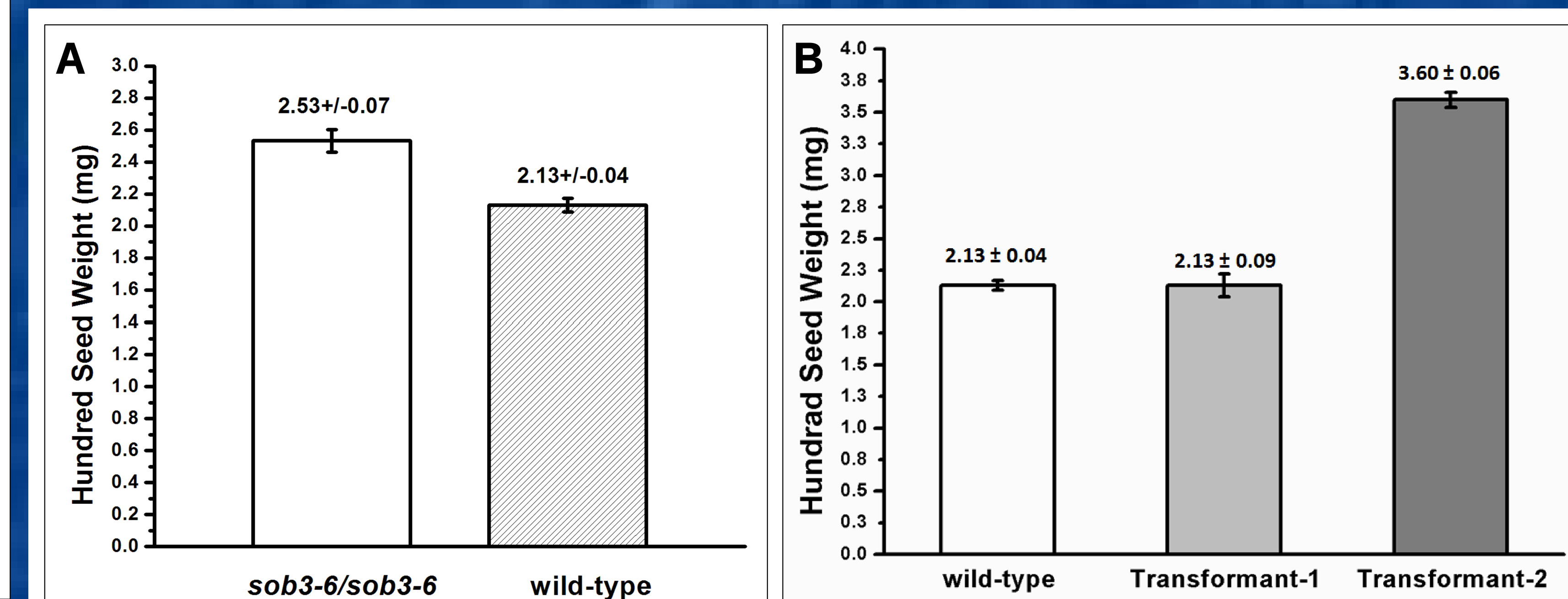

Figure 3 The ***sob3-6*** **allele confers larger seeds in** ***Arabidopsis*****. (A)** The weight of 100 homozygous *Arabidopsis sob3-6* mutant seeds (left/open) is heavier when compared to a wild-type control (right/hatched). **(B)** The weight of 100 T3 generation transgenic *Arabidopsis* seeds over-expressing *Atsob3-6* compared to the wild type. Transformant-2 (far-right/dark) is heavier when compared to the wild type (far-left/open) and Transformant-1 (center/light). Transformant-1 confers a hypocotyl phenotype that is the same as the wild type. Transformant-2 confers a longer hypocotyl than the wild-type. Raw values are presented above the bars along with ±SEM.

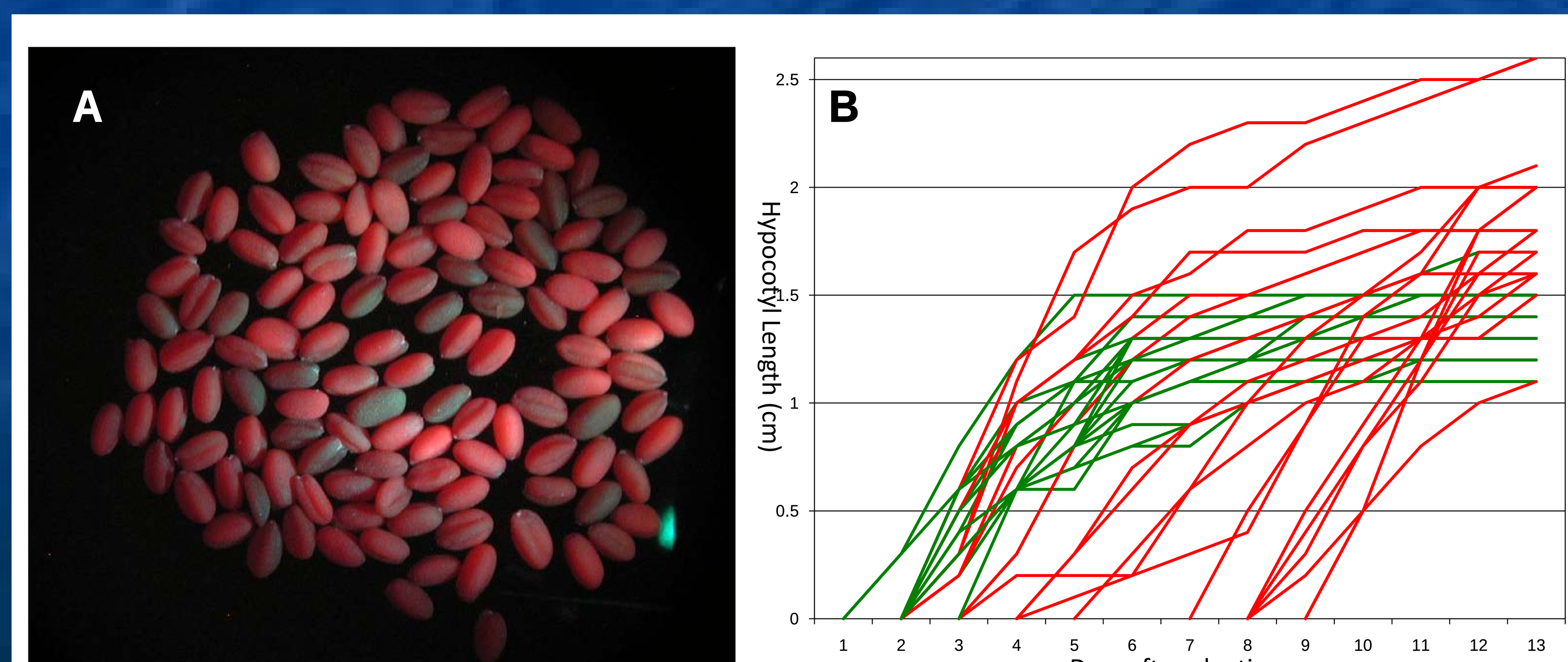

Figure 4 Transgenic ***Camelina*** **expressing the** ***Arabidopsis sob3-6*** **allele have longer hypocotyls. (A)** Transgenic *Camelina* seeds were identified using a DsRed fluorescent marker protein **(B)** Primary (T1) *Camelina* transformants expressing the *Arabidopsis sob3-6* allele (red lines) are generally taller and faster growing than non-transformed sibling seedlings (green lines) despite often germinating later than non-transformed sibling seedlings controls.

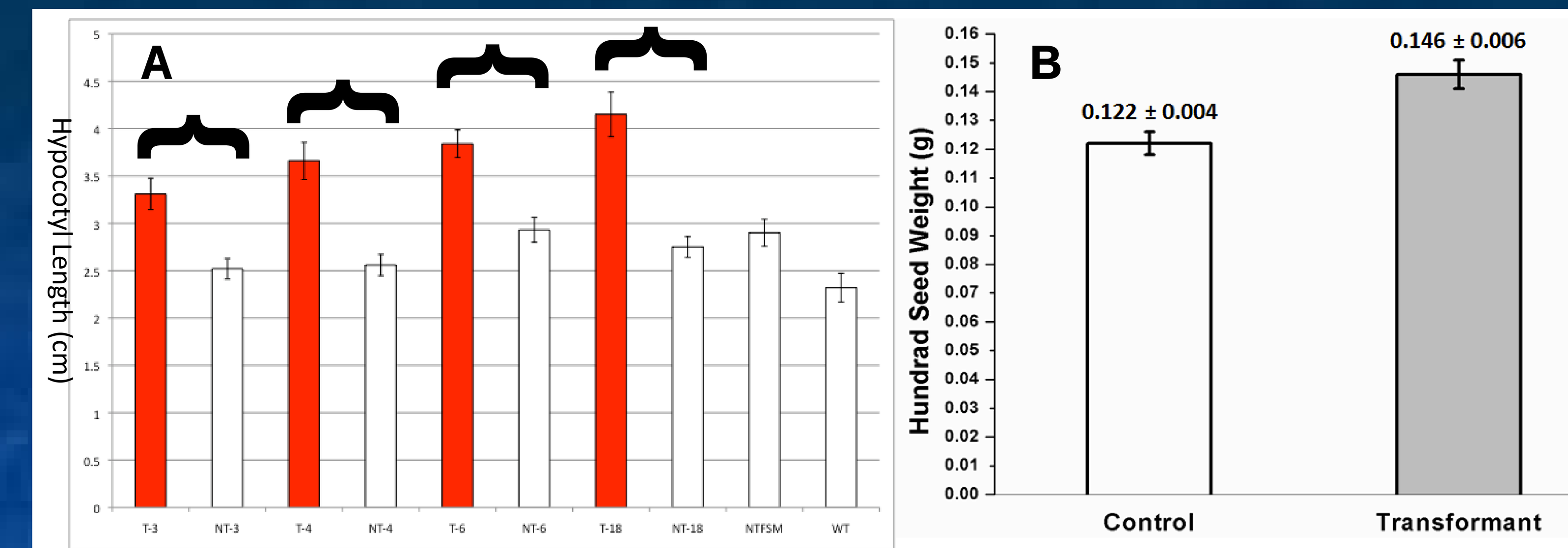

Figure 5 The tall hypocotyl phenotype of transgenic ***Camelina*** **expressing the** ***Arabidopsis sob3-6*** **is still seen in the next generation. (A)** Transgenic T2 seedlings (red bars) maintain the tall hypocotyl phenotype compared to their non-transgenic siblings (open bars). Brackets denote sibling pairs. The right two bars represent non-transformed wild-type controls. **(B)** The weight of 100 T4 generation transgenic *Camelina* seeds over-expressing *Atsob3-6* (right/shaded) is heavier when compared to a transgenic line expressing the empty-vector (left/open). The "transformant" line (right) also yields seedlings with longer hypocotyls than empty-vector control line. Raw values are presented above the bars along with ± SEM.

Figure 6 Transgenic ***Camelina*** **expressing the** ***Arabidopsis sob3-6*** **can emerge better than the wild type when planted deeply in dry soils.** Ten T3 transgenic *Camelina* seeds (right), and ten wild-type siblings (left) after being planted on 1 cm of moist Palouse silt-loam and then covered with 8 cm of dry Palouse silt-loam. All ten seeds germinated in each pot. No wild-type seedlings emerged where as five transgenic seedlings did, with three surviving. This experiment has been repeated twice with the same results. A U.S. currency quarter is shown as a size comparison.

Conclusion

- *SOB3/AHL29* and *ESC/AHL 27* negatively regulate hypocotly growth in *Arabidopsis* seedlings. **(Figure 1)**
- The *sob3-6* and *esc-11* dominant-negative alleles confer a longer hypocotyl in transgenic *Arabidopsis* seedlings. **(Figure 2)**
- The longer seedling and larger cotyledon phenotype conferred by *sob3-6* leads to larger seeds. **(Figure 3)**
- The *Arabidopsis sob3-6* allele also confers longer hypocotyls and larger seeds in *Camelina*. **(Figure 4 and 5)**
- The *Arabidopsis sob3-6* allele, when expressed in *Camelina* allows emergence when planted deeply in dry soils. **(Figure 6)**

Future Directions

- Identify and clone similar genes from the *Camelina* genome.
- Generate *sob3-6-like* mutations in these genes and express in transgenic plants.
- Identify family members that are expressed at high levels in seeds and seedlings.
- Screen TILLING populations for induced *sob3-6-like* mutations in these genes.

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