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

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Abstract

In low rainfall, dryland-cropping areas of Eastern Washington, such as the regions around Washucama, 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 seedlings have access to ground water. In areas with higher rainfall, canola and Camelina are often used in rotations where they are planted in wheast 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 hypocotyls that are up to twice as long as the wild type. We have shown that a similar mutation in another AVL 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

**A** 5-day-old *Arabidopsis* seedlings grown under continuous light. *sob3-2* seedlings show a suppressed hypocotyl growth phenotype. Three intragenic suppressor seedlings plants, sob3-2 s10b-5 and sob3-6, have longer hypocotyl than sob3-2 phyD-4 plants. Scale bar=2mm. **B** Recapitulation of the suppressed hypocotyl growth phenotype of sob3-6 over-expression. Scale bar=2mm. **C** Wild-type and sob3-6 seedlings over a 44-day period under long-day (16h light:8h dark) condition. sob3-6 plant shows an adult phenotype with elongated leaves, delayed growth and flowering time. **D** Fluence-rate response assay of 5-day-old seedlings grown in continuous light, sob3-6 ec12 double-null seedlings have longer hypocotyl than wild-type seedlings, while sob3-2 D gain-of-function seedlings have shorter hypocotyl than wild-type seedlings. Asterisk represents a p-value<0.005 by student’s t-test. (E) sob3-6 suppresses the short hypocotyl phenotype conferred by the sob3-6 allele suggesting sob3-6 functions as a dominant-negative allele. Street et al. (2008). Plant J, 54(1):1-14

Figure 2

**A** *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-12, 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 AVL members.

Figure 3

**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 esc-12 (left/open) is heavier when compared to the wild type (far-right/open). Transformant 1 (center/light) is heavier when compared to the wild type (far-left/open) and Transformant 2 (center/dark) 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

**A** Transgenic *Camelina* expressing the *Arabidopsis* sob3-6 allele have longer hypocotyls. (A) Transgenic *Camelina* seeds were identified using a labeled 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

**A** The tall hypocotyl phenotype of transgenic *Camelina* expressing the *Arabidopsis* sob3-6 allele is still seen in the next generation. **B** The weight of 100 T4 generation transgenic *Camelina* seeds over-expressing sob3-6 (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 sibling seeds (left) after being planted on 1 cm of moist soil were sown into the same pots and then covered with 8 cm of dry Palouse silt-loam. All transgenic seedlings germinated in each pot. No wild-type seedlings emerged whereas 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/AHL27 negatively regulate hypocotyl 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 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 mutants 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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