frontiers

*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. Jarvis[1], Trevor B. Romsdahl[2], Michaela McGinn[1], Tara J. Nazarenus[3], Edgar B. Cahoon[3], Kent D. Chapman[2], and John C. Sedbrook[1*]**

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

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

[3]Department 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 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:1 | 22:1 | 24:1 |
|---|---|---|---|---|---|---|---|---|---|
| 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 |
| *fad2-4* (-2bp) | 2.1±0.1** | 0.1±0.1** | 0.4±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±0.0** | 0.6±0.0** | 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±0.0** | 0.5±0.0** | 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 |
| *fae1-3* (-4bp) | 3.5±0.1** | 0.3±0.0 | 0.8±0.0** | 47.8±1.9** | 28.5±1.4** | 17.8±0.6** | 0.9±0.1** | 0.0±0.0** | 0.3±0.0** |
| *fad2-4 fae1-3* | 2.5±0.2** | 0.2±0.1 | 0.6±0.0** | 90.6±0.6** | 0.5±0.1** | 2.6±0.3** | 1.5±0.1** | 0.0±0.0** | 0.2±0.2** |
| *rod1-4 fae1-3* | 3.4±0.0* | 0.0±0.0** | 0.6±0.0** | 59.7±0.3** | 17.1±0.2* | 19.3±0.3** | 0.0±0.0** | 0.0±0.0** | 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 |
| *fae1-3* (-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 ºC** | 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 ºC** | 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 | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **28 ºC** | 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 ºC** | 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 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **28 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***rod1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **12 ºC** | 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 | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **28 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***fad2-5*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 ºC** | 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 | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***rod1-4 fae1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **28 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***fad2-4 fae1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **22 ºC** | 0.0±0.0 | 14.7±0.6 | 6.0±3.5 | 3.3±3.1 | 1.0±1.0 | 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±0.0 |
| **28 ºC** | 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 | 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 |

**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 ºC** | 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 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **28 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***fae1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 | 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 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***rod1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **22 ºC** | 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 | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***fad2-5*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **28 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***rod1-4 fae1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **22 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| ***fad2-4 fae1-3*** | | | | | | | | | | | | | | | | |
| **4 ºC** | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **22 ºC** | 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 | 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 ºC** | 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 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

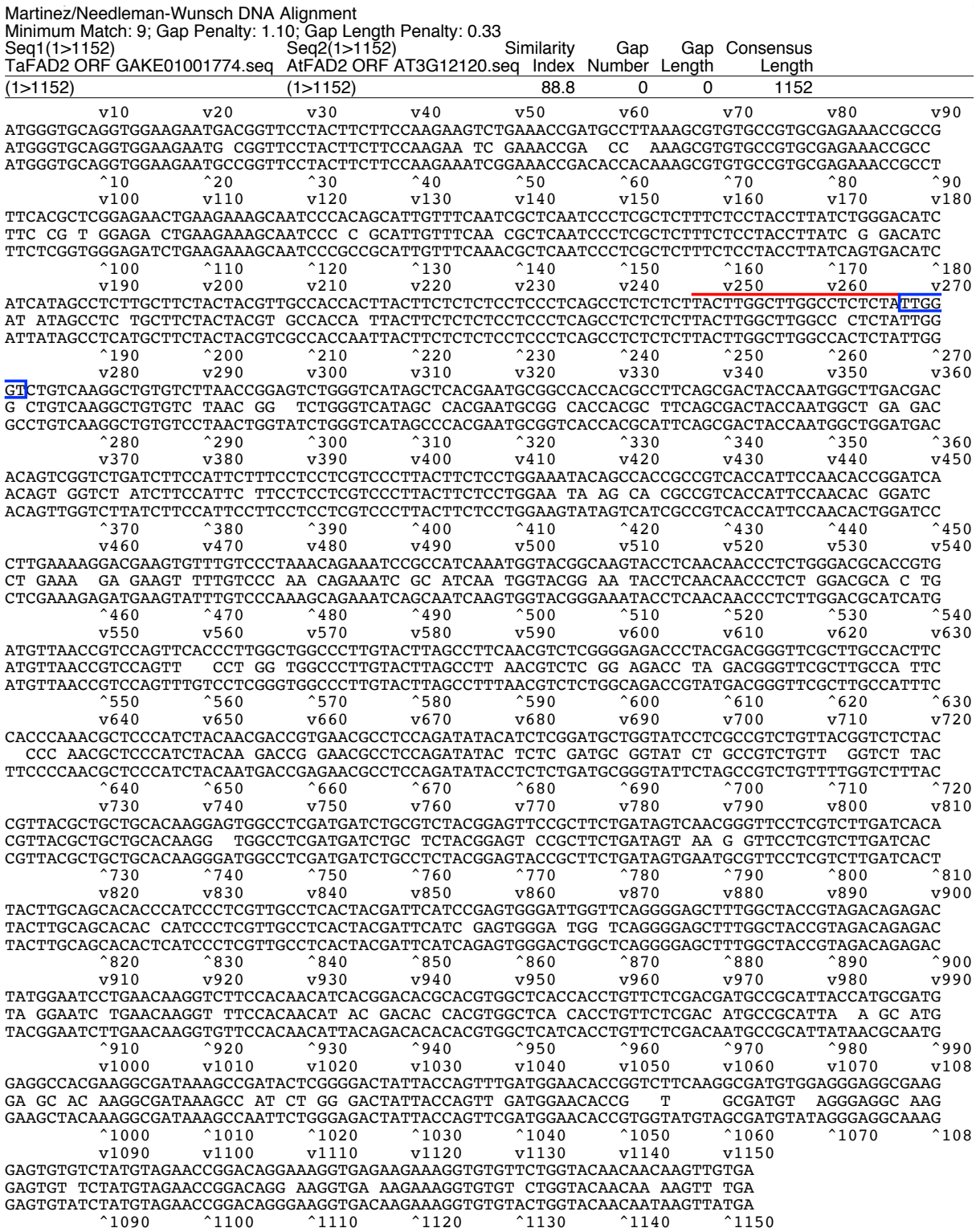


**Figure S1**. Nucleotide sequence alignment of the *Thlaspi arvense TaFAD2* ORF (top sequence) versus the *Arabidopsis thaliana AtFAD2* ORF (AT3G12120.1). The *TaFAD2* ORF sequence is derived from transcriptome assembly contig GAKE01001774.1 (Dorn et al., 2014), which shares 100% identity with *Thlaspi 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.

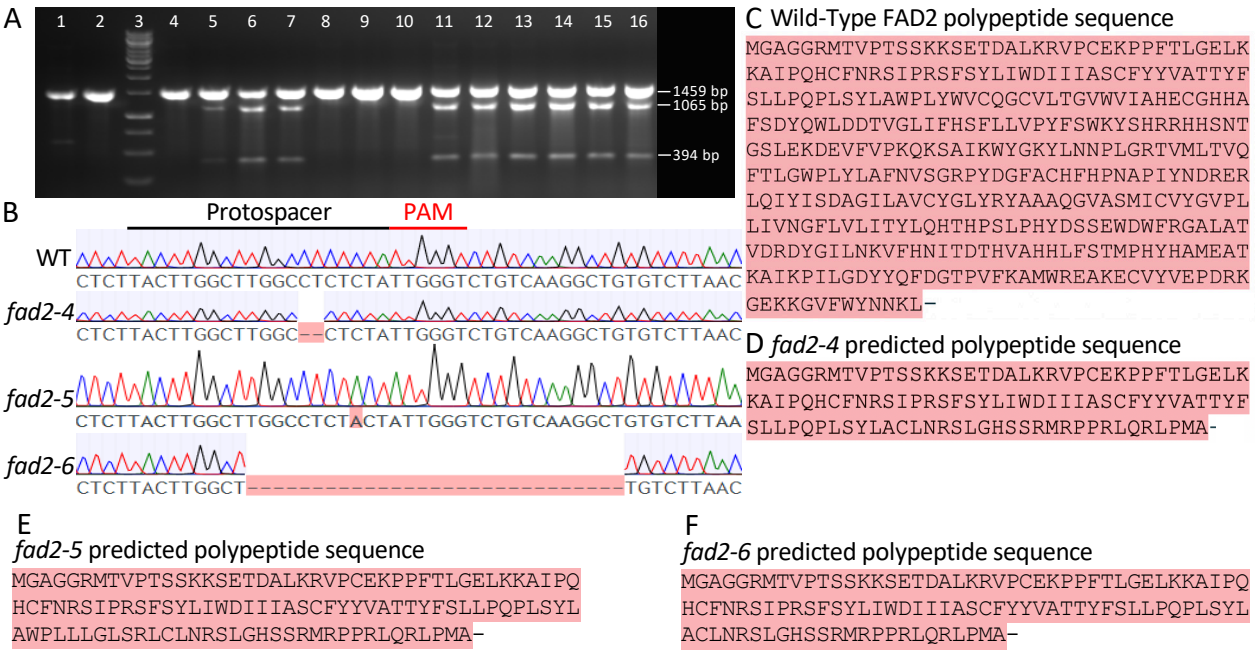


**Figure S2**. Characterization of the CRISPR-induced *Thlaspi arvense fad2* mutants' sequences. (**A**) Electrophoresed T7 endonuclease I-digested PCR products scoring wild type (WT) *FAD2* (1459 bp band) versus *fad2* mutations (partial digestion of 1459 bp band producing 1065 and 394 bp products). 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).

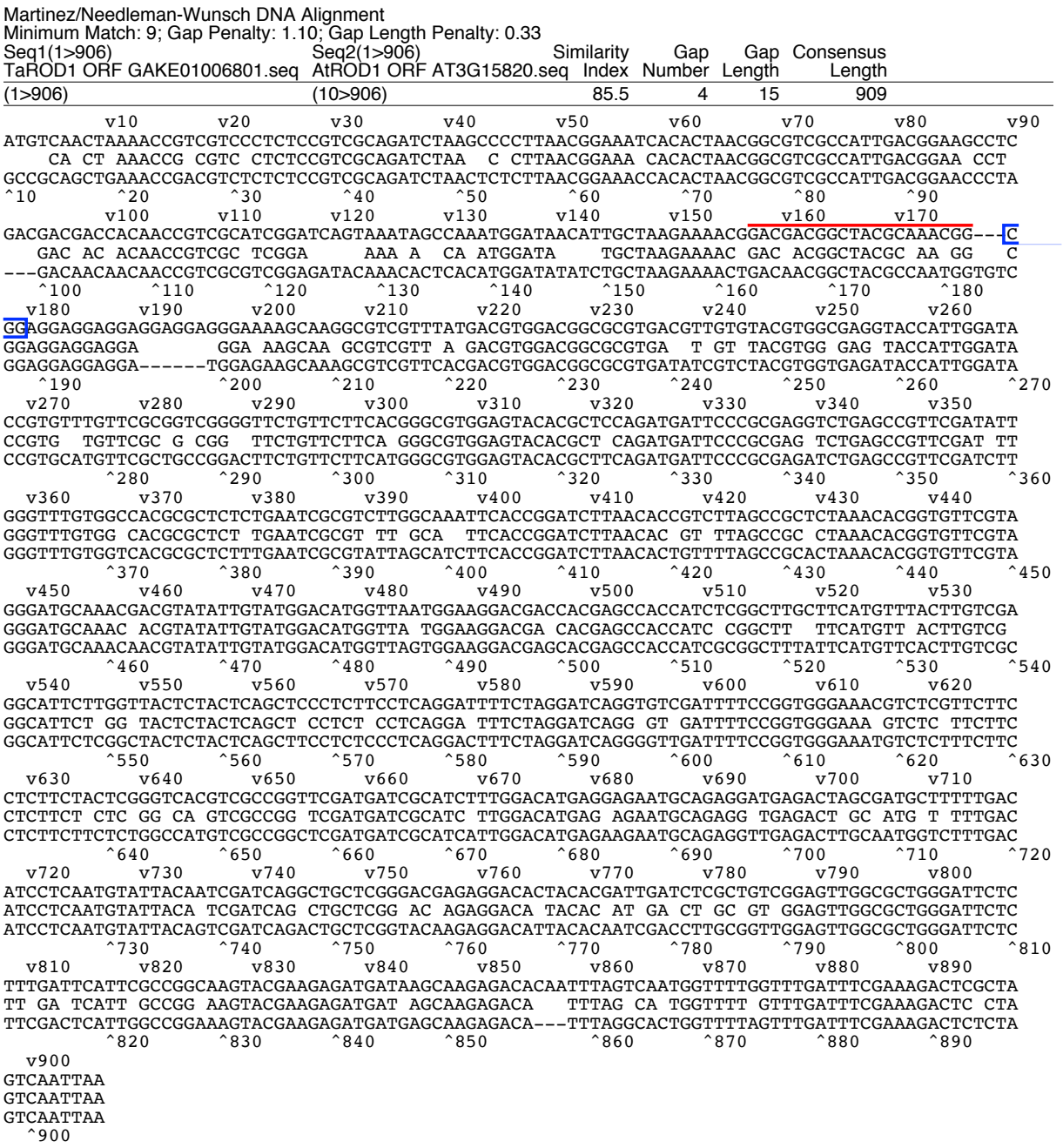


**Figure S3**. Nucleotide sequence alignment of the *Thlaspi arvense TaROD1* ORF (top sequence) versus the *Arabidopsis thaliana AtROD1* ORF (AT3G15820.1). The *TaROD1* ORF sequence was derived from transcriptome assembly contig GAKE01006801.1 (Dorn et al., 2014), which shares 100% identity with *Thlaspi 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.

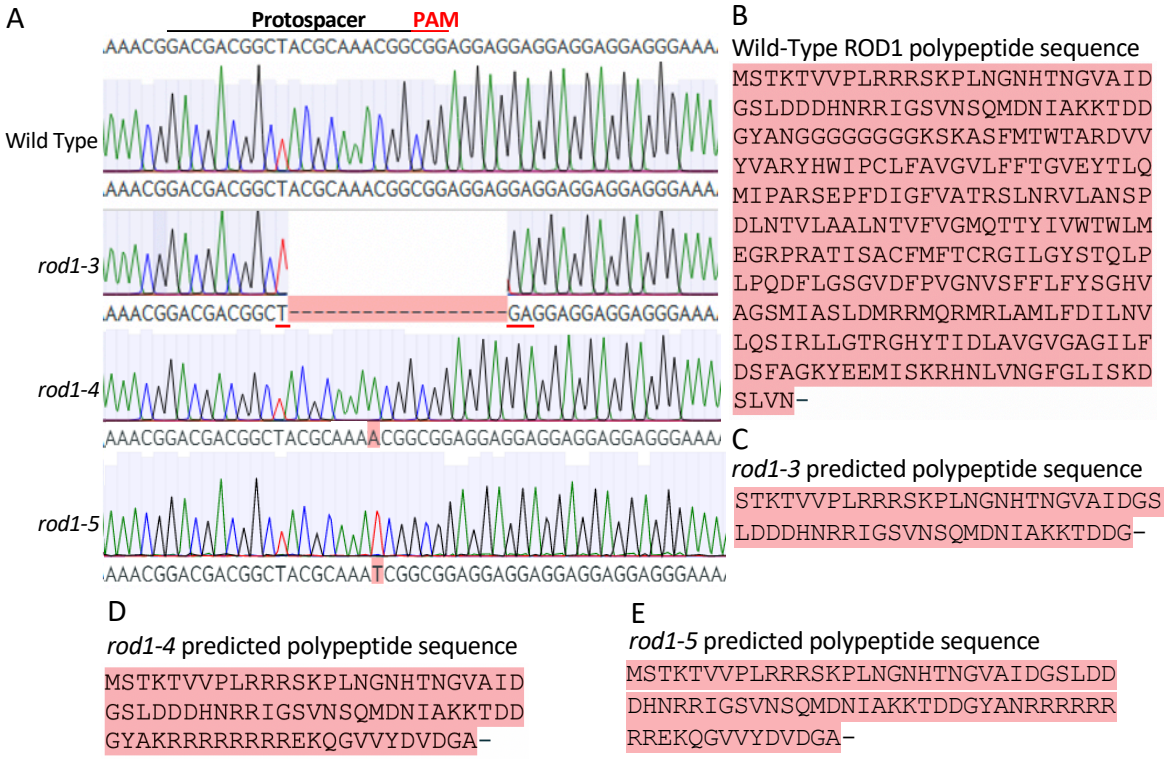


**Figure S4. C**haracterization of the CRISPR-induced *Thlaspi arvense rod1* mutants' sequences. (**A**) *Tarod1* DNA sequence chromatograms showing the nature of each mutation. *TaROD1* coding sequences homozygous for wild type (top sequence); 18 bp deletion (*rod1-3*); +A insertion (*rod1-4*); and +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).

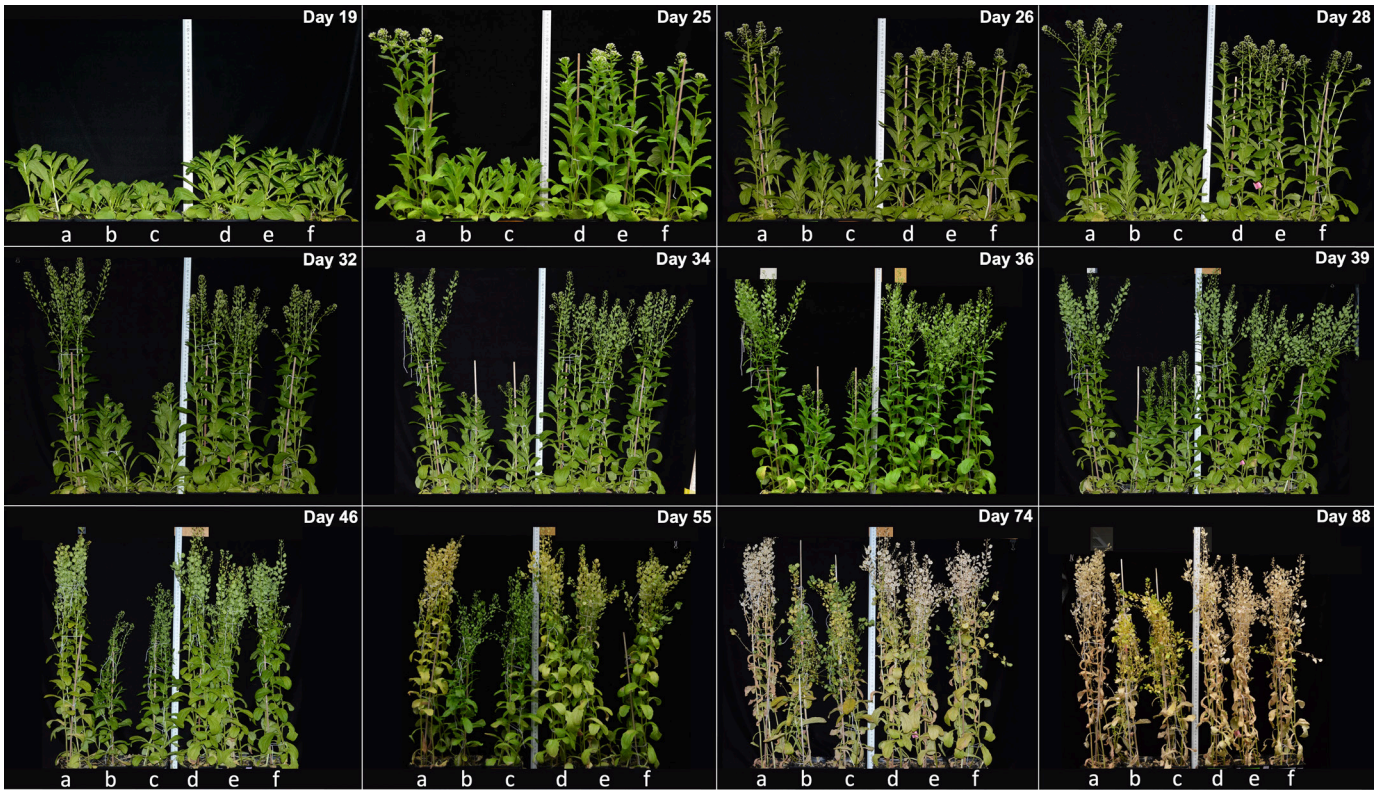


**Figure S5.** Growth time course of different lipid mutants and wild-type plants grown at the same time and conditions from Day 19 until Day 88 of their plant life cycles. Shown are four plants for each genotype growing in four-inch pots. (a) *rod1 fae1*, (b) *fad2 fae1*, (c) *fad2*, (d) Wild-type Spring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

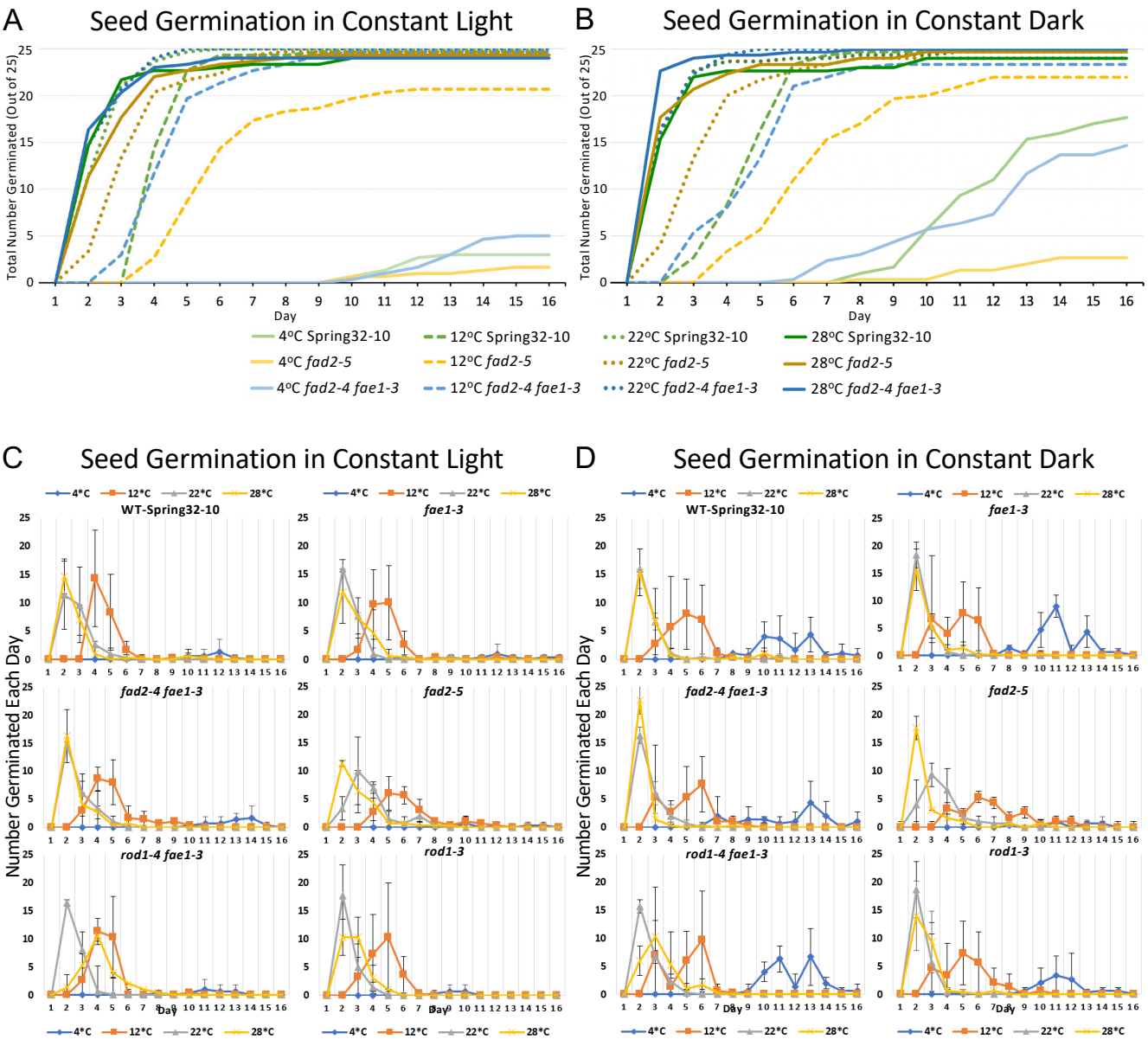


**Figure S6A-D**. Average amounts of seed germination over a 16-day period under different temperatures and light regimes. Sets of 75 seeds for each genotype were plated onto three agar growth media plates (25 seeds per plate; n = 3) and incubated at 4 °C, 12 °C, 22 °C, or 28 °C either in constant florescent 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.

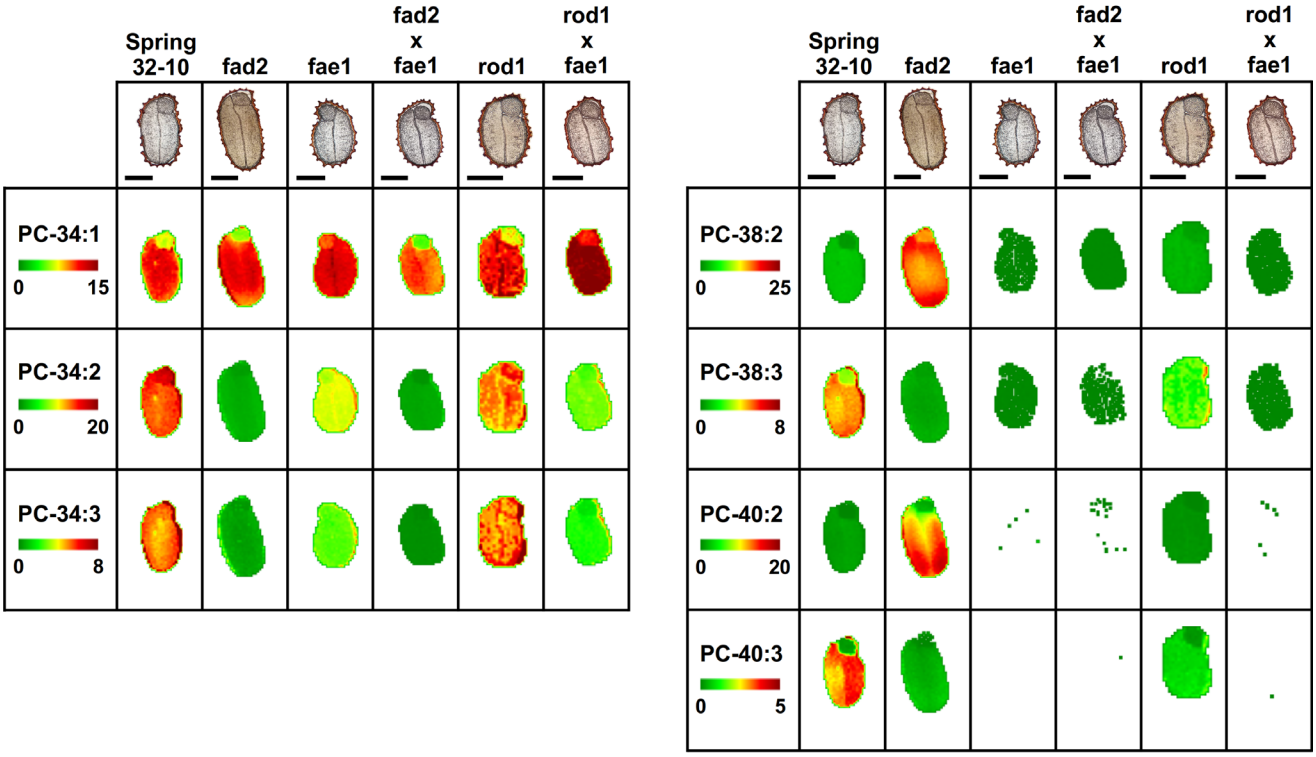


**Figure S7.** MS imaging of other PC molecular species detected in wild-type Spring 32-10, the various mutants and mutant combinations.

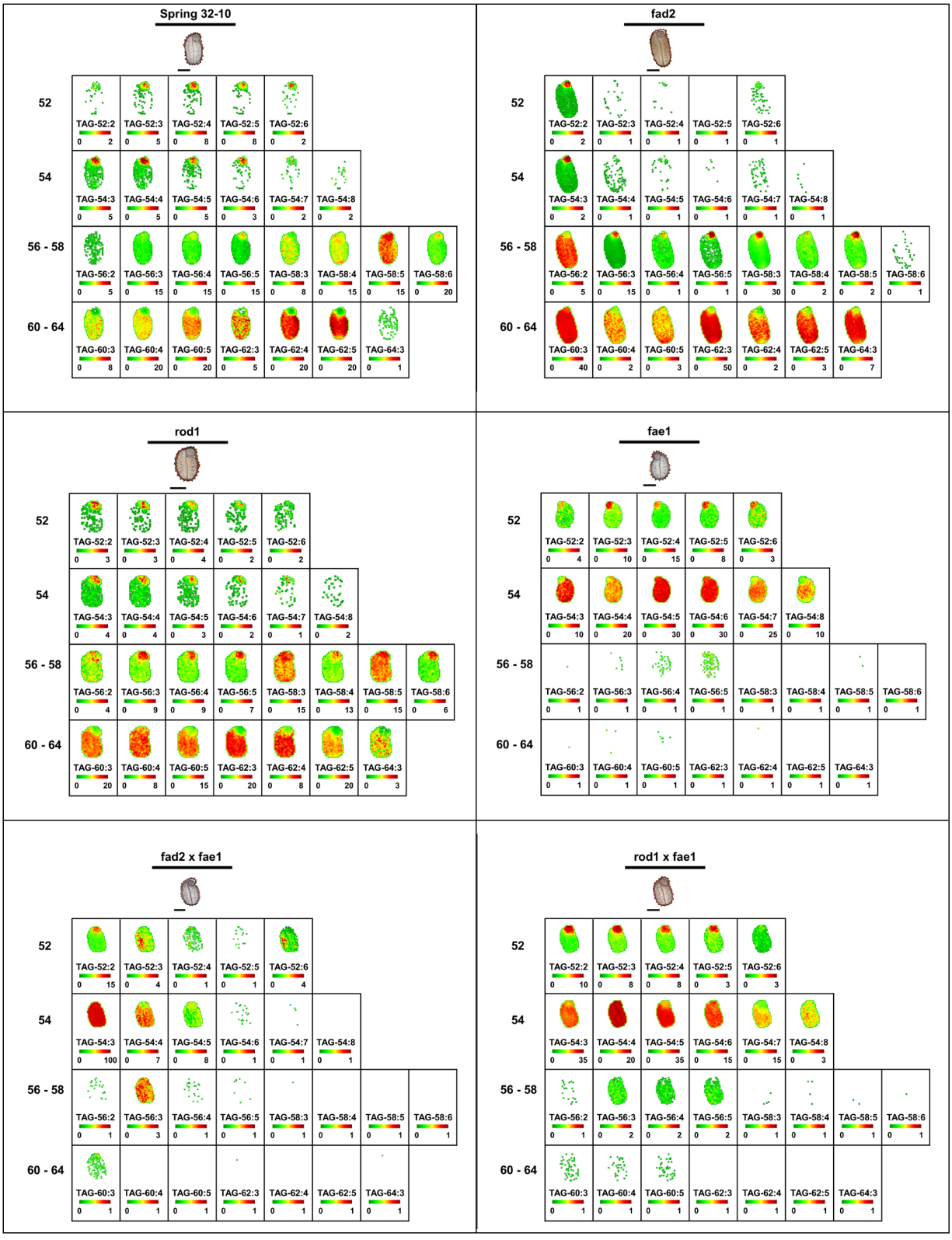


**Figure S8.** MS imaging of each TAG molecular species detected for wild type and mutant seeds.