

4/3/2024

Bernadette Juarez

U.S. Department of Agriculture

APHIS Deputy Administrator

Biotechnology Regulatory Services

**RECEIVED**

By ajdrummond for BRS Document Control Officer at 2:50 pm, Apr 03, 2024

Dear Deputy Administrator Juarez,

With this letter we respectfully request a Regulatory Status Review from USDA-APHIS's Biotechnology Regulatory Services (BRS) for the following Plant Trait Mechanism of Action: *Thlaspi arvense* L. (pennycress; field pennycress) CRISPR/Cas9 generated mutant lines, featuring disruption of any combination of: *FAE1* to produce a low erucic acid phenotype; *TT8* to produce a lower fiber phenotype; and [ ] to produce a lower glucosinolate phenotype. These PTMOA's are described in previously submitted RSR's 22-069-01rsr, 22-292-01rsr, 23-264-01rsr, 23-342-01rsr, 23-355-01rsr, 24-064-01rsr, and 24-065-01rsr.

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USDA has previously evaluated pennycress lines with the same phenotypes as the PTMOA's in this RSR via the AIR and RSR processes. Disruption of *TT8* and *FAE1* were reviewed in several AIR letters<sup>1</sup>, and in the recently reviewed 22-069-01rsr and 22-292-01rsr, the USDA did not identify any plausible pathway by which the modified plants would pose an increased plant pest risk. Lines containing disruptions in genes (*AOP2*, [ ]) that are in the same biosynthesis pathway and that exhibit the same phenotype as our third trait [ ], were evaluated in AIR requests<sup>2</sup>. *AOP2*, [ ] are examples of several genes that, when disrupted, reduce glucosinolate levels in pennycress. This RSR requests evaluation of pennycress with mutations in one of the aforementioned genes [ ] in addition to two other genes [ ] resulting in the same low glucosinolate phenotype, in combination with the previously evaluated disruptions in *TT8* and *FAE1*. In response to each of several previously submitted AIR letters, BRS deregulated pennycress lines harboring disruptions in *AOP2*, [ ], and other genes, concluding that: "...your genome edited pennycress lines are not themselves plant pests". USDA additionally stated in several AIR response letters that pennycress is not listed as a Federal noxious weed pursuant to 7 CFR part 360 and USDA has no reason to believe that the intended phenotypes of the pennycress lines would increase the weediness of pennycress.

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Further, via the confirmation of exemption process, single edits in the [ ] (22-336-01cr), [ ] (23-107-01cr), and [ ] (22-336-02cr) genes have been deemed "achievable by conventional breeding and unlikely to pose an increased plant pest risk relative to their conventionally bred counterparts".

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<sup>1</sup> Illinois State University, USDA response August 8, 2018; Illinois State University, USDA response April 9, 2019; Cover Cress, Inc., USDA response January 29, 2020; CoverCress, Inc., USDA response May 7, 2020; CoverCress, Inc., USDA response August 31, 2020

<sup>2</sup> Cover Cress, Inc., USDA response January 29, 2020; CoverCress, Inc., USDA response May 7, 2020; CoverCress, Inc., USDA response August 31, 2020; Illinois State University, USDA response August 24, 2020

**1. Information about Requestor****First Name:** Marcia**Last Name:** Weldon**Position:** Regulatory & Stewardship Manager**Organization Name (if applicable):** CoverCress, Inc.**Contact information (choose one or both)****Telephone:** [                      ]**Email address:** mweldon@covercress.com

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**2. Does the request contain Confidential Business Information (CBI)?**

Yes, this RSR request contains CBI.

This RSR for gene-edited pennycress contains confidential business information that could harm CoverCress, Inc. if publicly disclosed prior to publication of patent applications or other disclosures with that information. The specific information that could cause competitive harm are the names and sequences of the low glucosinolate target genes, as well as the specific modifications and the mechanism of action. Premature disclosure of that information could allow competitors to develop competitive products much more quickly than if the information was kept as CBI and/or trade secret. CoverCress, Inc. treats this information as private both customarily and actually and provides this information to the government under an assurance of privacy. The information is confidential within the meaning of 5 U. S. C. §552(b)(4), the Freedom of Information Act's Exemption 4.

**3. Description of the comparator plant:****Scientific name (genus, species):** *Thlaspi arvense***Common Name:** pennycress; field pennycress; CoverCress®**Subspecies / Cultivar / Breeding Line:**

Field pennycress is an oilseed crop undergoing domestication for use as an alternative source of biofuel and feedstock. Besides the economic benefit(s), the crop will provide ecosystem services as a cover crop in a two-year corn-soybean rotation. Although a prolific seed producer, interspecific hybridization is uncommon in the genus *Thlaspi* and there are no reports of interspecific hybrids with *Thlaspi arvense* (Al-Shehbaz, 1986; Best & McIntyre, 1975; Warwick et al., 2002).

CoverCress, Inc. has developed, through selection and breeding, several elite germplasm pennycress lines that have optimized agronomic characteristics. The improved germplasm are referred to as CoverCress® lines. It is envisioned that current and future lines from the breeding program, optimized for commercial production, will undergo late stage editing to produce the low erucic acid, lower fiber, lower glucosinolate phenotypes.

#### 4. Genotype of the modified plant (if genetic material is not inserted into the genome):

##### Nature of modification(s):

Mutations will be introduced into pennycress cultivars using a CRISPR/SpCas9 DNA construct designed to target genomic edits to the *FAE1*, *TT8*, [ ] genes. This transgene construct will be delivered to the plant using a disarmed *Agrobacterium tumefaciens* strain (GV3101) and a standard floral dip transformation method. When integrated into the plant genome, the expressed *Streptococcus pyogenes* CRISPR-associated protein 9 (SpCas9) endonuclease will be guided to five unique, targeted loci (the *FAE1*, *TT8*, [ ] genes). At these locations, the SpCas9 endonuclease will catalyze double-stranded DNA breaks, which would then be repaired by the plant's error-prone endogenous non-homologous end joining (NHEJ DNA) repair mechanisms, resulting in heritable mutations at the targeted loci.

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The design pipeline has two steps to ensure the gRNA is specific to the gene and to reduce off-targeting. First, an online gRNA design tool that has an inbuilt pennycress genome is used to identify gRNA target sites and provide a list of putative off-target sites. Furthermore, a blast search is performed with a candidate protospacer sequence against the pennycress genome to avoid the use of a protospacer with potential off targets.

DsRED fluorescent protein from *Discosoma* will be included in the plasmid to confer red fluorescence in plants that successfully take up the plasmid introduced by *A. tumefaciens*. The presence of the edits in T<sub>1</sub> plants will be confirmed through visualization of red fluorescent protein under a light system and confirmatory PCR screening of a fragment of the T-DNA. Seed from the progeny T<sub>2</sub> generation will then be screened for segregants that do not have the transgene as indicated by lack of red fluorescence. Resulting seedlings in the T<sub>2</sub> generation will be screened again for negative presence of DsRED and Cas, as well as homozygous edits to *FAE1*, *TT8*, [ ].

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Examples of these edits are listed in the following section.

##### Sequence and Comparison of each Modification

###### *FAE1, Fatty Acid Elongation 1*

The top line of the following sequence comparison is the unmodified sequence of *FAE1*. One representative modified gene sequence of *FAE1* is included for comparison. One site in the gene is edited and includes a single A insertion, highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

```
Ta_FAE1_WT      GCCTTCACCGTTTTTCGGTTTGGCTCTCTACATCGTAA-CCCGGCCCAAACCGGTTTACCT
Ta_fae1_Mut     GCCTTCACCGTTTTTCGGTTTGGCTCTCTACATCGTAAACCGGCCCAAACCGGTTTACCT
*****
```

###### *TT8, Transparent Testa 8*

The top line of the following sequence comparison is the unmodified sequence of *TT8*. A representative modified gene sequence of *TT8* is included for comparison. One site in the

gene is edited and includes a single G insertion, highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

```
Ta_TT8_WT      GGGAGAATGGATACTACAACGGTGCAATAAAG-ACGAGGAAGACAAC TCAGCCGGCGGAA
Ta_tt8_Mut     GGGAGAATGGATACTACAACGGTGCAATAAAGGACGAGGAAGACAAC TCAGCCGGCGGAA
*****
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The top line of the following sequence comparison is the unmodified sequence of the [ ] **CBI-Deleted**  
gene. One representative modified gene sequence of [ ] is included for comparison. One **CBI-Deleted**

site in the gene is edited and includes a single T insertion, highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

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The top line of the following sequence comparison is the unmodified sequence of the [ ] **CBI-Deleted**  
gene. One representative modified gene sequence of [ ] is included for comparison. **CBI-Deleted**

One site in the gene is edited and includes a 2 bp deletion, highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

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The top line of the following sequence comparison is the unmodified sequence of [ ]. **CBI-Deleted**  
One representative modified gene sequence of [ ] is included for comparison. One site **CBI-Deleted**

in the gene is edited and includes a single T insertion, highlighted in red. The full gene sequences and sequence comparison are included in Appendix 1.

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## 5. Description of new trait

**Intended trait #1:** low erucic acid seeds

**Intended phenotype #1:** low accumulation of erucic acid in seeds

Erucic acid in seed oil in homozygous *fae1* mutants is consistently <2% of total fatty acids, compared with >35% of total fatty acids in wild-type pennycress seeds (Chopra et al., 2018a;

Chopra et al., 2020; McGinn et al., 2019). The fatty acid profile of homozygous *fae1* mutants is comprised predominantly of oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3), which have known nutritional and energy value.

**Intended trait #2:** lower fiber seeds

**Intended phenotype #2:** yellow seed (as a marker for lower fiber)

In homozygous *tt8* mutants, the seed coats of pennycress are light yellow colored in contrast to the naturally dark seeds produced by wild-type pennycress, signifying the absence or reduction of condensed tannins in the seed coat. These seeds contain lower levels of undigestible fiber, and thus, higher metabolizable energy for animal feed.

In a composition study of several light-colored pennycress mutants versus 95 wild type pennycress accessions harvested at various locations across the USA, NIR spectroscopy analysis revealed that the light-colored pennycress contained 10-19.7% Acid Detergent Fiber (ADF) and 13.1-24.1% Neutral Detergent Fiber (NDF), while the dark-colored pennycress contained 20.8-37.9% ADF and 26.3-35.1% NDF (Ulmasov et al., 2020). Additional composition studies consistently show a 25-37% reduction in various fiber components relative to the dark wild type seed (Ulmasov et al., 2020). The lower fiber (yellow seed) phenotype is also associated with more consistent germination and reduced seed dormancy, a major factor in the weediness of this plant (Chopra et al., 2018a; Koirala et al., 2023; Ott et al., 2021).

**Intended trait #3:** reduced glucosinolate seeds

**Intended phenotype #3:** low accumulation of total glucosinolates in seeds; reduction of total glucosinolate levels to below 30  $\mu\text{mol/g}$  of total seed weight

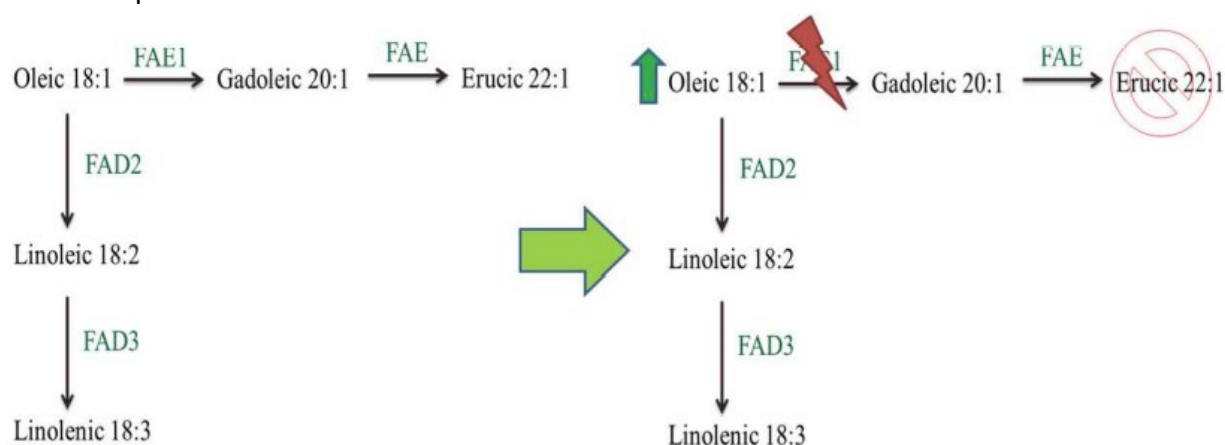
Pennycress contains high levels of oil (~25-35%) that makes it a desirable ultra-low carbon fuel feedstock (Altendorf et al. 2019; McGinn et al. 2019; Moser et al. 2009). In addition to this primary value for fuel, the seed could provide an energy source for animal feeds such as chicken feed. Field pennycress or CoverCress<sup>®</sup> also contains 80-110 $\mu\text{mol/g}$  glucosinolates derived through the aliphatic glucosinolate pathway (Chopra et al., 2018a; Chopra et al., 2020; Sedbrook et al., 2014). Glucosinolates are biologically active compounds found in the Brassicaceae family of plants, including broccoli, cabbage, cauliflower, rapeseed, mustard, and horseradish, and provide defense mechanisms for plants. Over 200 types of glucosinolates are found in brassicas (Prieto et al., 2019); pennycress contains the glucosinolate sinigrin (Chopra et al., 2020). Sinigrin and its metabolite allyl isothiocyanate can reduce palatability of food and feed at lower levels and result in toxic effects at higher levels. Therefore, presence of glucosinolates (which is almost all sinigrin) in field pennycress or CoverCress<sup>®</sup> impacts the ability to include the seed or meal in feed applications above limited inclusion rates (Chopra et al., 2020).

## Description of each MOA:

### Low erucic acid MOA

Field pennycress contains high levels of oil (~25-35%) that makes it a desirable ultra-low carbon fuel feedstock (McGinn et al., 2019; Moser et al., 2009; Sedbrook et al., 2014). In addition to this primary value for fuel, the seed, meal and/or oil could provide an energy source for animal feeds. The utility of pennycress for this use, however, is limited by the fact that the oil contains >35% erucic acid (Altendorf et al., 2019; McGinn et al., 2019). Erucic acid is a 22-carbon monounsaturated acid that is absorbed, distributed, and metabolized like other fatty acids involving primarily metabolism via mitochondrial beta-oxidation and, to a lesser extent, peroxisomal beta-oxidation. Like other longer-chain fatty acids, the rate of mitochondrial beta oxidation is comparatively lower for erucic acid; however, elevated erucic acid levels induce liver peroxisomal oxidation pathways as a mechanism of compensation. Interest in the safety of erucic acid occurred when results of studies in rats associated the dietary intake of high doses of erucic acid with myocardial lipidosis and heart lesions. Oilseed rape conventionally contains similarly high levels of erucic acid. Low erucic acid varieties were identified and marketed as canola, which have been shown to be safe for inclusion in animal feed.

Reduction in erucic acid is achieved through disruption of *fatty acid elongation 1* (*FAE1*) (Figure 1), resulting in higher levels of oleic (18:1) (Qiu et al., 2006). It is through this same mechanism that erucic acid levels are lowered in pennycress (McGinn et al., 2019). The edit to *FAE1* causes a frameshift which results in a premature stop codon and a truncated, non-functional protein.



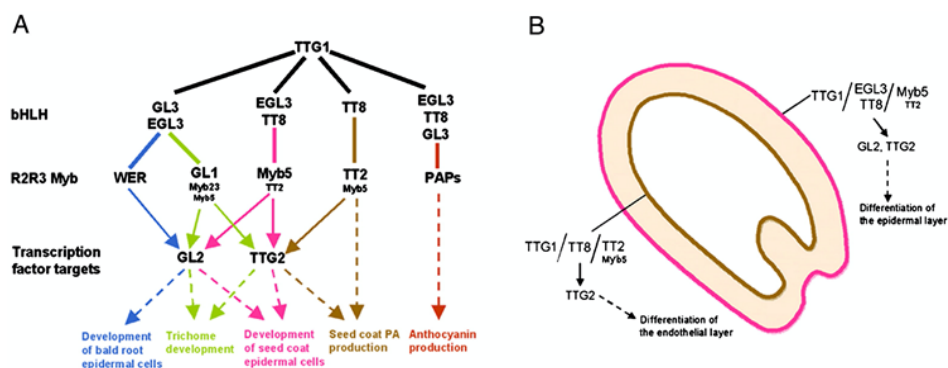
**Figure 1:** Fatty Acid Pathway to Reduce Erucic Acid Levels

## Lower fiber MOA

Field Pennycress is high in fiber (level is variable, but Acid Detergent Fraction values of up to 40% mass on a dry weight basis have been observed (Ulmasov et al., 2020), which can impact digestibility as a feed ingredient.

The production of seed coat fiber was first characterized in the model plant *Arabidopsis*. *Arabidopsis* seed coats derive their brown color from the accumulation of proanthocyanidins (PAs), a class of flavonoid chemicals (polymerized flavan-3-ols, or condensed tannins) that protect against a variety of biotic and abiotic stresses and help maintain seed dormancy and viability (Debeaujon et al., 2003). PAs start out as colorless epicatechin compounds until they are transported to the vacuole where they are polymerized and oxidized as the seed desiccates. In *Arabidopsis*, PAs are only produced in a narrowly defined cell layer in the endothelium of the seed, and TTG1, TT8/bHLH042, and TT2/MYB123 have been demonstrated as being the three main regulators of PA biosynthesis in seed coat (Baudry et al., 2004; Lepiniec et al., 2006). Gonzalez et al. (2009) described how TTG1 works in a complex with a particular combination of MYB class and bHLH class transcription factors to regulate epidermal development of the seed coat (Figure 2).

Loss-of-function mutants in these genes exhibit the transparent “testa” phenotype as a result of low levels of oxidized PAs in the seed coat (Chopra et al., 2018b). The transparent testa phenotype has been observed in brassicas, including canola, and is characterized by yellow seeds that have more oil because of the resulting thinner seed coat and larger embryo (Abraham and Bhatia, 1986). Meal from these brassicas have also been shown to be useful in animal feed because of the relatively lower fiber and higher metabolizable energy (Simbaya et al., 1995; Slominski et al., 1994 and 1999). Similarly, the transparent testa phenotype was observed with loss-of-function mutations in orthologs of these genes in pennycress, resulting in reduced fiber content (Chopra et al., 2018b). In CoverCress® the *TT8* gene was edited for loss of function. The edit to *TT8* causes a frameshift which results in a premature stop codon and a truncated, non-functional protein.

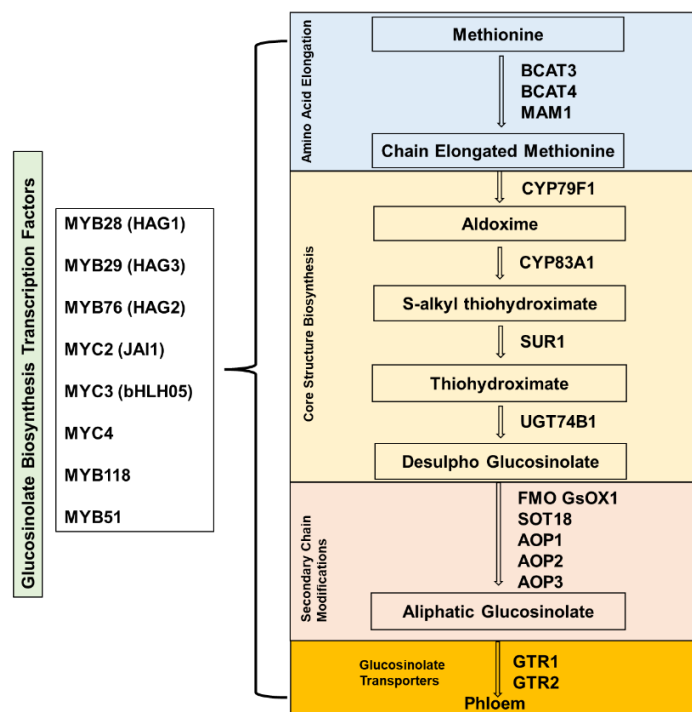


**Figure 2:** Models for the regulation of TTG-1 dependent pathways in seed coat development. (A) A regulatory network for the positive control of TTG1-dependent epidermal cell fates.

Solid lines indicate interactions between members of a complex. Solid arrows indicate direct regulation of *GL2* or *TTG2* targets. Dashed arrows indicate a multi-step differentiation pathway. Colored lines and arrows indicate specific regulator combinations and the pathway controlled. Text size in the case of the MYBs indicates their relative contributions to cell fate regulation. (B) A regulatory model for the differentiation of the seed coat outer and inner layers specified by specific TTG1-dependent transcriptional complexes. Text size in the case of MYB5 and TT2 MYBs indicates their relative contributions to the development of the outer and inner testa layers (Figure from Gonzalez et al., 2009).

### Low total glucosinolate MOA

Glucosinolates are secondary metabolites important for plant defense during development and exposure to biotic and abiotic environmental conditions, as well as helpful in the suppression of weeds. S nderby et al. (2010) gives an overview of the glucosinolate biosynthesis pathway. Glucosinolate biosynthesis in brassicas requires three steps, outlined in Figure 3: 1) amino acid chain elongation, 2) oxidation and sulfation to generate the core glucosinolate structure and 3) secondary side chain modifications to generate unique forms of glucosinolates. Levels of glucosinolate in the seed are regulated by transporters. There are more than 200 glucosinolates identified and classified in brassicas. Field pennycress or CoverCress<sup>®</sup> contains 80-100 mol/g glucosinolates in the form of sinigrin (>99% of the glucosinolate found in pennycress) derived through the aliphatic glucosinolate pathway (Chopra et al., 2018a; Chopra et al., 2020; Sedbrook et al., 2014).



**Figure 3:** Major steps in glucosinolate biosynthesis

The rationale for gene target selection was to identify those genes that could result in reduced glucosinolate levels in the seed through a combination of reduced production and mobilization, but do not impact other biosynthetic pathways or cause accumulation of glucosinolates in other parts of the plant. To reach the desired 70-80% reduction in glucosinolate levels will require loss of function mutations in a combination of gene targets in this RSR request.

These three targets include transcription factors ([ ]), and transporter ([ ]). The genes and their respective contribution to achieve reduced glucosinolate levels are described below.

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[ ] encodes a high-affinity, proton-dependent glucosinolate-specific transporter that is crucial for the transport of both methionine- and tryptophan-derived glucosinolates to seeds [ ]. Gene edited alleles for loss of function of [ ] in pennycress resulted in a reduction in sinigrin levels compared to wild type, with no impact on other biosynthetic pathways or observed accumulation of glucosinolates in other parts of the plant. The representative edited allele for [ ] provided has a 1-bp insertion that causes a frameshift which results in a premature stop codon and a truncated, non-functional protein. Evidence from both greenhouse and field studies demonstrates that plants with this loss of function grow normally throughout their lifecycle unlike observations in *Brassica juncea* (Tan et al. 2022).

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[ ] is a MYB protein that directly regulates the expression of aliphatic glucosinolates biosynthesis in brassicas [ ]. The representative edited allele for [ ] has a 2-bp deletion that causes a frameshift which results in a premature stop codon and a truncated, non-functional protein. This results in a reduction in sinigrin levels compared to wild type. Evidence from greenhouse and field studies demonstrates that plants grow normally throughout their lifecycle and no apparent reduction in plant height or changes in leaf morphology have been observed.

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[ ] is a bHLH protein that is known to interact directly with glucosinolate-related transcription factors, and it helps coordinate expression of several biosynthesis genes required to accumulate glucosinolate [ ]. We generated alleles in pennycress to test this hypothesis and found via RT-PCR that genes involved in glucosinolate biosynthesis were reduced significantly in various stages of plant development. The representative edited allele for [ ] has a 1-bp insertion that causes a frameshift which results in a premature stop codon and a truncated, non-functional protein. [ ] pennycress edits will have a reduction in sinigrin levels compared to its co-grown wild type. Evidence from both greenhouse and field studies demonstrates that plants grow normally throughout their lifecycle and no apparent reduction in plant height or changes in leaf morphology have been observed.

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To achieve meaningful reduction in glucosinolate levels, knockouts in multiple gene targets across the various stages of production and transport are needed to reach the phenotype goal of below 30  $\mu\text{mol/g}$  of total seed weight.

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**Appendix 1 – Full Sequences and Sequence Comparisons**

**Unmodified and Modified Sequences of FAE1, TT8, [ ]**  
Protospacer Sequence Highlighted Yellow, Edit Highlighted Red

CBI-Deleted

*FAE1* Wild Type, Unmodified Sequence*Ta FAE1* WT CDS:

ATGACGTCCGTTAACGTTAAGCTCCTTTACCATTACGTCATCACCAACTTTTTCAACCTTTGCTTCTTCCCG  
TTAGCGGCGATCGTTGCCGAAAAGCCTCTCGGCTTACCACAAACGATCTTCACCACTTCTACTATTCCCTA  
TCTCCAACACAACCTAATAACCATATCTCTACTCTTTGCCTTACCGTTTTCGGTTTGGCTCTCTACATCGT  
AACC CGGCCCAAACCGGTTTACCTCGTTGACCATTCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTA  
TCTCTAAGGTCATGGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGGCAAGCGATGA  
TTCGTCCTGGCTTGATTTCTTGAGGAAGATTGAGGAGCGGTCTGGTCTAGGCGATGAAACCCACGGCCC  
CGAGGGACTGCTTCAGGTCCCTCCACGGAAGACTTTTGCCGCGGCGGTGAAGAAACAGAGCAAGTGA  
TCATCGGTGCGCTCGAAAACTATTCGAGAACACCAAAGTTAACCTAAAGAGATTGGTATACTTGTGG  
TGAAGTCAAGCATGTTTAATCCGACTCCTTCGCTCTCGGCGATGGTTGTTAATACTTTCAAGCTCCGAAGC  
AACATCAGAAGCTTTAATCTTGGAGGAATGGGTTGTAGTGCCGCGGTTATAGCCATTGATCTGGCTAAG  
GACTTGTTGCATGTCCATAAAAACACTTATGCTCTTGTGGTGAGCACAGAGAACATCACTTACAACATTT  
ATGCTGGTGATAACAGATCCATGATGGTTTGAATTGCTTGTTCGTTGTTGGTGGGGCCGCGATTTTGCT  
CTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCAGCTACTTCACACGGTTCGGACGCATACCG  
GAGCTGACGACAAGTCTTTCGATGTGTGCAACAAGAAGACGACGAGAGCGGTAAAACCGGGGTGTGT  
TTGTCCAAGGACATAACCGGTGTTGCCGGGAGAACTGTTGAGAAAAACATAACAACATTGGGTCCGTTG  
GTTCTTCTTTTAGCGAGAAATTTCTTTTTTCGTTACCTTCATCGCCAAGAACTCTTTAAAGACAAGATC  
AAACATTACTACGTCCCGGATTTCAAGCTTGCTATCGACCATTTTTGTATTATGCCGGAGGCAGAGCCG  
TGATCGATGTGCTACAGAAGAACTTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTTAC  
ATAGATTTGGGAACACTTCGTCTAGCTCAATTTGGTATGAATTGGCGTACATAGAGGCAAAAGGAAGGA  
TGAAGAGAGGGAACAAAGTTTGGCAGATTGCTTAGGGTCAGGGTTTAAGTGTAAAGTGCGGTTTGG  
GTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGTCCTTGGAACATTGCATTGATAGATATCCAGAT  
GCAATTGATTCTGATTCCGGTAAGTCAGAGACTCGTGTCCAAAACGGTCGGTCCTAA

*FAE1* Modified Sequence, Representative Sequence*Ta fae1* Mutant CDS:

ATGACGTCCGTTAACGTTAAGCTCCTTTACCATTACGTCATCACCAACTTTTTCAACCTTTGCTTCTTCCCG  
TTAGCGGCGATCGTTGCCGAAAAGCCTCTCGGCTTACCACAAACGATCTTCACCACTTCTACTATTCCCTA  
TCTCCAACACAACCTAATAACCATATCTCTACTCTTTGCCTTACCGTTTTCGGTTTGGCTCTCTACATCGT  
AA(A)CCCGGCCCAAACCGGTTTACCTCGTTGACCATTCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTA  
TCTCTAAGGTCATGGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGGCAAGCGATGA  
TTCGTCCTGGCTTGATTTCTTGAGGAAGATTGAGGAGCGGTCTGGTCTAGGCGATGAAACCCACGGCCC  
CGAGGGACTGCTTCAGGTCCCTCCACGGAAGACTTTTGCCGCGGCGGTGAAGAAACAGAGCAAGTGA  
TCATCGGTGCGCTCGAAAACTATTCGAGAACACCAAAGTTAACCTAAAGAGATTGGTATACTTGTGG  
TGAAGTCAAGCATGTTTAATCCGACTCCTTCGCTCTCGGCGATGGTTGTTAATACTTTCAAGCTCCGAAGC  
AACATCAGAAGCTTTAATCTTGGAGGAATGGGTTGTAGTGCCGCGGTTATAGCCATTGATCTGGCTAAG  
GACTTGTTGCATGTCCATAAAAACACTTATGCTCTTGTGGTGAGCACAGAGAACATCACTTACAACATTT

ATGCTGGTGATAACAGATCCATGATGGTTTCGAATTGCTTGTTCCGTGTTGGTGGGGCCGCGATTTTGCT  
CTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCAGCTACTTCACACGGTTCGGACGCATACCG  
GAGCTGACGACAAGTCTTTCCGATGTGTGCAACAAGAAGACGACGAGAGCGGTAAAACCGGGGTGTGT  
TTGTCCAAGGACATAACCGGTGTTGCCGGGAGAACTGTTGAGAAAAACATAACAACATTGGGTCCGTTG  
GTTCTTCCTTTTAGCGAGAAATTTCTTTTTTCGTTACCTTCATCGCCAAGAACTCTTTAAAGACAAGATC  
AAACATTACTACGTCCCGGATTTCAAGCTTGCTATCGACCATTTTTGTATTTCATGCCGGAGGCAGAGCCG  
TGATCGATGTGCTACAGAAGAACTTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTTAC  
ATAGATTTGGGAACACTTCGTCTAGCTCAATTTGGTATGAATTGGCGTACATAGAGGCAAAAGGAAGGA  
TGAAGAGAGGGGAACAAAGTTTGGCAGATTGCTTTAGGGTCAGGGTTAAGTGTAAATAGTGCAGTTTGG  
GTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGTCCTTGGAACATTGCATTGATAGATATCCAGAT  
GCAATTGATTCTGATTCCGGTAAGTCAGAGACTCGTGTCCAAAACGGTCGGTCCTAA

*TT8* Wild Type, Unmodified Sequence

*Ta TT8* WT CDS:

ATGGATGAATCAAGTATTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTTCAAGGGCT  
GCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAACTTTGTCCTCAACAAAGg  
ttcttttttttttaataaatttcatcgatctctcacaataaaaaccctaaattttatatcatttattattatatgtttaactacataattatcag  
tattttaaccgtccatgtgctttatttggtccatttctgtctcatattttacttgaggttcagactgccgagcacatctctcgtttgtctcgaat  
ctgtgagacttttctgttattggcacttctgtgtcaattgagttatactgaagtaattatatgtttaaatgaattagGGTTTTGCTGTG  
GGAGAATGGATACTACAACGGTGCAATAAAGACGAGGAAGACAACCTAGCCGGCGGAAGTGACGGCG  
GAAGAGGCTGCGTTAGAGAGGAGTCAGCAGCTAAGGGAACCTTACGAGGCCCTTTTGCCGGAGAGTC  
CTCATCGGAAGCTAGGGCATGCACGGCATTATCGCCGGAGGATCTGACGGAGACTGAATGGTTTTATCT  
AATGTGTGTCTCTTTCTCTTCCCTCCTCCTCCGGgtaccaactctctctctctctctctctctctctctctctctctctctctctctc  
tctctctctctttgtctatactgaagtttcttaatttatcttttatcatctctactgaagacaaaaatagattgtgtgttaaatagcgaatca  
cgaatatttggaagcattaaaaaactgaggaggttgagttactgaagaagaatgtattggagttgatgaacgtactccat  
tttagtgaacataattggaccgttgagattcttatttttctgctgattgattatctaaagtagaagcataaatagataatacataaatgcata  
acaaattgtgttagttatgggtatagttaatgcttttctctatgagaggaaaaaaaaaaaaatatataatgtggaagtaataattttgta  
gGATGCCAGGAAAGGCGTATGCGAGGAGGAAACACGTATGGCTATGTGGTGCAAATGAGGTTGACAGT  
AAAATCTTTCTAGGGCTATTCTCGCAAAGgtctatttcttttctattaccactactctatgcatctacttctctacattttat  
atatctcatcttcaaattaattaatttctgtctttattttcttgatgctccttctacatcggtcggttccttaatggtagAGTGCCAAA  
ATCCAGgtaaacgttgtctttattgattaattctaatttgagtaattttacattattttacatgtttgaaaattgtttgtgataaaaaa  
aaagCAGACAGTGGTTTGATTCCCATGCTTGATGGCGTTGTGGAACCTAGGCACAACGAACAAGgtacggc  
gtagttatcttttatatatgcataaccaaaggttaagaaaaaggttagaagagaaatagatcatgcttaagtttatcagttaaattaa  
aatgtaaaaataagatattatgttcattaataatgtatagccctgttagttaaaaaaagaataaaatatttaaccatttgaagtcataat  
ataaaaaatattgttttggagatagtacataattctcacaataaaaaaaataacaaagggtgattaagggaaggagttggatacatgtt  
gtttgtctgtgtgtgaagGTAAAAGAAGATATAGCGTTTGTTGAGCTCATAAAGAGTTTTTTCCATAACCACC  
CCAAGTCAAACCCAAAAGCTGCTCTTTCTGAACACTCCATCAACGAAGAGCACGAAGAAGACGAAGAAC  
AAGAAGAAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCTC  
TCCTGATGATGATGACGTCTCAATCAAACCTACTCTCTGATTTCCATGTAGAATCAACCCACACTTTAG  
gtatacacttatacattaaattagttaacgatatacattacgtatctattttttgtaacaagaaatttaaaaaatattcgccatttcttgt  
tatgtctaaagaaaatctataaaatttatgaatagACACACACATGGACATGATGAATCTAATGGAGGAGGGTGA  
AACTATTCTCAGACAGTATCAACACTTCTATGTCACAACCCACGAGTCTTTTTTCAGATTGAGTTTCCACA  
TCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAATTTTAAAGAGCATCAGCGAGTGGA

CTAAATCGACGTCGTCGTCGCAATGGATGCTCAAACACATAATCTTGAGAGTTCCTTTACTCCACGACCA  
CACTAAAGAAAAGAGGCTGCCTCGAGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGAGGAGAGAG  
AAGCTGAATGAGAGATTCAACACTGAGATCATTGGTTCCTTTGTGACCAAGATGGATAAAGTCTCA  
ATTCTTGAGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCATC  
ACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGTTGAGGTTTCC  
ATCATAGAGAGTGATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGGTCTATTGCTCGACATCCTTC  
AGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCATACCGCGGTGAACGAGCGTGATTTTCGAGG  
CCGAGATAAGGGCTATGGTGAGAGGGAAGAAACCAAGCATTGCTGAGGTCAAAGAGCCATCCATCAA  
ACTATATCCAATATTAACTATAG

### *TT8* Modified Sequence, Representative Sequence

*Ta tt8* Mutant CDS:

ATGGATGAATCAAGTATTTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTTCAAGGGCT  
GCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTTCTGGCAACTTTGTCCTCAACAAAGg  
Ttcttttttttttaataaatttcatcgatctctcacaataaaaaaccctaaattttatatcatattattattatgtttaactacataaattatcag  
Tattttaaccgtccatgtgctttatttgggtccatttctgtctcatattttacttgaggttcagactgccgagcacatctctcgtttgtctcgaat  
ctgtgagactttttcgtttattggcacttctgtgtcaattgagttatactgaagtaattatatgtttaaatgaattagGGTTTTGCTGTG  
GGAGAATGGATACTACAACGGTGCAATAAAG(G)ACGAGGAAGACAACCTAGCCGGCGGAAGTGACGGCG  
GAAGAGGCTGCGTTAGAGAGGAGTCAGCAGCTAAGGGAACTTACGAGGCCCTTTGGCCGGAGAGTC  
CTCATCGGAAGCTAGGGCATGCACGGCATTATCGCCGGAGGATCTGACGGAGACTGAATGGTTTTATCT  
AATGTGTGTCTCTTTCTCTTTCCCTCCTCCTCCGGgtaccaactctctctctctctctctctctctctctctctctctctctctctctc  
Tctctctctctttgtctatactgaagtttctaatttatcttttatcatctcctactgaagacaaaaatagtattgtgtgttaaatgcgaatca  
Cgaatattgtggaagcattaaaaaactgaggaggttgagttactgaaagaagaatgtattggagttgatgaaacgtacactccat  
Ttagtgaacataattggaccgttgagattctattttttgctgattgattatctaagtagaagcataaataagataataacataaattgcata  
acaaattgtgttagttatgggtatagttaatgcttttctctatgagaggaaaaaataataaatgtggaagtaataattttgt  
agGATGCCAGGAAAGGCGTATGCGAGGAGGAAACACGTATGGCTATGTGGTGCAATGAGGTTGACAGT  
AAAATCTTTTCTAGGGCTATTCTCGCAAAGgtctatttcttttctattaccactactctatgcatctacttctctacatttat  
atatctcatctttcaaattaattaatttctgtctttattttcttgatgctcttctacatcggtcggttccttaatggttagAGTGCCAAA  
ATCCAGgtaaacgttgtctttattgattaattctaatttgagtaataatttaccattattttacatgtttgaaaattgtttgtgataaaaaa  
aaagCAGACAGTGGTTTGATTCCCATGCTTGATGGCGTTGTGGAAGTGGCACAACGAACAAGgtacggc  
gtagttatcttttatatatgcataaccaaatgggttaagaaaaaggttagaagagaaatagatcatgcttaagttttatcagttaaattaaa  
aatgtaaaaaataagatattatgttcattaataatgtatagtcctgttagttaaaaaaagaataaaatatttaaccatttgaagtcataat  
ataaaaaattgttttggagatagtagataattctcacaataaaaaaataacaaagggtatgattaagggaaggagttggatacatgtt  
gtttgtctgtgtgtgaagGTAAAAGAAGATATAGCGTTTGTTGAGCTCATAAAGAGTTTTTCCATAACCACC  
CCAAGTCAAACCCAAAAGCTGCTCTTTCTGAACACTCCATCAACGAAGAGCACGAAGAAGACGAAGAAC  
AAGAAGAAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCTC  
TCCTGATGATGATGACGTCTCAATCAAACCTACTCTGATTTCCATGTAGAATCAACCACACTTTAG  
Gtatacattatacattaaattagttacgatatacattacgtatctatttttgttaacaagaatttaaaaaatattcgccatttctttgt  
tatgtctaaagaaaatctataaattttatgaatagACACACACATGGACATGATGAATCTAATGGAGGAGGGTGGGA  
AACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTTCAGATTCAGTTTCCACA  
TCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAATTTTAAAGAGCATCAGCGAGTGGA  
CTAAATCGACGTCGTCGTCGCAATGGATGCTCAAACACATAATCTTGAGAGTTCCTTTACTCCACGACCA  
CACTAAAGAAAAGAGGCTGCCTCGAGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGAGGAGAGAG

AAGCTGAATGAGAGATTCATAAACTGAGATCATTGGTTCCTTTGTGACCAAGATGGATAAAGTCTCA  
ATTCTTGGAGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCATC  
ACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGTTGAGGTTTCC  
ATCATAGAGAGTGATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGGTCTATTGCTCGACATCCTTC  
AGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCATACCGCGGTGAACGAGCGTGATTTGAGG  
CCGAGATAAGGGCTATGGTGAGAGGGAAGAAACCAAGCATTGCTGAGGTCAAAGAGCCATCCATCAA  
ACTATATCCAATATTAAGTATAG

[ ] Wild Type, Unmodified Sequence

CBI-Deleted

*Ta* [ ] WT CDS:

CBI-Deleted

[

CBI-Deleted

]

[ ] Modified Sequence, Representative Sequence

CBI-Deleted

*Ta* [ ] Mutant CDS:

CBI-Deleted

[

CBI-Deleted

**CBI-Deleted**

]

[ ] Wild Type, Unmodified Sequence  
*Ta* [ ] WT CDS:  
[

**CBI-Deleted****CBI-Deleted****CBI-Deleted**

]

[       ] Modified Sequence, Representative Sequence

**CBI-Deleted**

*Ta* [       ] Mutant CDS:

**CBI-Deleted**

[

**CBI-Deleted**

]

[       ] Wild Type, Unmodified Sequence

**CBI-Deleted**

*Ta* [       ] WT CDS:

**CBI-Deleted**

[

**CBI-Deleted**

**CBI-Deleted**

]

[ ] Modified Sequence, Representative Sequence

**CBI-Deleted***Ta* [ ] Mutant CDS:**CBI-Deleted**

[

**CBI-Deleted**

]

## Sequence Comparisons of the unmodified and modified genes

### *FAE1*

The top lines of the following sequence comparison are the unmodified sequence of *FAE1* (Ta\_FAE1\_WT). One representative modified gene sequence of *FAE1* (Ta\_fae1\_Mut) is included for comparison. One site in the gene is edited and includes a single A insertion, highlighted in red.

|             |                                                                                    |
|-------------|------------------------------------------------------------------------------------|
| Ta_FAE1_WT  | ATGACGTCCGTTAACGTTAAGCTCCTTTACCATTACGTCATCACCAACTTTTTCAACCTT                       |
| Ta_fae1_Mut | ATGACGTCCGTTAACGTTAAGCTCCTTTACCATTACGTCATCACCAACTTTTTCAACCTT<br>*****              |
| Ta_FAE1_WT  | TGCTTCTTCCCCTTAGCGGCGATCGTTGCCGGAAGCCTCTCGGCTTACCACAAACGAT                         |
| Ta_fae1_Mut | TGCTTCTTCCCCTTAGCGGCGATCGTTGCCGGAAGCCTCTCGGCTTACCACAAACGAT<br>*****                |
| Ta_FAE1_WT  | CTTCACCACTTCTACTATTCTATCTCCAACACAACCTAATAACCATATCTCTACTCTTT                        |
| Ta_fae1_Mut | CTTCACCACTTCTACTATTCTATCTCCAACACAACCTAATAACCATATCTCTACTCTTT<br>*****               |
| Ta_FAE1_WT  | GCCTTCACCGTTTTTCGGTTTGGCTCTCTACATCGTAA-CCCGGCCAAACCGGTTTACCT                       |
| Ta_fae1_Mut | GCCTTCACCGTTTTTCGGTTTGGCTCTCTACATCGTAA <sup>A</sup> CCCGGCCAAACCGGTTTACCT<br>***** |
| Ta_FAE1_WT  | CGTTGACCATTCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTATCTCTAAGGTCAT                        |
| Ta_fae1_Mut | CGTTGACCATTCTGCTACCTTCCACCATCGCATCTTAGAAGCAGTATCTCTAAGGTCAT<br>*****               |
| Ta_FAE1_WT  | GGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGGCAAGCGATGATTC                       |
| Ta_fae1_Mut | GGATATCTTCTATCAAGTAAGATTAGCCGATCCTTTACGGAACGCGGCAAGCGATGATTC<br>*****              |
| Ta_FAE1_WT  | GTCCTGGCTTGATTCTTGGAGGAAGATTAGGAGCGGTCTGGTCTAGGCGATGAAACCCA                        |
| Ta_fae1_Mut | GTCCTGGCTTGATTCTTGGAGGAAGATTAGGAGCGGTCTGGTCTAGGCGATGAAACCCA<br>*****               |
| Ta_FAE1_WT  | CGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAGACTTTTGCCGCGGCGGTGAAGA                        |
| Ta_fae1_Mut | CGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAGACTTTTGCCGCGGCGGTGAAGA<br>*****               |
| Ta_FAE1_WT  | AACAGAGCAAGTGATCATCGGTGCGCTCGAAAACTATTTCGAGAACACCAAAGTTAACCC                       |
| Ta_fae1_Mut | AACAGAGCAAGTGATCATCGGTGCGCTCGAAAACTATTTCGAGAACACCAAAGTTAACCC<br>*****              |
| Ta_FAE1_WT  | TAAAGAGATTGGTATACTTGTGGTGAAGTCAAGCATGTTTAAATCCGACTCCTTCGCTCTC                      |
| Ta_fae1_Mut | TAAAGAGATTGGTATACTTGTGGTGAAGTCAAGCATGTTTAAATCCGACTCCTTCGCTCTC<br>*****             |
| Ta_FAE1_WT  | GGCGATGGTTGTTAATACTTTCAAGCTCCGAAGCAACATCAGAAGCTTTAATCTGGAGG                        |
| Ta_fae1_Mut | GGCGATGGTTGTTAATACTTTCAAGCTCCGAAGCAACATCAGAAGCTTTAATCTGGAGG<br>*****               |
| Ta_FAE1_WT  | AATGGGTTGTAGTGCCGCGCTTATAGCCATTGATCTGGCTAAGGACTTGTTCATGTCCA                        |
| Ta_fae1_Mut | AATGGGTTGTAGTGCCGCGCTTATAGCCATTGATCTGGCTAAGGACTTGTTCATGTCCA<br>*****               |

|             |                                                               |
|-------------|---------------------------------------------------------------|
| Ta_FAE1_WT  | TAAAAACACTTATGCTCTTGTGGTGAGCACAGAGAACATCACTTACAACATTTATGCTGG  |
| Ta_fae1_Mut | TAAAAACACTTATGCTCTTGTGGTGAGCACAGAGAACATCACTTACAACATTTATGCTGG  |
|             | *****                                                         |
| Ta_FAE1_WT  | TGATAACAGATCCATGATGGTTTCGAATTGCTTGTTCCGTGTTGGTGGGGCCGCGATTTT  |
| Ta_fae1_Mut | TGATAACAGATCCATGATGGTTTCGAATTGCTTGTTCCGTGTTGGTGGGGCCGCGATTTT  |
|             | *****                                                         |
| Ta_FAE1_WT  | GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCAGCTACTTCACACGGTTTCG |
| Ta_fae1_Mut | GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCAGCTACTTCACACGGTTTCG |
|             | *****                                                         |
| Ta_FAE1_WT  | GACGCATACCGGAGCTGACGACAAGTCTTTCCGATGTGTGCAACAAGAAGACGACGAGAG  |
| Ta_fae1_Mut | GACGCATACCGGAGCTGACGACAAGTCTTTCCGATGTGTGCAACAAGAAGACGACGAGAG  |
|             | *****                                                         |
| Ta_FAE1_WT  | CGGTAAAACCGGGGTGTGTTTGTCCAAGGACATAACCGGTGTTGCCGGGAGAACTGTTCA  |
| Ta_fae1_Mut | CGGTAAAACCGGGGTGTGTTTGTCCAAGGACATAACCGGTGTTGCCGGGAGAACTGTTCA  |
|             | *****                                                         |
| Ta_FAE1_WT  | GAAAAACATAACAACATTGGGTCCGTTGGTTCTTCCTTTTAGCGAGAAATTTCTTTTTTT  |
| Ta_fae1_Mut | GAAAAACATAACAACATTGGGTCCGTTGGTTCTTCCTTTTAGCGAGAAATTTCTTTTTTT  |
|             | *****                                                         |
| Ta_FAE1_WT  | CGTTACCTTCATCGCCAAGAACTCTTTAAAGACAAGATCAAACATTACTACGTCCCGGA   |
| Ta_fae1_Mut | CGTTACCTTCATCGCCAAGAACTCTTTAAAGACAAGATCAAACATTACTACGTCCCGGA   |
|             | *****                                                         |
| Ta_FAE1_WT  | TTTCAAGCTTGCTATCGACCATTTTTGTATTTCATGCCGAGGCAGAGCCGTGATCGATGT  |
| Ta_fae1_Mut | TTTCAAGCTTGCTATCGACCATTTTTGTATTTCATGCCGAGGCAGAGCCGTGATCGATGT  |
|             | *****                                                         |
| Ta_FAE1_WT  | GCTACAGAAGAACTTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTTACA  |
| Ta_fae1_Mut | GCTACAGAAGAACTTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTTACA  |
|             | *****                                                         |
| Ta_FAE1_WT  | TAGATTTGGGAACACTTCGTCTAGCTCAATTTGGTATGAATTGGCGTACATAGAGGCCAAA |
| Ta_fae1_Mut | TAGATTTGGGAACACTTCGTCTAGCTCAATTTGGTATGAATTGGCGTACATAGAGGCCAAA |
|             | *****                                                         |
| Ta_FAE1_WT  | AGGAAGGATGAAGAGAGGGAACAAAGTTTGGCAGATTGCTTTAGGGTCAGGGTTAAGTG   |
| Ta_fae1_Mut | AGGAAGGATGAAGAGAGGGAACAAAGTTTGGCAGATTGCTTTAGGGTCAGGGTTAAGTG   |
|             | *****                                                         |
| Ta_FAE1_WT  | TAATAGTGCGGTTTGGGTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGTCCTTGGA   |
| Ta_fae1_Mut | TAATAGTGCGGTTTGGGTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGTCCTTGGA   |
|             | *****                                                         |
| Ta_FAE1_WT  | ACATTGCATTGATAGATATCCAGATGCAATTGATTCTGATTCCGGTAAGTCAGAGACTCG  |
| Ta_fae1_Mut | ACATTGCATTGATAGATATCCAGATGCAATTGATTCTGATTCCGGTAAGTCAGAGACTCG  |
|             | *****                                                         |
| Ta_FAE1_WT  | TGTCCAAAACGGTCGGTCCTAA                                        |
| Ta_fae1_Mut | TGTCCAAAACGGTCGGTCCTAA                                        |
|             | *****                                                         |

## TT8

The top lines of the following sequence comparison are the unmodified sequence of *TT8* (Ta\_TT8\_WT). A representative modified gene sequence of *TT8* (Ta\_tt8\_Mut) is included

for comparison. One site in the gene is edited and includes a single G insertion, highlighted in red.

|            |                                                                                 |
|------------|---------------------------------------------------------------------------------|
| Ta_TT8_WT  | ATGGATGAATCAAGTATTTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT                    |
| Ta_tt8_Mut | ATGGATGAATCAAGTATTTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT<br>*****           |
| Ta_TT8_WT  | CAAGGGCTGCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAA                    |
| Ta_tt8_Mut | CAAGGGCTGCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAA<br>*****           |
| Ta_TT8_WT  | CTTTGTCCTCAACAAAGGTTCTTTTTTTTTTTAATAAATTTTCATCGATCTCTCACAATA                    |
| Ta_tt8_Mut | CTTTGTCCTCAACAAAGGTTCTTTTTTTTTTTAATAAATTTTCATCGATCTCTCACAATA<br>*****           |
| Ta_TT8_WT  | AAAACCCTAAATTTTATATCATTTATTATTATATGTTAACTACATAATTATCAGTATTT                     |
| Ta_tt8_Mut | AAAACCCTAAATTTTATATCATTTATTATTATTATGTTAACTACATAATTATCAGTATTT<br>*****           |
| Ta_TT8_WT  | TAACCGTCCATGTGCTTTATTTGGTTCATTTCTGTCTCATATTTTACTTGAGGTTTCAGA                    |
| Ta_tt8_Mut | TAACCGTCCATGTGCTTTATTTGGTTCATTTCTGTCTCATATTTTACTTGAGGTTTCAGA<br>*****           |
| Ta_TT8_WT  | CTGCCGAGCACATCTCTCGTTTGTCTCGAATCTGTGAGACTTTTTCGTTTATTGGCACTT                    |
| Ta_tt8_Mut | CTGCCGAGCACATCTCTCGTTTGTCTCGAATCTGTGAGACTTTTTCGTTTATTGGCACTT<br>*****           |
| Ta_TT8_WT  | CTGTGTCAATTGAGTTATACTGAAGTAATTATATGTTTAAATGAATTAGGGTTTTGCTGT                    |
| Ta_tt8_Mut | CTGTGTCAATTGAGTTATACTGAAGTAATTATATGTTTAAATGAATTAGGGTTTTGCTGT<br>*****           |
| Ta_TT8_WT  | GGGAGAATGGATACTACAACGGTGCAATAAAG-ACGAGGAAGACAACCTCAGCCGGCGGAA                   |
| Ta_tt8_Mut | GGGAGAATGGATACTACAACGGTGCAATAAAG <b>G</b> ACGAGGAAGACAACCTCAGCCGGCGGAA<br>***** |
| Ta_TT8_WT  | GTGACGGCGGAAGAGGCTGCGTTAGAGAGGAGTCAGCAGCTAAGGGAACCTTTACGAGGCC                   |
| Ta_tt8_Mut | GTGACGGCGGAAGAGGCTGCGTTAGAGAGGAGTCAGCAGCTAAGGGAACCTTTACGAGGCC<br>*****          |
| Ta_TT8_WT  | CTTTTGCCCGGAGAGTCCTCATCGGAAGCTAGGGCATGCACGGCATTATCGCCGGAGGAT                    |
| Ta_tt8_Mut | CTTTTGCCCGGAGAGTCCTCATCGGAAGCTAGGGCATGCACGGCATTATCGCCGGAGGAT<br>*****           |
| Ta_TT8_WT  | CTGACGGAGACTGAATGGTTTTATCTAATGTGTGTCTCTTTCTCTTCCCTCCTCCTTCC                     |
| Ta_tt8_Mut | CTGACGGAGACTGAATGGTTTTATCTAATGTGTGTCTCTTTCTCTTCCCTCCTCCTTCC<br>*****            |
| Ta_TT8_WT  | GGGTACCCAACCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT                     |
| Ta_tt8_Mut | GGGTACCCAACCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT<br>*****            |
| Ta_TT8_WT  | CTCTTTTGTCTATACTGAAGTTTCTTAATTTATCTTTTTATCATCTCCTACTGAAGACAA                    |
| Ta_tt8_Mut | CTCTTTTGTCTATACTGAAGTTTCTTAATTTATCTTTTTATCATCTCCTACTGAAGACAA<br>*****           |
| Ta_TT8_WT  | AAATAGTATTGTGTGTTAAATGCGAATCACGAATATTGTGGAAGCATTAAAAACAAACTG                    |
| Ta_tt8_Mut | AAATAGTATTGTGTGTTAAATGCGAATCACGAATATTGTGGAAGCATTAAAAACAAACTG<br>*****           |

|            |                                                                  |
|------------|------------------------------------------------------------------|
| Ta_TT8_WT  | AGGAGGTTGAGTTACTGAAAGAAGAAATGTATTGGAGTTGATGAAACGTACACTCCATTT     |
| Ta_tt8_Mut | AGGAGGTTGAGTTACTGAAAGAAGAAATGTATTGGAGTTGATGAAACGTACACTCCATTT     |
|            | *****                                                            |
| Ta_TT8_WT  | TAGTGAACATAAATTGGACCGTTGAGATTCTTATTTTTTTGCTGATTGATTATCTAAAGTA    |
| Ta_tt8_Mut | TAGTGAACATAAATTGGACCGTTGAGATTCTTATTTTTTTGCTGATTGATTATCTAAAGTA    |
|            | *****                                                            |
| Ta_TT8_WT  | GAAGCATAAATAGATAATACATAAATGCATAACAAATTGTGTTAGTTATGGGTATAGTTA     |
| Ta_tt8_Mut | GAAGCATAAATAGATAATACATAAATGCATAACAAATTGTGTTAGTTATGGGTATAGTTA     |
|            | *****                                                            |
| Ta_TT8_WT  | ATGCTTTTTCTCTATGAGAGGAAAAAAAAAAAAAAAAATATATAAATGTGGAAGTAATAATTTT |
| Ta_tt8_Mut | ATGCTTTTTCTCTATGAGAGGAAAAAAAAAAAAAAAAATATATAAATGTGGAAGTAATAATTTT |
|            | *****                                                            |
| Ta_TT8_WT  | GTAGGATGCCAGGAAAGGCGTATGCGAGGAGGAAACACGTATGGCTATGTGGTGCAAATG     |
| Ta_tt8_Mut | GTAGGATGCCAGGAAAGGCGTATGCGAGGAGGAAACACGTATGGCTATGTGGTGCAAATG     |
|            | *****                                                            |
| Ta_TT8_WT  | AGGTTGACAGTAAATCTTTTTCTAGGGCTATTCTCGCAAAGGTCTATTTTCCTTTTTTCATT   |
| Ta_tt8_Mut | AGGTTGACAGTAAATCTTTTTCTAGGGCTATTCTCGCAAAGGTCTATTTTCCTTTTTTCATT   |
|            | *****                                                            |
| Ta_TT8_WT  | TACCACTACTCTATGCATCTACTTCTCTACCTATTTATATATCTCATCTTTCAAATTAAT     |
| Ta_tt8_Mut | TACCACTACTCTATGCATCTACTTCTCTACCTATTTATATATCTCATCTTTCAAATTAAT     |
|            | *****                                                            |
| Ta_TT8_WT  | TAATTTTCTGTCTTTATTTTCTTGGATGCTCCTTCTACATCGGTCGGTTCCTTAATGGTT     |
| Ta_tt8_Mut | TAATTTTCTGTCTTTATTTTCTTGGATGCTCCTTCTACATCGGTCGGTTCCTTAATGGTT     |
|            | *****                                                            |
| Ta_TT8_WT  | AGAGTGCCAAAATCCAGGTAAACGTTGTCTTTATTGATTAATTCTAATTTTGAGTAATAT     |
| Ta_tt8_Mut | AGAGTGCCAAAATCCAGGTAAACGTTGTCTTTATTGATTAATTCTAATTTTGAGTAATAT     |
|            | *****                                                            |
| Ta_TT8_WT  | TTTACATTTATTTTACATGTTTGAAAATTGTTTGTGATAAAAAAAAAAGCAGACAGTGGT     |
| Ta_tt8_Mut | TTTACATTTATTTTACATGTTTGAAAATTGTTTGTGATAAAAAAAAAAGCAGACAGTGGT     |
|            | *****                                                            |
| Ta_TT8_WT  | TTGCATTCCCATGCTTGATGGCGTTGTGGAAC TAGGCACAACGAACAAGGTACGCGCTAG    |
| Ta_tt8_Mut | TTGCATTCCCATGCTTGATGGCGTTGTGGAAC TAGGCACAACGAACAAGGTACGCGCTAG    |
|            | *****                                                            |
| Ta_TT8_WT  | TTATCTTTTTATATATGCATAACCAAATGGTTAAGAAAAAGGTTAGAAGAGAAATAGATC     |
| Ta_tt8_Mut | TTATCTTTTTATATATGCATAACCAAATGGTTAAGAAAAAGGTTAGAAGAGAAATAGATC     |
|            | *****                                                            |
| Ta_TT8_WT  | ATGCTTAAGTTTTATCAGTTAAATTAAAAATGTAAAAATAAGATATTATGTTTCATTAATA    |
| Ta_tt8_Mut | ATGCTTAAGTTTTATCAGTTAAATTAAAAATGTAAAAATAAGATATTATGTTTCATTAATA    |
|            | *****                                                            |
| Ta_TT8_WT  | ATGTATAGTCCCTGTTAGTTAAAAAAAAGAATAAAATATTTAACCATTGGAAGTCATAAT     |
| Ta_tt8_Mut | ATGTATAGTCCCTGTTAGTTAAAAAAAAGAATAAAATATTTAACCATTGGAAGTCATAAT     |
|            | *****                                                            |
| Ta_TT8_WT  | ATAAAAAATATTGTTTTTGGAGATAGTACATAATTCTCACAATAAAAAAATAACAAAGGG     |
| Ta_tt8_Mut | ATAAAAAATATTGTTTTTGGAGATAGTACATAATTCTCACAATAAAAAAATAACAAAGGG     |
|            | *****                                                            |

|            |                                                              |
|------------|--------------------------------------------------------------|
| Ta_TT8_WT  | ATGATTAAGGGAAGGAGTTGGATACATGTTGTTTGTCTGTGTGTGAAGGTAAAAGAAG   |
| Ta_tt8_Mut | ATGATTAAGGGAAGGAGTTGGATACATGTTGTTTGTCTGTGTGTGAAGGTAAAAGAAG   |
|            | *****                                                        |
| Ta_TT8_WT  | ATATAGCGTTTGTGAGCTCATAAAGAGTTTTTTCCATAACCACCCCAAGTCAAACCCAA  |
| Ta_tt8_Mut | ATATAGCGTTTGTGAGCTCATAAAGAGTTTTTTCCATAACCACCCCAAGTCAAACCCAA  |
|            | *****                                                        |
| Ta_TT8_WT  | AAGCTGCTCTTTCTGAACACTCCATCAACGAAGAGCACGAAGAAGACGAAGAACAAGAAG |
| Ta_tt8_Mut | AAGCTGCTCTTTCTGAACACTCCATCAACGAAGAGCACGAAGAAGACGAAGAACAAGAAG |
|            | *****                                                        |
| Ta_TT8_WT  | AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCT    |
| Ta_tt8_Mut | AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTTGGCT    |
|            | *****                                                        |
| Ta_TT8_WT  | CTCCTGATGATGATGACGTCTCCAATCAAAACCTACTCTCTGATTTCATGTAGAATCAA  |
| Ta_tt8_Mut | CTCCTGATGATGATGACGTCTCCAATCAAAACCTACTCTCTGATTTCATGTAGAATCAA  |
|            | *****                                                        |
| Ta_TT8_WT  | CCCACACTTTAGGTATACACTTATACATTAAATTAGTTAACGATATCATTACAGTATCT  |
| Ta_tt8_Mut | CCCACACTTTAGGTATACACTTATACATTAAATTAGTTAACGATATCATTACAGTATCT  |
|            | *****                                                        |
| Ta_TT8_WT  | ATTTATTTTGTTAACAAGAAATTTAAAAATATTCGCCATTTCTTTGTTATGTCTAAAGAA |
| Ta_tt8_Mut | ATTTATTTTGTTAACAAGAAATTTAAAAATATTCGCCATTTCTTTGTTATGTCTAAAGAA |
|            | *****                                                        |
| Ta_TT8_WT  | AATCTATAAAATTTTATGAATAGACACACACATGGACATGATGAATCTAATGGAGGAGGG |
| Ta_tt8_Mut | AATCTATAAAATTTTATGAATAGACACACACATGGACATGATGAATCTAATGGAGGAGGG |
|            | *****                                                        |
| Ta_TT8_WT  | TGGAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTC  |
| Ta_tt8_Mut | TGGAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTTTC  |
|            | *****                                                        |
| Ta_TT8_WT  | AGATTTCAGTTTCACATCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAA |
| Ta_tt8_Mut | AGATTTCAGTTTCACATCTTCTTACATCCAATCATCATTTGCCACATGGAAGGCTGATAA |
|            | *****                                                        |
| Ta_TT8_WT  | TTTTAAAGAGCATCAGCGAGTGGAAGCTAAATCGACGTCGTCGTCGCAATGGATGCTCAA |
| Ta_tt8_Mut | TTTTAAAGAGCATCAGCGAGTGGAAGCTAAATCGACGTCGTCGTCGCAATGGATGCTCAA |
|            | *****                                                        |
| Ta_TT8_WT  | ACACATAATCTTGAGAGTTCCTTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG |
| Ta_tt8_Mut | ACACATAATCTTGAGAGTTCCTTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG |
|            | *****                                                        |
| Ta_TT8_WT  | AGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGCAGGAGAGAGAAGCTGAATGAGAGATT |
| Ta_tt8_Mut | AGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGCAGGAGAGAGAAGCTGAATGAGAGATT |
|            | *****                                                        |
| Ta_TT8_WT  | CATAAACTGAGATCATTGGTTCCCTTTGTGACCAAGATGGATAAAGTCTCAATTCTTGG  |
| Ta_tt8_Mut | CATAAACTGAGATCATTGGTTCCCTTTGTGACCAAGATGGATAAAGTCTCAATTCTTGG  |
|            | *****                                                        |
| Ta_TT8_WT  | AGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA |
| Ta_tt8_Mut | AGACACCATCAACTACGTAAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA |
|            | *****                                                        |

|            |                                                               |
|------------|---------------------------------------------------------------|
| Ta_TT8_WT  | TCACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGT  |
| Ta_tt8_Mut | TCACGAACAAAAACATAAGCGGATGCGTAGCTGTAAGGGAAAAACGTGGGAAGAGGTCGT  |
|            | *****                                                         |
| Ta_TT8_WT  | TGAGGTTTCCATCATAGAGAGTGATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGG  |
| Ta_tt8_Mut | TGAGGTTTCCATCATAGAGAGTGATGTTTTGTTAGAGATGAGATGCGAGTACCGAGATGG  |
|            | *****                                                         |
| Ta_TT8_WT  | TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA  |
| Ta_tt8_Mut | TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA  |
|            | *****                                                         |
| Ta_TT8_WT  | TACCGCGGTGAACGAGCGTGATTTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAGAA |
| Ta_tt8_Mut | TACCGCGGTGAACGAGCGTGATTTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAGAA |
|            | *****                                                         |
| Ta_TT8_WT  | ACCAAGCATTGCTGAGGTCAAAAGAGCCATCCATCAAACATATATCCAATATTAACTATA  |
| Ta_tt8_Mut | ACCAAGCATTGCTGAGGTCAAAAGAGCCATCCATCAAACATATATCCAATATTAACTATA  |
|            | *****                                                         |
| Ta_TT8_WT  | G                                                             |
| Ta_tt8_Mut | G                                                             |
|            | *                                                             |

[      ]

The top lines of the following sequence comparison are the unmodified sequence of the [      ] gene. One representative modified gene sequence of [      ] is included for comparison. One site in the gene is edited; the modified sequence includes a single T insertion. The edit is in red.

[

CBI-Deleted

CBI-Deleted

CBI-Deleted (2)

CBI-Deleted



**CBI-Deleted Copy**

**CBI-Deleted**



**CBI-Deleted Copy**

**CBI-Deleted**

**CBI-Deleted**

]

[ ]

The top lines of the following sequence comparison are the unmodified sequence of the [ ] gene. One representative modified gene sequence of [ ] is included for comparison. One site in the gene is edited; the modified sequence includes a 2 bp deletion. The edit is in red.

**CBI-Deleted****CBI-Deleted****CBI-Deleted (2)**

[

**CBI-Deleted**

**CBI-Deleted**

]

[ ]

The top lines of the following sequence comparison are the unmodified sequence of the [ ] gene. One representative modified gene sequence of [ ] is included for comparison. One site in the gene is edited; the modified sequence includes a single T insertion. The edit is in red.

[

**CBI-Deleted****CBI-Deleted****CBI-Deleted (2)****CBI-Deleted**



**CBI-Deleted Copy**

**CBI-Deleted**



CBI-Deleted Copy

CBI-Deleted