Manipulating the *AT-Hook Motif Nuclear Localized* (*AHL*) Gene Family for Bigger Camelina Seeds with Improved Stand Establishment

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In low rainfall dryland-cropping areas of eastern Washington, 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 water in the soil. 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 novel approach to improve stand establishment by uncoupling seedling and adult phenotypes through the manipulation of members of the AT-hook motif nuclear localized (AHL) family. The DNA-binding AHL proteins contain two functional units, the AT-hook motif and the PPC domain. Over-expression of one *Arabidopsis thaliana AHL* allele (*sob3-6*), with abolished DNA-binding capacity, leads to a long hypocotyl seedling phenotype with normal adult stature. Similarly, over-expression of the SOB3/AtAHL29 PPC domain alone, as well as alleles with specific mutations in the PPC domain, also confers similar long-hypocotyl seedlings with normal adult stature in *Arabidopsis* (Figure 1). All successful transformants with the long-hypocotyl phenotype also showed increased seed size and weight. We further over-expressed the *Arabidopsis sob3-6* allele in the oilseed crop *Camelina sativa*. These transgenic *Camelina* lines had larger seeds and seedlings with longer hypocotyls than their non-transgenic siblings. These transgenic *Camelina* seedlings can successfully emerge from deep planting in dry soil (Figure 2). These results demonstrate that transgenic manipulation of the *AHL* genes can improve stand establishment in dryland cropping systems. This approach may also be used in non-transgenic breeding strategies that employ specific mutations in members of the *AHL* gene family. We have recently been awarded a USDA/NIFA grant ($498,000/three years) to continue this project. Half of this award is for working with wheat, the other half for *Camelina*.

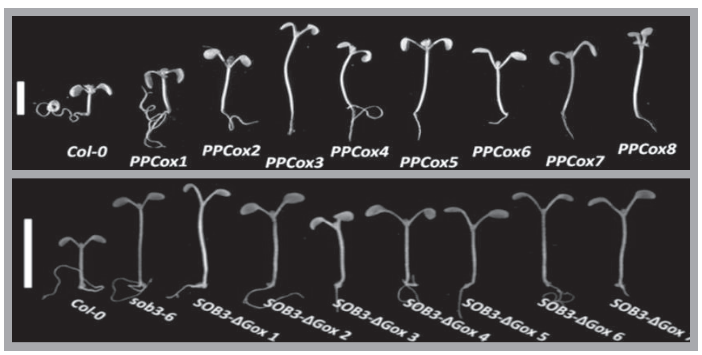

Figure 1. Over-expressing an AHL protein in Arabidopsis lacking the AT-hook domain leads to taller seedlings (top). The same phenotype can be obtained with an AHL protein that lacks six amino acids (bottom). Scale Bars= 1 cm.

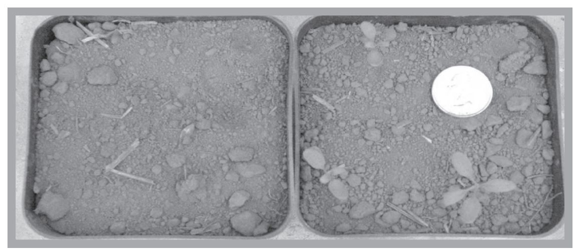

Figure 2. *Camelina* expressing the *sob*3-6 mutation emerge from deep planting in dry soil. Non-transgenic (left) and transgenic (right) seeds were planted on 1 cm of moist Palouse silt/loam and covered with 8 cm of dry silt/loam. All seeds germinated. 5 of 10 transgenic seeds emerged, 3 survived.

Rotational Influence of Spring Grown Brassica Biofuel and Other Crops on Winter Wheat

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Growing Brassica oilseed crops in eastern Washington must fit within the regional rotational cropping systems. When grown, broadleaf crops usually precede winter wheat in rotation and studies worldwide have shown the benefit to winter wheat when following a broadleaf crop. The potential benefit of any crop should include the rotational effects on following crops.

These studies are two year crop sequence studies on eight spring crops (spring wheat, spring barley, dry pea, lentil, camelina, yellow mustard, oriental mustard, and canola) planted in year 1 followed by winter wheat (year 2) grown across all year 1 spring crops. The year 2 winter wheat planted within each of the previous spring crop areas is divided into sub-plots receiving fertilizer rates of 32, 64, 96, 128, 160 lbs N/acre applied in a split-plot, factorial design.

Results and Conclusions from five years of spring crop comparisons:

- Performance variability among spring crop within a year is high and varies over years.
- Some crops, particularly barley and camelina, are more consistent across years.
- Market prices and yields will guide spring crop selection for growers.
- Winter wheat performance following spring crops is best after pea and lentil, followed by brassicas, and both are better than after wheat or barley.
- Wheat fertilizer response is best after legumes followed closely by brassica crops.
- These studies show the tangible rotational benefits attributable to biofuel and other spring crops that are followed by winter wheat.

Results and Conclusions from rotation effect on winter wheat in 2013:

Winter wheat yield, test weight, plant height, and grain protein from 2013 plots following eight previous spring crops are presented in Table 1. Although there were no significant (p=0.05) differences among the previous crops for winter wheat yields, highest yields followed pea and lentil, similar to earlier results that showed that legume crops grown the year pervious increased performance of winter wheat. Protein levels were also highest following spring legume crops. Thus, from an input amount, resource utilization, and food produced per unit of greenhouse gas emissions, the legume crops are standouts from the spring brassica and cereal crops because they do not require N fertilizer, they increased following winter wheat crop yields, and supported higher grain protein in the following crops. In similar experiments, the brassica crops produced higher following winter wheat performance than when winter wheat followed spring wheat or barley. All spring crops, except the grain legumes, received similar amounts of N fertilizer for their growing season. Both yield and protein increased along with increasing N fertilizer rates and the largest responses were in middle increments of N fertilizer rates, 64 to 96 to 128 lb/acre of applied N. Similar to previous results, there was no significant interaction of N rate and previous crop indicating similar N response after all spring crops. The 160 lb/acre N fertilizer rate did not increase yields compared to the 128 lb/acre rate, but did increase grain protein.

Previous Spring Crop	Wheat Yield	Test wt.	Height	Protein
	bu/acre	lb/bu	inches	%
Spring Wheat	80	61.3	28	11.0
Spring Barley	85	61.5	29	10.8
Dry Pea	92	61.8	29	11.5
Lentil	89	61.9	29	11.3
Camelina	80	61.9	30	10.5
Yellow Mustard	84	61.7	29	10.3
Oriental Mustard	86	61.8	30	10.9
Canola	79	61.9	29	11.0
Average	84	61.7	29	10.9
LSD (0.05)	n.s.	0.4	1	0.8

N Fertilizer Rate (lbs/acre)	Seed Yield	Test wt.	Height	Protein
	bu/acre	lb/bu	inches	%
32	80	61.6	29	10.3
64	81	61.8	29	10.6
96	85	61.9	29	10.8
128	89	61.7	30	11.3
160	88	61.7	30	11.5
Average	84	61.7	29	10.9
LSD (0.05)	3	n.s.	1	0.2