**Title:** Development of Camelina Lines Resistant to Group 2 Herbicides

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**Technical Support:** Ron Sloot

**Duration:** Five years; 2007-2011.

**Background:** Our initial experiments found that all camelina varieties tested were similar to canola varieties in their sensitivity to even residual soil levels of imidazolinone and sulfonylurea herbicides that are commonly used in the Pacific Northwest (PNW). In other plant species, resistance to these herbicides have typically occurred by specific mutations at loci that code for the enzyme acetolactate synthase (ALS) which is necessary for synthesis of certain essential amino acids (Chaleff & Mauvais, 1984; Haughn & Somerville, 1986; Hattori et al., 1992; Tranel and Wright 2002). Plant breeders working on other crops, like canola and wheat, have been successful in identifying genetic variants that are more resistant to these herbicides by mutagenesis and selection (Tan et al. 2005). We therefore initiated a program to identify mutant alleles and identify the best genetic backgrounds to incorporate these alleles into germplasm for superior varieties.

Camelina is poorly characterized genetically, but some advances have been made recently (Gehringer et al. 2006, Hutcheon et al. 2010). Its chromosome number (2n=40) indicates it is probably a polyploidy since basic chromosome numbers in the *Brassica* tribe are typically small. It also appears to have mostly diploid inheritance, though most of the markers studied were AFLPs which are not ideal for these analyses since they are multilocus markers and typically scored as dominant alleles. Most SSR markers also detected more than one locus (Gehringer et al. 2006). In addition, Hutcheon et al. found three different sequences for two genes coding for proteins involved in fatty acid biosynthesis, supporting the idea that the genome is polyploid. In addition, the genome size was found to be approximately three times that of diploid relatives.

Several other breakthroughs have recently been made that should help camelina get established as a viable oilseed crop in eastern Washington. An herbicide (Poast®) has been approved to help control grassy weeds (http://agr.mt.gov/camelina/Poast%20Label.pdf). Progress has also been made in gaining approvals for the use of camelina meal as a feed ingredient. The FDA has now written a “letter of no objection” for the feeding of camelina meal to broiler chickens, laying hens and cattle fed in confinement for slaughter at rates of no more than 10 percent of the final diet.

**Objectives:** The main objective is to identify lines that are less sensitive to imidazolinone herbicides like Pursuit® and Beyond®. Pursuit is used widely in legume rotations and Beyond is used increasingly in Clearfield® wheat production. A secondary objective is to find lines with increased resistance to sulfonylurea-based herbicides, like Maverick®. The ultimate objective is to release these lines to camelina breeders so that the herbicide resistant traits are incorporated into commercial cultivars.
**Methods:** Construct camelina populations that are mutagenized with ethyl-methane sulfonate and amplify these populations by self fertilization. Screen these populations in field plots with commercial rates of herbicides. Determine the inheritance of resistance while moving the resistance into camelina lines with good yield potential. Examine the performance of camelina cultivars to determine suitable backgrounds for the mutations.

**Results:** To identify mutations in camelina that confer tolerance to group 2 herbicides, we constructed EMS mutagenized populations of the camelina cultivars Calena and Cheyenne. The mutagenized seed was advanced by self-fertilization one generation without selection to allow mutant alleles to become homozygous to better reveal recessive or codominant alleles. Our initial field experiment identified several putative mutants resistant to the herbicide imazethapyr (Pursuit®). The putative mutants were transplanted to the greenhouse and seed was recovered from most. We tested the progeny with imazethapyr by spraying recommended field rates (4 oz/acre) to determine which of the families are derived from true herbicide resistant mutations. We also tested progeny seedlings of the putative mutants for tolerance to imazamox (Beyond® herbicide). Four of the lines, designated IM1-IM4, showed significant levels of resistance to both herbicides and no morphological abnormalities. One of the mutants (IM1) was from the Cheyenne population and three were from the Calena population. All of the progeny from each mutant appeared to have similar levels of increased resistance to both herbicides indicating the original selection was homozygous for the mutation and that the mutation caused cross resistance. Fertility and seed set and morphology were similar to the parental lines.

We also screened the mutagenized populations to identify resistance to the sulfonylurea herbicide sulfosulfuron (Maverick®). Seed from several possible mutants were collected and tested in the greenhouse for resistance. One of the lines, designated SM1, showed increased resistance. As with the IM1-IM4 mutants, seed from the SM4 mutant appeared true breeding for resistance indicating the original mutant plant selected was homozygous for the resistance gene.

The mutants were intercrossed to each other and to Calena. F2 populations of resistant x susceptible crosses were made for all but IM3, where no F2 seed was obtained. Examination of segregation in the F2 populations indicated that the resistances of the IM1, IM2, IM4 and SM4 were caused by single dominant or codominant genes (Table 1). The levels of damage varied among the segregating F2 seedlings so they were initially scored as resistant, susceptible and intermediate. When the intermediate and resistant classes were combined, all of the mutants segregated 3:1 as expected for a single dominant gene. When the frequencies of the three classes were tested against a 1:2:1 ratio, the IM1 and SM1 progeny showed an excess of seedlings and scored as intermediate. Testing progeny of some of these plants scored as intermediate indicated some bred true for resistance, indicating the F2 plants were actually homozygous. Taken together, the data suggest that resistance segregates as a co-dominant gene, but the homozygous resistant and heterozygous plants are difficult to distinguish.
Table 1. Segregation of resistance to imazethapyr in F2 families derived from resistant by susceptible crosses.

<table>
<thead>
<tr>
<th>F2</th>
<th>Number of Plants</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Susceptible</th>
<th>Chi-sq 1:2:1</th>
<th>Chi-sq 3:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calena/IM1</td>
<td>809</td>
<td>149</td>
<td>450</td>
<td>210</td>
<td>19.4351*</td>
<td>0.39596</td>
</tr>
<tr>
<td>Cheyenne/IM2</td>
<td>551</td>
<td>117</td>
<td>279</td>
<td>155</td>
<td>5.3303</td>
<td>2.8802</td>
</tr>
<tr>
<td>Cheyenne/IM4</td>
<td>646</td>
<td>145</td>
<td>354</td>
<td>147</td>
<td>5.9628</td>
<td>1.7358</td>
</tr>
<tr>
<td>Calena/SM4</td>
<td>821</td>
<td>173</td>
<td>452</td>
<td>196</td>
<td>9.6796*</td>
<td>0.5558</td>
</tr>
</tbody>
</table>

* indicate Chi-square values that deviate significantly (P<.01) from expected ratios.

F2 populations from crosses between the different mutants did not segregate for susceptibility or levels of resistance that were noticeably higher than the parent. This indicates that all of the resistances occurred at the same locus.

To better characterize their resistances, lines derived from all five mutants were examined with group 2 herbicides from three different chemical classes (Fig. 1). All of the lines showed increased resistance to imazethapyr and flucarbazone, but SM4 showed higher levels of resistance. In addition, SM4 showed increased resistance to the sulfosulfuron (a sulfonylurea), while the others appeared similarly sensitive to the control plants.

![Fresh biomass of Camelina 21 days after herbicide treatment](image)

**Figure 1.** Resistances of five Camelina mutants to three group 2 herbicides as compared to the sensitive cultivar Calena.

To determine the nature of the mutation in the SM4 line, ALS encoding genes were PCR amplified and sequenced using degenerate primers. Four different genomic clones and 15 different cDNAs were sequenced. Surprisingly, 11 different sequences were identified among the 19 clones (Figure 2). None of the genes showed nucleotide substitutions that had been previously observed in other plants which gained resistance to group 2 herbicides. One of these
(SM4-5-cDNA, Fig. 2) showed a substitution corresponding to a change identified in the Yeast ALS encoding gene that caused resistance. The substitution caused a phenylalanine to leucine replacement at the position corresponding to amino acid 578 in the yeast protein (Figure 3). To determine if this change was unique to the SM4 variant, PCR primers were generated that were specific to the nucleotide substitution. The primers efficiently amplified DNA fragments of the expected size from the SM4 mutant but not from the parental line Cheyenne.

**Figure 2.** Alignment of polymorphic nucleotides from genomic and cDNA clones of the ALS encoding genes from the SM4 mutant.

**Figure 3.** Nucleotide substitution in the ALS encoding gene causing the increased resistance in the SM4 mutant. The resulting phenylalanine to leucine substitution is also shown.

**Discussion:** We identified five mutations that confer partial resistance to different group 2 herbicides. Four of the mutants were very similar or identical in their responses to the herbicides tested, while the SM4 mutation appeared different and superior for practical purposes. SM4 showed higher levels of resistance to all three chemical classes tested and was the only mutant that showed increased resistance to sulfosulfuron (a sulfonylurea). Even the SM4 mutant, however, was not completely resistant to the herbicides when they were sprayed directly on the foliage because the seedlings typically are stunted at least temporarily. The incomplete resistance is probably due in part to the copy number of the ALS gene family in camelina. Eleven different sequences were identified in the SM4 line. Although this line was derived from a single M2 plant, it is possible it is heterozygous for some family members. The numbers of sequences generated, however, indicates there are at least six different ALS genes in camelina. Since most of the genes were identified as cDNA sequences, it appears that most of these genes are actively transcribed.
Since the SM4 mutant is partially resistant to three subclasses of the group 2 herbicides this is now the favorite mutant and is being bred for release. This mutant occurred in the Cheyenne background and we have crossed it to Calena. Several large F2 families were planted in the field in June and sprayed with Pursuit. Seed from 470 vigorous plants have been harvested and will be planted at Lind in duplicate plots in late winter. One plot will be used to estimate yield and the other will be sprayed to identify which lines are homozygous for the resistant allele or are still segregating. Seed from the highest yielding, homozygous resistant lines will be collected for advancement.

**Impact/Potential Outcomes:** Camelina varieties resistant to group 2 herbicides will help adoption of this crop in the intermediate rainfall area and possibly parts of the low and high rainfall areas. No large differences in yield potential were seen among varieties in our tests. We therefore do not think it will be difficult to create a cultivar with the herbicide resistant traits incorporated along with yield potentials similar to existing cultivars.

**Publications and Presentations:** We presented the project at the Pacific Northwest Direct Seed Association meeting January 2009 (short talk) and 2010 (poster) and at the Washington State Bioenergy Research Symposium in November 2010 (talk and poster).

**Future directions in the upcoming year:** Our main objective is to get the ALS-SM4 mutant allele homozygous into a good genetic background that can be released to breeders or grown as a variety. We will:

1) Select good yielding homozygous lines from our nursery in Lind.
2) Write and submit a germplasm release paper.
3) Write and submit a paper describing the mutants and their inheritance.
4) Amplify sufficient seed to begin field herbicide trials to test relative resistance to levels of group 2 herbicide carryover. These will be conducted in two locations and will begin with fall 2011 soil treatments and spring 2011 plantings at Davenport and Pullman. The cultivar Cheyenne will be compared to favorite ALS-SM4 lines.

**References:**


