

Supplemental Material

CRISPR/Cas9-Induced *fad2* and *rod1* Mutations Stacked with *fae1* Confer High Oleic Acid Seed Oil in Pennycress (*Thlaspi arvense* L.)

Brice A. Jarvis¹, Trevor B. Romsdahl², Michaela McGinn¹, Tara J. Nazarenus³, Edgar B. Cahoon³, Kent D. Chapman², and John C. Sedbrook^{1*}

¹School of Biological Sciences, Illinois State University, Normal, IL 61790, USA

²BioDiscovery Institute and Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA

³Department of Biochemistry and Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68588 USA

*Correspondence: John Sedbrook, Email: jcsedbr@ilstu.edu

Table S1. Seed TAG fatty acid compositions of the different pennycress CRISPR-induced oilseed mutants. Values represent means plus/minus standard deviations. Asterisks represent significant differences compared to wild type based on Student t-test analysis where p < 0.05. n = 3. These data are graphed in Figure 2.

Genotype	<u>16:0</u>	<u>16:1</u>	<u>18:0</u>	<u>18:1</u>	<u>18:2</u>	<u>18:3</u>	<u>20:1</u>	<u>22:1</u>	<u>24:1</u>
WT Spring 32-10	3.0±0.1	0.3 ± 0.0	0.4 ± 0.0	12.1±0.8	18.1 ± 0.5	11.7±0.4	12.0±0.5	34.9±0.9	3.3±0.1
<i>fad2-4</i> (-2bp)	2.1±0.1**	$0.1\pm0.1**$	$0.4{\pm}0.0$	34.9±0.7**	0.5±0.2**	1.7±0.1**	16.6±0.4**	39.3±0.7**	3.4±0.1**
fad2-5 (+A)	2.4	0.1	0.4	35.0	0.5	2.5	14.9	39.8	3.8
fad2-6 (-29bp)	2.5	0.1	0.4	34.0	0.5	2.7	14.2	41.4	3.6
rod1-3 (-18bp)	3.4±0.4*	$0.4\pm0.0**$	$0.6\pm0.0**$	22.7±1.0**	8.5±0.6**	11.8 ± 0.6	14.0±0.3**	35.3±2.0	2.8±0.2**
rod1-4 (+A)	3.2±0.3	$0.4{\pm}0.0**$	$0.5\pm0.0**$	22.4±0.7**	9.1±0.8**	11.2±0.2	14.4±0.2**	35.7±1.5	3.0±0.3
rod1-5 (+T)	4.3	0.0	0.5	23.1	10.5	12.5	14.3	31.7	2.8
<i>fae1-3</i> (-4bp)	3.5±0.1**	0.3 ± 0.0	$0.8\pm0.0**$	47.8±1.9**	28.5±1.4**	17.8±0.6**	0.9±0.1**	$0.0\pm0.0**$	0.3±0.0**
fad2-4 fae1-3	2.5±0.2**	0.2 ± 0.1	$0.6\pm0.0**$	90.6±0.6**	0.5±0.1**	2.6±0.3**	1.5±0.1**	$0.0\pm0.0**$	0.2±0.2**
rod1-4 fae1-3	3.4±0.0*	$0.0\pm0.0**$	$0.6\pm0.0**$	59.7±0.3**	17.1±0.2*	19.3±0.3**	$0.0\pm0.0**$	$0.0\pm0.0**$	0.0±0.0**

Table S2. Total seed germination on agar growth media plates of the different pennycress CRISPR-induced oilseed mutants. Values represent cumulative germination over a 10-day period (total means of 50). Standard deviations represent plus/minus the average daily new germination. Asterisks represent significant differences compared to wild type based on one-way ANOVA; Tukey test analysis versus wild type where $p < 0.05^*$ and $p < 0.01^{**}$. n=3 biological reps of 50 seeds each. These data are graphed in Figure 4.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
WT Spring32-10	0.0±0.0	13.7±4.0	44.3±5.7	46.0±1.5	46.7±0.6	47.0±0.6	47.0±0.0	47.0±0.0	47.3±0.6	47.3±0.0
<i>fae1-3</i> (-4bp)	0.0 ± 0.0	24.0±4.4*	49.0±5.2	49.3±0.6	49.7±0.6	49.7±0.0	49.7±0.0	49.7±0.0	49.7±0.0	49.7±0.0
rod1-4 (+A)	0.0 ± 0.0	14.3±4.2	44.3±5.6	46.7±0.6	47.3±0.6	48.0±1.2	48.0±0.0	48.0±0.0	48.3±0.6	48.3±0.0
fad2-5 (+A)	0.0 ± 0.0	1.7±1.5**	10.3±7.1**	25.3±0.6**	33.7±3.1*	37.3±1.7**	43.0±1.0	44.7±0.0	45.3±0.0	45.3±0.0
fad2-4 (-2bp)	0.0 ± 0.0	1.3±1.5**	31.0±4.2**	42.3±7.0	46.0±1.5	48.0±2.5	49.0±6.4	49.0±1.5	49.0±1.2	49.0±0.0
rod1-3 fae1-3	0.0 ± 0.0	36.7±4.9**	49.7±4.4	49.7±0.0	50.0±0.6	50.0±0.0	50.0±0.0	50.0±0.0	50.0±0.0	50.0±0.0
fad2-4 fae1-3	0.0 ± 0.0	0.7±0.6**	22.7±1.7**	38.3±0.6	45.0±1.2	46.7±1.5*	46.7±0.0	47.3±0.6	48.3±1.0	48.3±0.0
fad2-5 fae1-3	0.0 ± 0.0	0.0±0.0**	10.0±0.0**	24.0±6.2**	35.0±4.0*	42.0±5.0**	44.0±2.0	45.3±1.5	45.7±0.6	45.7±0.0

Table S3. Amounts of seed germination in constant light at different temperatures over a 16-day period. Data can be found in graph form in Figure S5. Values are the means of the number of seeds that germinated on the given day plus/minus standard deviations. 3 biological reps of 25 seeds each rep. Student T-test versus wild type, $p < 0.05^*$, $p < 0.01^{**}$.

WT Spring32-10	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16
4 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.7±0.6	0.7±1.2	1.3±2.3	0.3±0.6	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0
12 °C	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	14.3±8.5	8.3±6.7	1.7±1.5	0.0 ± 0.0	0.0 ± 0.0	0.3±0.6	0.0 ± 0.0						
22 °C	0.0 ± 0.0	11.3±6.0	9.7±6.7	2.7±0.6	1.00 ± 1.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0				
28 °C	0.0 ± 0.0	14.7±3.1	7.0±2.6	1.0±1.0	0.0±0.0	0.3±0.6	0.3±0.6	0.0 ± 0.0	0.0±0.0	0.67±1.2	0.33±0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0
fae1-3																
4 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.3±0.6	0.0±0.0	0.3±0.0	1.0±1.7	0.3±0.6	0.0 ± 0.0	0.3±0.6	0.3±0.6
12 °C	0.0 ± 0.0	0.0±0.0	1.7±2.9	9.7±6.1	10.0±6.6	2.7±2.3	0.0 ± 0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.3±0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22 °C	0.0 ± 0.0	16.0±0.0	7.7±0.6	1.00±1.0*	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.33±0.6	0.0±0.0	0.0±0.0	0.0 ± 0.0				
28 °C	0.0 ± 0.0	12.0±5.6	7.3±3.5	4.7±4.0	0.7±1.2	0.3±0.6	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0						
rod1-3																
4 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.7±1.2	0.7±1.2	0.0±0.0	0.0 ± 0.0				
12 °C	0.0 ± 0.0	0.0±0.0	3.3±5.8	7.3±7.1	10.3±9.6	3.7±3.2	0.3±0.6	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0						
22 °C	0.0 ± 0.0	17.7±3.2	5.0±3.6	1.3±2.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0				
28 °C	0.0 ± 0.0	10.3±5.5	10.3±4.2	3.00±1.0	1.00 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0						
fad2-5																
4 °C	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.7±1.2	0.0 ± 0.0	0.3±0.6	0.0 ± 0.0	0.3±0.6	0.3±0.6	0.0 ± 0.0
12 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	2.7±2.5*	6.0±3.0	5.7±1.5	3.0±2.0	1.0±0.0	0.3±0.6	1.0±1.0	0.7±0.6	0.3±0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22 °C	0.0 ± 0.0	3.3±2.1	10.0 ± 6.0	7.0±1.0*	1.3±1.5	0.7±1.2	2.0±1.0*	0.3±0.6	0.0±0.0	0.0 ± 0.0						
28 °C	0.0 ± 0.0	11.3±0.6	6.3±3.8	4.3±3.2	0.7±0.6	0.7±1.2	0.3±0.6	0.3±0.6	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.0±0.0				
rod1-4 fae1-3																
4 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.3±0.6	1.0±1.7	0.7±1.2	0.7±1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
12 °C	0.0 ± 0.0	0.0±0.0	2.7±4.6	11.3±2.3	10.3±7.2	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0
22 °C	0.0 ± 0.0	16.3±2.3	8.0±2.0	0.7±1.2*	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0				
28 °C	0.0 ± 0.0	1.3±0.6*	5.3±3.2	10.7±4.5*	4.0±3.0	2.0±1.0*	1.0 ± 1.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0 ± 0.0					
fad2-4 fae1-3																
4 °C	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.3±0.6	0.7±1.2	0.7±1.2	1.3±1.2	1.7±2.1	0.3±0.6	0.0 ± 0.0
12 °C	0.0 ± 0.0	0.0±0.0	3.0±5.2	8.7±2.1	8.0±4.0	1.7±2.1	1.3±1.5	0.7±0.6	1.0±0.0	0.3±0.6	0.0 ± 0.0					
22 °C	0.0 ± 0.0	14.7±0.6	6.0±3.5	3.3±3.1	1.0 ± 1.0	0.0 ± 0.0										
28 °C	0.0 ± 0.0	16.3±4.7	4.0±2.0	2.7±1.2	0.3±0.6	0.7±1.2	0.0 ± 0.0									

Table S4. Amounts of seed germination in constant darkness at different temperatures over a 16-day period. Data can be found in graph form in Figure S5. Values are the means of the number of seeds that germinated on the given day plus/minus standard deviations. 3 biological reps of 25 seeds each rep. Student T-test versus wild type, $p<0.05^*$, $p<0.01^{**}$.

WT Spring32-10	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16
4 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	1.0±0.0	0.7±1.2	4.0±2.6	3.7±4.0	1.7±2.9	4.3±3.1	0.7±0.6	1.0±1.7	0.7±1.2
12 °C	0.0±0.0	0.0±0.0	2.7±4.6	5.7±9.0	8.0±6.2	7.0±6.1	1.0 ± 1.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0
22 °C	0.0 ± 0.0	16.0±3.5	6.7±1.5	1.0 ± 0.0	0.0 ± 0.0	0.3±0.6	0.0±0.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.33±0.0	0.0 ± 0.0				
28 °C	0.0 ± 0.0	15.3±4.2	6.7±5.9	0.7±1.2	0.0±0.0	0.00 ± 0.0	0.0±0.0	0.3±0.6	0.0±0.0	1.0±1.0	0.0 ± 0.0					
fae1-3																
4 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	1.3±0.6	0.3±0.0	4.7±3.2	9.0±2.0	0.7±1.2	4.3±2.9	0.7±0.6	0.7±0.6	0.0 ± 0.0
12 °C	0.0 ± 0.0	0.0±0.0	6.7±11.5	4.0±3.0	7.7±5.8	6.3±6.0	0.3±0.6	0.0 ± 0.0								
22 °C	0.0±0.0	18.3±2.3	5.3±2.1	0.7±0.6	0.0±0.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0
28 °C	0.0 ± 0.0	15.7±3.8	5.0±2.6	1.0 ± 1.0	1.3±1.2	0.0 ± 0.0	0.3±0.6	0.0 ± 0.0	0.0±0.0	0.3±0.6	0.0 ± 0.0					
rod1-3																
4 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.7±0.6	2.0±2.6	3.3±3.5	2.7±4.6	0.3±0.6	0.7±0.6	0.7±0.6	0.0±0.0
12 °C	0.0 ± 0.0	0.0±0.0	4.7±8.1	3.3±5.8	7.3±5.7	5.7±5.5	2.0±2.6	1.3±2.3	0.0±0.0	0.7±1.2	0.0 ± 0.0	0.0±0.0				
22 °C	0.0 ± 0.0	18.7±5.0	5.7±5.1	0.3±0.6	0.3±0.6	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0						
28 °C	0.0 ± 0.0	14.0±6.2	9.3±5.5	0.7±0.6	0.3±0.6	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0						
fad2-5																
4 °C	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0 ± 0.0	1.0±1.0	0.0 ± 0.0	0.7±0.6	0.7±1.2	0.0 ± 0.0	0.0 ± 0.0
12 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	3.3±4.9	2.3±2.1	5.3±2.1	4.33±1.5**	1.7±1.5	2.7±0.6**	0.3±0.6	1.0±1.0	1.0±1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0
22 °C	0.0 ± 0.0	4.0±4.4*	9.3±2.1	6.7±3.8	1.7±1.2	1.0±1.0	0.7±1.2	0.7±1.2	0.0±0.0	0.0 ± 0.0	0.0±0.0					
28 °C	0.0 ± 0.0	17.7±2.1	3.0±0.0	1.7±2.1	1.0±1.0	0.0±0.0	0.0±0.0	0.7±0.6	0.0±0.0	0.7±0.6	0.0 ± 0.0					
rod1-4 fae1-3																
4 °C	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.7±1.2	4.0±1.7	6.3±2.3	1.3±2.3	6.7±5.0	2.0±1.0	0.7±0.6	0.7±1.2
12 °C	0.0 ± 0.0	0.0 ± 0.0	7.0±12.1	1.3±2.3	6.0±5.3*	9.7±8.7	0.7±1.2	0.3±0.6	0.0±0.0	0.0±0.0	0.0 ± 0.0					
22 °C	0.0 ± 0.0	15.7±1.2	6.7±1.2	2.3±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0±0.0
28 °C	0.0 ± 0.0	6.0±2.6*	10.3±2.9	5.3±5.9	1.0±1.0	1.7±1.2	0.3±0.6	0.0 ± 0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0					
fad2-4 fae1-3																
4 °C	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.0	2.0±0.0	0.7±0.0	1.3±0.0	1.3±0.0	0.7±0.0	1.0 ± 0.0	4.3±0.0	2.0±0.0	0.0 ± 0.0	1.0±0.0
12 °C	0.0 ± 0.0	0.0 ± 0.0	5.3±0.0	2.7±0.0	5.3±0.0	7.7±0.0	1.0±0.0	1.0 ± 0.0	0.3±0.0	0.0±0.0	0.0 ± 0.0					
22 °C	0.0 ± 0.0	16.3±0.0	6.0±0.0	2.0±0.0	0.7±0.0	0.0±0.0	0.0±0.0	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0						
28 °C	0.0 ± 0.0	22.7±0.0*	1.3±0.0	0.3±0.0	0.0±0.0	0.3±0.0	0.0±0.0	0.3±0.0	0.0±0.0	0.0 ± 0.0						

Martinez/Needleman-Wun Minimum Match: 9: Gap P	sch DNA A	Alignment	Penalty: 0.3	3				
Seq1(1>1152) TaFAD2 OBE GAKE01001	1774 sea	Seq2(1>1152) AT3G12120	Similarity	Gap	Gap Consens	us 1th	
(1>1152)	1774.009	(1>1152)	///0012120.	88.8	0	0 11	52	
v10 v	720	v30	v40	v50	v60	v70	v80	v90
ATGGGTGCAGGTGGAAGAA	IGACGGTT	CCTACTTCTT	CCAAGAAGTC	TGAAACCGA	IGCCTTAA.	AGCGTGTGCCGT	GCGAGAAACC	GCCG
ATGGGTGCAGGTGGAAGAA	rg CGGTT	CCTACTTCTT	CCAAGAA TC	GAAACCGA	CC AA	AGCGTGTGCCGT	GCGAGAAACC	GCC
ATGGGTGCAGGTGGAAGAA	rgccggtt	°CCTACTTCTT	CCAAGAAATC	GGAAACCGA		AGCGTGTGCCGT	GCGAGAAACC	GCCT
10 x100 x	20 7110	30 v120	40 v130	50 v140	v150	70 v160	80 v170	90 v180
TTCACGCTCGGAGAACTGA	AGAAAGCA	ATCCCACAGC	ATTGTTTCAA	TCGCTCAAT	CCTCGCT	CTTTCTCCTACC	TTATCTGGGA	CATC
TTC CG T GGAGA CTGA	AGAAAGCA	ATCCC C GC	ATTGTTTCAA	CGCTCAAT	CCTCGCT	CTTTCTCCTACC	TTATC G GA	CATC
TTCTCGGTGGGAGATCTGA	AGAAAGCA	ATCCCGCCGC	ATTGTTTCAA	ACGCTCAAT	CCTCGCT	CTTTCTCCTACC	TTATCAGTGA	CATC
^100	`110	^120	^130	^140	^150	^160	^170	^180
v190 v	7200	v210	v220	v230	v240	v250	v260	v270
ATCATAGCCTCTTGCTTCTA	ACTACGTT	GCCACCACTT		CCTCCCTCA	GCCTCTCT	CTTACTTGGCTT	GGCCTCTCTA	TTGG
		GCCACCA II.		CCTCCCTCA			GGCC CICIA	ATTGG ATTGG
^190	200	^210	^220	^230	^240	^250	^260	^270
v280 v	7290	v300	v310	v320	v330	v340	v350	v360
GTCTGTCAAGGCTGTGTCT	TAACCGGA	GTCTGGGTCA	TAGCTCACGA	ATGCGGCCA	CCACGCCT	TCAGCGACTACC	AATGGCTTGA	CGAC
G CTGTCAAGGCTGTGTC	FAAC GG	TCTGGGTCA	TAGC CACGA	ATGCGG CAG	CCACGC T	TCAGCGACTACC	AATGGCT GA	GAC
GCCTGTCAAGGCTGTGTCCT	TAACTGGT	ATCTGGGTCA	TAGCCCACGA	ATGCGGTCA	CCACGCAT	TCAGCGACTACC	AATGGCTGGA	TGAC
	-290						-350	-360
	୵୰୰୰ ୵୷୷୷୷୷୷୷		୰୳୰୰ ୵୴୴୵୵୴୴୵୴୵	V410 CTCC77777		V43U CCCCTCACCATT		V45U
ACAGT GGTCT ATCTTCC	ATTC TTC	CTCCTCGTCC		CTGGAA TA	AG CA C	GCCGTCACCATT	CCAACACCGG	ATC
ACAGTTGGTCTTATCTTCCA	ATTCCTTC	CTCCTCGTCC	CTTACTTCTC	CTGGAAGTA	TAGTCATC	GCCGTCACCATT	CCAACACTGG	ATCC
^370	`380	^390	^400	^410	^420	^430	^440	^450
v460 v	7470	v480	v490	v500	v510	v520	v530	v540
CTTGAAAAGGACGAAGTGT	TGTCCCT	AAACAGAAAT	CCGCCATCAA	ATGGTACGG	CAAGTACC	TCAACAACCCTC	TGGGACGCAC	CGTG
CT GAAA GA GAAGT T	TGTCCC	AA CAGAAAT	C GC ATCAA	TGGTACGG	AA TACC	TCAACAACCCTC	T GGACGCA	C TG
CTCGAAAGAGATGAAGTAT	PTGTCCCA	AAGCAGAAA'I'		CGTGGTACGG	SAAATACC	TCAACAACCCTC ^520	1 TIGGACGCAT	CATG ^540
¥00 x550 x	7560	¥30 ¥570	v580	v590	v600	v610	v620	v630
ATGTTAACCGTCCAGTTCAG	CCTTGGC	TGGCCCTTGT	ACTTAGCCTT	CAACGTCTC	GGGGAGAC	CCTACGACGGGT	TCGCTTGCCA	CTTC
ATGTTAACCGTCCAGTT	CCT GG	TGGCCCTTGT	ACTTAGCCTT	AACGTCTC	GG AGAC	C TA GACGGGT	TCGCTTGCCA	TTC
ATGTTAACCGTCCAGTTTG	ICCTCGGG	TGGCCCTTGT	ACTTAGCCTT	TAACGTCTC	FGGCAGAC	CGTATGACGGGT	TCGCTTGCCA	TTTC
^550	`560	^570	^580	^590	^600	^610	^620	^630
v640 v	7650	v660	v670	v680	v690	v700	v710	v720
	ACAACGAC	CGTGAACGCC	TCCAGATATA	CATCTCGGA	IGCIGGIA	TCCTCGCCGTCT	GTTACGGTCT	
	CAA GAC	CGAGAACGCC	ТССАВАТАТА ТССАСАТАТА		TGC GGIA		GII GGICI GTTTTCGTCT	
^640	`650	^660	^670	^680	^690	^700	^710	^720
v730 v	740	v750	v760	v770	v780	v790	v800	v810
CGTTACGCTGCTGCACAAG	GAGTGGCC	TCGATGATCT	GCGTCTACGG	AGTTCCGCT	FCTGATAG	TCAACGGGTTCC	TCGTCTTGAT	CACA
CGTTACGCTGCTGCACAAG	G TGGCC	TCGATGATCT	GC TCTACGG	AGT CCGCT	ICTGATAG	T AA G GTTCC	TCGTCTTGAT	CAC
CGTTACGCTGCTGCACAAG	GATGGCC	TCGATGATCT	GCCTCTACGG	AGTACCGCT	rctgatag	TGAATGCGTTCC	TCGTCTTGAT	CACT
/30	740	750	760	770	780	790	1008	118
TACTTGCAGCACACCCATC	CTCGTTG	CCTCACTACG	ATTCATCCGA	GTGGGATTG	V070 GTTCAGGG	GAGCTTTGGCTA	CCGTAGACAG	AGAC
TACTTGCAGCACAC CATCO	CTCGTTG	CCTCACTACG	ATTCATC GA	GTGGGA TG	G TCAGGG	GAGCTTTGGCTA	CCGTAGACAG	GAGAC
TACTTGCAGCACACTCATC	CTCGTTG	CCTCACTACG	ATTCATCAGA	GTGGGACTG	GCTCAGGG	GAGCTTTGGCTA	CCGTAGACAG	GAGAC
^820	`830	^840	^850	^860	^870	^880	^890	^900
v910 v	7920	v930	v940	v950	v960	v970	v980	v990
TATGGAATCCTGAACAAGG		AACATCACGG	ACACGCACG'I	GGCTCACCA	CTGTTCT	CGACGATGCCGC	ATTACCATGC	GATG
			ACAC CACGI	GGCTCA CA			ATTA A GC	ATG
^910	`920	^930	^940	^950	^960	^970	^980	^990
v1000 v	71010	v1020	v1030	v1040	v1050	v1060	v1070	v108
GAGGCCACGAAGGCGATAA	AGCCGATA	CTCGGGGACT	ATTACCAGTT	TGATGGAAC	ACCGGTCT	TCAAGGCGATGT	GGAGGGAGGC	GAAG
GA GC AC AAGGCGATAAA	AGCC AT	CT GG GACT	ATTACCAGTT	GATGGAACA	ACCG T	GCGATGT	AGGGAGGC	AAG
GAAGCTACAAAGGCGATAAA	AGCCAATT	CTGGGAGACT	ATTACCAGTT	CGATGGAAC	ACCGTGGT	ATGTAGCGATGT	ATAGGGAGGC	AAAG
^1000 <i>(</i>	1010	^1020	^1030	^1040	^1050	^1060	^1070	^108
		VIIIO	VII20					
GAGTGT TCTATGTAGAAC	CGGACAGG	AAGGTGA A	AGAAAGGIGI	GTICIGGIA	CAACAA A	AGTT TGA		
GAGTGTATCTATGTAGAAC	CGGACAGG	GAAGGTGACA	AGAAAGGTGT	GTACTGGTA	CAACAATA	AGTTATGA		
^1090	`1100	^1110	^1120	^1130	^1140	^1150		

Figure S1. Nucleotide sequence alignment of the *Thlaspi arvense TaFAD2* ORF (top sequence) versus the *Arabidopsis thaliana AtFAD2* ORF (AT3G12120.1). The *TaFAD2* ORF sequence is derived from transcriptome assembly contig GAKE01001774.1 (Dorn et al., 2014), which shares 100% identity with *Thlaspi arvense* MN106 reference genome sequences. The red line delineates the 20 nucleotide protospacer sequence used in the CRISPR/SaCas9 construct. Outlined in blue is the NNGGGT protospacer adjacent motif (PAM) recognized by SaCas9.



Figure S2. Characterization of the CRISPR-induced *Thlaspi arvense fad2* mutants' sequences. (A) Electrophoresed T7 endonuclease I-digested PCR products scoring wild type (WT) *FAD2* (1459 bp band) versus *fad2* mutations (partial digestion of 1459 bp band producing 1065 and 394 bp products). Lanes 1, 2, 4, 8-10 are WT segregants, whereas lanes 5-7 and 11-16 harbor the *fad2-3* mutation. Lane 3: Fermentas 1kb GeneRuler. (B) DNA sequence chromatograms. WT *TaFAD2* (top); *fad2-4* allele (2 bp deletion); *fad2-5* allele (A insertion); *fad2-6* allele (29 bp deletion). All three mutations are located as expected within the protospacer binding site of the CRISPR/*Sa*Cas9 guide RNA. (C, D) Predicted FAD2 polypeptide sequence in (C) WT pennycress versus the (D) *fad2-4* (E) *fad2-5*, and (F) *fad2-6* mutants. Note the predicted polypeptides encoded by each mutant are predicted to be truncated due to the frameshift mutations in the open reading frame (ORF).

Martinez/Needleman-Wunsch DNA Alignment	
Minimum Match: 9; Gap Penalty: 1.10; Gap Length Penalty: 0.33	
TaROD1 ORF GAKE01006801.seq AtROD1 ORF AT3G15820.seq Index Number Length Length	
(1>906) (10>906) 85.5 4 15 909	_
v10 v20 v30 v40 v50 v60 v70 v80 v90)
ATGTCAACTAAAACCGTCGTCCCTCTCCGTCGCAGATCTAAGCCCCTTAACGGAAATCACACTAACGGCGTCGCCATTGACGGAAGCCTC	
CA CT AAACCG CGTC CTCTCCGTCGCAGATCTAA C CTTAACGGAAA CACACTAACGGCGTCGCCATTGACGGAA CCT	
GCCGCAGCTGAAACCGACGTCTCTCTCCGCCGCAGATCTAACTCTCTTTAACGGAAACCACAACTAACGGCGTCGCCATTGACGGAACCCCTA	
10 20 30 40 50 60 70 80 90	
$v_{100} v_{110} v_{120} v_{130} v_{140} v_{150} v_{160} v_{160} v_{170}$	
GACGACGACCACCACACCGTCGCATCGGATCAGTAAATAGCCAAATGGATAACATTGCTAAGAAAACGGACGACGACGACGACGACGACGACGACGACG	
GAC AL ALAALUGTUGU TUGUA AAA A CA ATGUATA IGUTAAGAAAAL GAL ALGGUTALGU AA GG C	
GACAACAACAACAACACCEGGGGGGGGGGGGGGGGGGGG	
100 110 120 130 140 130 100	
GCAGGAGGAGGAGGAGGAGGAGGAAAAGCAAGGCGTCGTTTATGACGTGGACGCGCGCG	
GGAGGAGGAGGAGGA GGA AAGCAA GCGTCGTT A GACGTGGACGGCGCGTGA T GT TACGTGG GAG TACCATTGGATA	
GGAGGAGGAGGATGGAGAAGCAAAGCGTCGTTCACGACGTGGACGCGCGCGTGATATCGTCTACGTGGAGATACCATTGGATA	
^190 ^200 ^210 ^220 ^230 ^240 ^250 ^260 ^27	0
v270 v280 v290 v300 v310 v320 v330 v340 v350	
CCGTGTTTGTTCGCGGTCGGGGTTCTGTTCTTCACGGGCGTGGAGTACACGCTCCAGATGATTCCCCGCGAGGTCTGAGCCGTTCGATATT	
CCGTG TGTTCGC G CGG TTCTGTTCTTCA GGGCGTGGAGTACACGCT CAGATGATTCCCGCGAG TCTGAGCCGTTCGAT TT	
CCGTGCATGTTCGCTGCCGGACTTCTGTTCTTCATGGGCGTGGAGTACACGCTTCAGATGATTCCCGCGAGATCTGAGCCGTTCGATCTT	
^280 ^290 ^300 ^310 ^320 ^330 ^340 ^350 ^36	0
v360 v370 v380 v390 v400 v410 v420 v430 v440	
GGGTTTGTGGCCACGCGCTCTCTGAATCGCGTCTTTGGCAAATTCACCGGGATCTTAACACCGGTCTTTAGCGCGCTCTTAAACACGGTGTTCGTA	
GGGTTTGTGG CACGCGCTCT TGAATCGCGT TT GCA TTCACCGGATCTTAACAC GT TTAGCCGC CTAAACACGGTGTTCGTA	
GGGTTTGTGTCACGCGCTCTTTGAATCGCGTATTAGCATCTTTACCCGGATCTTAACACTGTTTTAGCCGCACTAAACACGGTGTTCGTA	
370 380 390 400 410 420 430 440 43	0
GGGATGCAAACTAACGTATTGTATGGACATGGTTGGAAGGACGACGACCACCACCACCACCGCCTTTATTCATGTTCATCGTCGC	
<u>^460</u> <u>^470</u> <u>^480</u> <u>^490</u> <u>^500</u> <u>^510</u> <u>^520</u> <u>^530</u> <u>^54</u>	0
v540 v550 v560 v570 v580 v590 v600 v610 v620	
GGCATTCTTGGTTACTCTACTCAGCTCCCCCTCTTCCTCAGGATTTTCTAGGATCAGGTGTCGATTTTCCGGTGGGAAACGTCTCGTTCTTC	
GGCATTCT GG TACTCTACTCAGCT CCTCT CCTCAGGA TTTCTAGGATCAGG GT GATTTTCCGGTGGGAAA GTCTC TTCTTC	
GGCATTCTCGGCTACTCTACTCAGCTTCCTCTCCCCCAGGACTTTCTAGGATCAGGGGTTGATTTTCCGGTGGGAAATGTCTCTTTCTT	
^550 ^560 ^570 ^580 ^590 ^600 ^610 ^620 ^63	0
v630 v640 v650 v660 v670 v680 v690 v700 v710	
CTCTTCTACTCGGGTCACGTCGCCGGTTCGATGATCGCCTTTTGGACATGAGGAGAATGCAGAGACTAGCGATGACGATGCTTTTTGAC	
CTCTTTCT CTC GG CA GTCGCCGG TCGATGATCGCATC TTGGACATGAG AGAATGCAGAGG TGAGACT GC ATG T TTTTGAC	
CTCTTCTTCTTCGCCATGCCGCCCGGCTCGATGATCGCATCGCATGGACATGCAGAGGGTTGACAGGGCTTGCAATGGTCTTTGAC	
640 650 660 670 680 690 700 710 72	0
V / 20 V / 20 V / 40 V / 20 V / 60 V / 70 V / 70 V / 70 V / 90 V 800	
AICCICANTGIAITACAAICGAITAGGGCGCGGGGGGGGGGGGGGGGG	
^730 ^740 ^750 ^760 ^770 ^780 ^790 ^800 ^81	0
v810 v820 v830 v840 v850 v860 v870 v880 v890	. •
TTTGATTCATTCGCCGGCAAGTACGAAGAGAGAGAGAGAG	
TT GA TCATT GCCGG AAGTACGAAGAGATGAT AGCAAGAGACA TTTAG CA TGGTTTT GTTTGATTTCGAAAGACTC CTA	
TTCGACTCATTGGCCGGAAAGTACGAAGAGATGATGAGCAAGAGACATTTAGGCACTGGTTTTAGTTTGATTTCGAAAGACTCTCTA	
^820	
v900	
GTCAATTAA	
GTCAATTAA	
GTCAATTAA	
^900	

Figure S3. Nucleotide sequence alignment of the *Thlaspi arvense TaROD1* ORF (top sequence) versus the *Arabidopsis thaliana AtROD1* ORF (AT3G15820.1). The *TaROD1* ORF sequence was derived from transcriptome assembly contig GAKE01006801.1 (Dorn et al., 2014), which shares 100% identity with *Thlaspi arvense* MN106 reference genome sequences. The red line delineates the 20 nucleotide protospacer sequence used in the CRISPR/SpCas9 construct. Outlined in blue is the NGG protospacer adjacent motif (PAM) recognized by SpCas9.



Figure S4. Characterization of the CRISPR-induced *Thlaspi arvense rod1* mutants' sequences. (A) *Tarod1* DNA sequence chromatograms showing the nature of each mutation. *TaROD1* coding sequences homozygous for wild type (top sequence); 18 bp deletion (*rod1-3*); +A insertion (*rod1-4*); and +T insertion (*rod1-5*). All three mutations are located as expected at the CRISPR/*Sp*Cas9 guide RNA binding site ("Protospacer" location delineated with a black line). (**B**, **C**, **D**, **E**) Predicted ROD1 polypeptide sequence in (**B**) wild-type pennycress versus the (**C**) *rod1-3* (**D**) *rod1-4*, and (**E**) *rod1-5* mutants. Note the predicted polypeptides encoded by *rod1-4* and *rod1-5* are truncated due to frameshifts in the open reading frame (ORF), whereas the 18 bp deletion in *rod1-4* introduces a premature stop codon (underlined in red).



Figure S5. Growth time course of different lipid mutants and wild-type plants grown at the same time and conditions from Day 19 until Day 88 of their plant life cycles. Shown are four plants for each genotype growing in four-inch pots. (a) *rod1 fae1*, (b) *fad2 fae1*, (c) *fad2*, (d) Wild-type Spring 32-10, (e) *fae1*, and (f) *rod1*.



Figure S6A-D. Average amounts of seed germination over a 16-day period under different temperatures and light regimes. Sets of 75 seeds for each genotype were plated onto three agar growth media plates (25 seeds per plate; n = 3) and incubated at 4 °C, 12 °C, 22 °C, or 28 °C either in constant florescent light (A, C) or constant darkness (B, D). Seed germination in (A) and (B) are graphed as cumulative, whereas as (C) and (D) are graphed as the number that germinated each day. Values and significant differences can be found in Tables S2 and S3. Error bars in (C) and (D) are standard deviations.



Figure S7. MS imaging of other PC molecular species detected in wild-type Spring 32-10, the various mutants and mutant combinations.



Figure S8. MS imaging of each TAG molecular species detected for wild type and mutant seeds.