

3/26/2024

Bernadette Juarez
U.S. Department of Agriculture
APHIS Deputy Administrator
Biotechnology Regulatory Services

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By ajdrummond for BRS Document Control Officer at 2:26 pm, Mar 26, 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; [] to produce a lower glucosinolate phenotype; [] to produce a reduced-shatter phenotype; and [] to produce a lower PUFA/higher oleic acid phenotype. The first two PTMOAs are described in 22-069-01rsr. The third PTMOA is described in 22-241-01rsr. This RSR requests the evaluation of those previously submitted PTMOAs in combination with two additional PTMOAs.

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USDA has previously evaluated pennycress lines with the same phenotypes as three of the PTMO's in this RSR via the AIR process. Disruption of *TT8* and *FAE1* were reviewed in several AIR letters¹. Additionally, lines containing disruption in genes (*AOP2*, []) that are in the same biosynthesis pathway and that exhibit the same phenotype as our third trait [], were evaluated in some of these and other AIR requests². *AOP2*, []

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[] are examples of several genes that, when disrupted, reduce glucosinolate levels in pennycress. This RSR requests evaluation of pennycress with mutations in an alternate gene that results in the same low glucosinolate phenotype, []. In response to each of several previously submitted AIR letters, BRS deregulated pennycress lines harboring disruptions in *FAE1*, *TT8*, *AOP2* and other genes, concluding that: "...your gene-edited pennycress lines are not themselves plant pests". In other AIR response letters, USDA additionally stated 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 [] (22-336-02cr), [] (22-107-02cr), and [] (23-208-01cr) 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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¹ 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

² 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



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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)

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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, reduced-shatter, and higher oleic acid/lower PUFA 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, reduced-shatter, and higher oleic acid/lower PUFA 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 T₁ 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 T₂ generation will then be screened for segregants that do not have the transgene as indicated by lack of red fluorescence. Resulting seedlings in the T₂ 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
Ta_fael_Mut1

GCCTTCACCGTTTCGGTTGGCTCTCACATCGTAA-CCCGGCCCAAACCGGTTACCT
GCCTTCACCGTTTCGGTTGGCTCTCACATCGTAA A CCCGGCCCAAACCGGTTACCT

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	GGGAGAATGGATACTACAACGGTGCAATAAG-ACGAGGAAGACAACTCAGCCGGCGGAA
Ta_tt8_Mut1	GGGAGAATGGATACTACAACGGTGCAATAAG C ACGAGGAAGACAACTCAGCCGGCGGAA

[]

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

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The top line of the following sequence comparison is the unmodified sequence of []. One representative modified gene sequence of [] 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.

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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., 2018b; 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., 2018b; 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 80 µmol/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® also contains 80-110µmol/g glucosinolates derived through the aliphatic glucosinolate pathway (Chopra et al., 2018b; 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® impacts the ability to include the seed or meal in feed applications above limited inclusion rates (e.g. 4% in broiler diets) (Chopra et al., 2020).

Intended trait #4: reduced shatter seedpods

Intended phenotype #4: reduced pod dehiscence, reduced seed dispersal

Pennycress seedpods often shatter due to high winds or mechanical harvest. This premature breakage can reduce yields by more than 50% in certain environmental conditions and limits economic viability in the domestication of pennycress for use in biofuels and bioproducts. Mutant pennycress lines, with reduced [] activity may have 60-90% reduction in pod dehiscence compared to its co-grown wild type resulting in improved agronomic properties (Chopra et. al., 2020). The reduction in premature shatter and decreased loss during harvest also reduces the soil seed bank, easing management as a domesticated crop.

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Intended trait #5: lower PUFA/higher oleic acid seeds

Intended phenotype #5: reduction of polyunsaturated fatty acids (PUFAs) in seeds, increase of oleic acid in seeds

A by-product of reducing erucic acid in pennycress oil via editing of the *FAE1* gene (trait #1), is an increase in polyunsaturated fatty acids (PUFAs), in particular linolenic acid with three double bonds (18:3) and linoleic acid with two double bonds (18:2). Higher levels of PUFAs in seed oil can reduce the stability of the oil, hence reducing its shelf life. The [] gene was identified as a gene target that, if loss of function occurs, results in []

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[], but does not impact other biosynthetic pathways.

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.

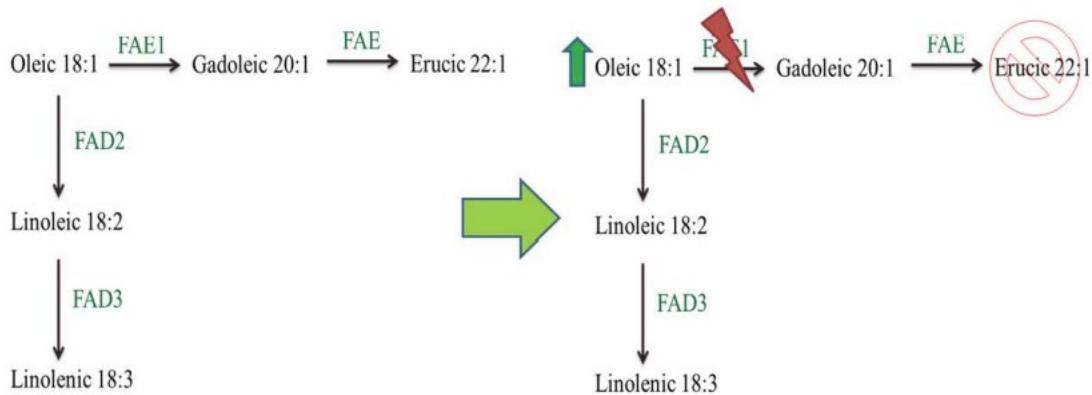


Figure 1: Fatty Acid Pathway to Reduce Erucic Acid Levels

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., 2018a). 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., 2018a). 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.

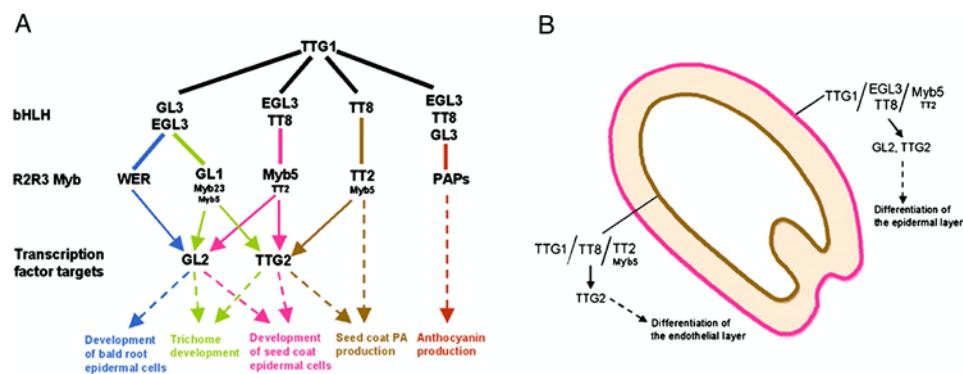


Figure 2: Models for the regulation of TTG-1 dependent pathways in seed coat development. (A) A regulatory network for the positive control of TTG1-dependent epidermal cell fates. Solid lines indicate interactions between members of a complex. Solid arrows indicate direct regulation of *GL2* or *TTG2* 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 resistance to insects and serve as defense compounds in different tissues of the plant and can help with suppressing weeds. Sønderby 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® contains 80-100 μ mol/g glucosinolates in the form of sinigrin derived through the aliphatic glucosinolate pathway (Chopra et al., 2018b; Chopra et al., 2020; Sedbrook et al., 2014).

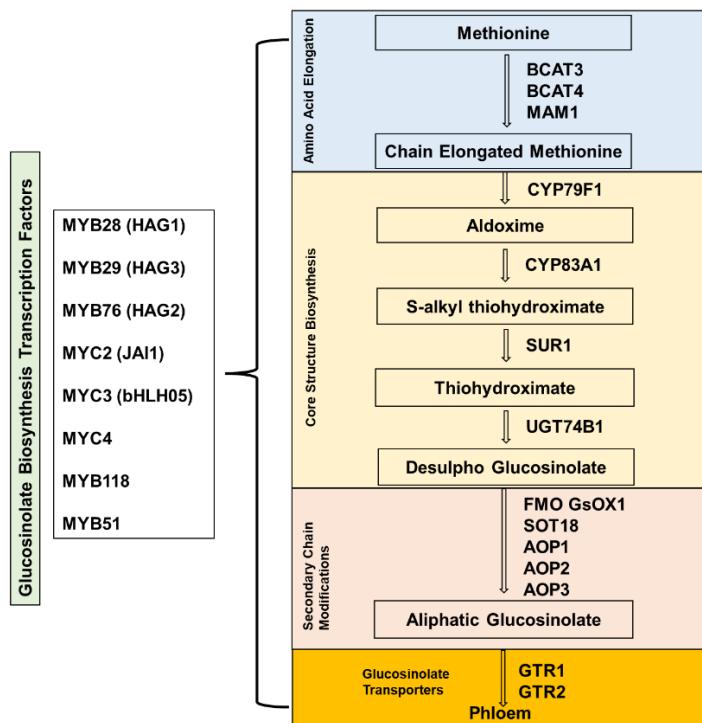


Figure 3: Major steps in glucosinolate biosynthesis

The rationale for gene target selection was to identify those genes that 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. The transcription factor [] was identified as CBI-Deleted the gene target in this RSR request.

[] is a bHLH protein that is known to interact directly with glucosinolate-related CBI-Deleted transcription factors, and it helps coordinate expression of several biosynthesis genes CBI-Deleted required to accumulate glucosinolate []. We generated alleles in CBI-Deleted pennycress to test this hypothesis and found via RT-PCR that genes involved in glucosinolate CBI-Deleted biosynthesis were reduced significantly in various stages of plant development. The CBI-Deleted representative edited allele for [] has a 1-bp insertion that causes a frameshift which CBI-Deleted results in a premature stop codon and a truncated, non-functional protein. Resulting sinigrin CBI-Deleted levels in [] pennycress mutants are reduced compared to its co-grown wild type. CBI-Deleted

Reduced Seedpod Shatter MOA

Wildtype pennycress seedpods often exhibit premature breakage by wind or during mechanical harvest. In the domestication of pennycress, improvements are necessary as this premature seedpod shatter can result in yield losses of over 50%. Mutants were identified with mutations in the pennycress candidate ortholog of the *Arabidopsis* [] gene. The seedpod contains a separation layer of cells flanking the septum (also called a replum) which divides the seedpod into two halves []. [] encodes a [] regulating the formation of this separation layer, mediating pod breakage, and mutations in this gene generated mutants with a varying reduction in breakage of the dehiscence zones. The representative edited allele for [] has a 1-bp insertion that causes a frameshift resulting in a truncated, non-functional protein. [] pennycress mutants may have 60-90% reduction in pod dehiscence, compared to its co-grown wild type, reducing seed dispersal and yield loss (Chopra et al., 2020).

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Figure 4: An intact seedpod compared to a broken seedpod with released seeds (both are wild-type). The arrows show the septum of an intact pod (left) and the septum structure that remains after pod shatter. The arrows highlight the septum remaining after pod shatter. (Chopra et al., 2020)

Higher Oleic Acid/Lower PUFAs MOA

Pennycress *FAE1* mutants containing the desired decrease in erucic acid also contain a desirable elevation in oleic acid and an accumulation of higher levels of polyunsaturated fatty acids (PUFAs), in particular linolenic acid with three double bonds (18:3) and linoleic acid with two double bonds (18:2). Although linolenic acid, belonging to the omega3 class of fatty acids, is associated with reduced heart disease, reduced bone fracture risk and reduced childhood obesity (Perng et al., 2014; Rajaram, 2014), for many applications, PUFAs are not desirable. The extra bonds reduce the stability of the oil, shortening the shelf life of products containing the oil (Gordon, 1990).

A reduction in PUFA levels can be achieved by disrupting the activity of the [] gene, resulting in higher levels of oleic acid []

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], modulates polyunsaturated fatty acid (PUFA) content by interconverting PC and DAG, transferring 18:1 into PC for desaturation, and 18:2 and 18:3 into the triacylglycerol (TAG) biosynthetic pathway thereby increasing PUFA content in TAG (Figure 5). An edit to [] in pennycress may result in a frameshift leading to a premature stop codon and a truncated, non-functional protein. The seeds of [] pennycress edits may have a []

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[], compared to its co-grown wild type, resulting in improved product quality and stability.

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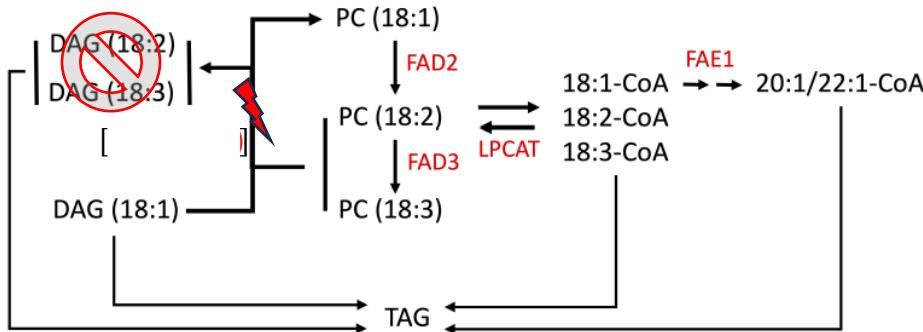


Figure 5: Simplified scheme for fatty acid modification in Brassicas illustrating reduction of polyunsaturated fatty acids via interruption of [] activity.

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In conclusion, CoverCress™ is currently being developed as a new rotational oilseed crop that has the benefits of a cover crop that could be grown between the fall harvest and spring establishment of traditional crops in temperate regions. Edits and/or mutations to *FAE1*, *TT8*, [] genes have resulted in the low erucic acid, lower fiber, reduced total glucosinolates, reduced shatter, and lower PUFAs/higher oleic acid seed traits, respectively. The mechanism of action for the genetic changes leading to each phenotype is well-understood and has a history of publication. Based on this information, we request USDA's review of the combination of these previously reviewed and new PTMOAs and confirmation that the traits, produced through simultaneous or sequential editing of any combination of *FAE1*, *TT8*, [] genes in pennycress, are not subject to regulation under Part 340.

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References

- Abraham, V., Bhatia, C. R., (1986). Development of Strains with Yellow Seedcoat in Indian Mustard (*Brassica juncea* Czern. & Coss.). *Plant Breeding*, 97, 86–88.
- Al-Shehbaz, I. A. (1986). The genera of Lepidieae (Cruciferae; Brassicaceae) in the southeastern United States. *J. Arnold Arbor.*, Harv. Univ., 67, 265–311.
- Altendorf, K., Isbell, T., Wyse, D. L., Anderson, J. A. (2019). Significant variation for seed oil content, fatty acid profile, and seed weight in natural populations of field pennycress (*Thlaspi arvense* L.). *Industrial crops and products*, 129, 261-268. DOI:10.1016/j.indcrop.2018.11.054
- Baudry, A., Heim, M. A., Dubreucq, B., Caboche, M., Weisshaar, B., Lepiniec, L. (2004). *TT2, TT8, and TTG1* synergistically specify the expression of *BANYULS* and proanthocyanidin biosynthesis in *Arabidopsis thaliana*. *Plant J.*, 39, 366-380.
- Best, K. F., & McIntyre, G. I. (1975). The Biology of Canadian Weeds. *Can. J. Plant Sci.*, 55, 279–292.
- Chopra, R., Johnson, E. B., Daniels, E., McGinn, M., Dorn, K. M., Esfahanian, M., Folstad, N., Amundson, K., Altendorf, K., Betts, K., Frels, K., Anderson, J. A., Wyse, D. L., Sedbrook, J. C., Marks, M. D. (2018a). Translational genomics using *Arabidopsis* as a model enables the characterization of pennycress genes through forward and reverse genetics. *Plant J.*, Dec, 96(6), 1093-1105. DOI:10.1111/tpj.14147. PMID: 30394623
- Chopra, R., Folstad, N., Lyons, J., Ulmasov, T., Gallaher, C., Sullivan, L., McGovern, A., Mitacek, R., Frels, K., Altendorf, K., Killam, A., Ismail, B., Anderson, J. A., Wyse, D. L., Marks, M. D. (2018b). The adaptable use of *Brassica* NIRS calibration equations to identify pennycress variants to facilitate the rapid domestication of a new winter oilseed crop. *Industrial crops and products*, 128, 55-61. DOI:10.1016/j.indcrop.2018.10.079
- Chopra, R., Johnson, E. B., Emenecker, R., Cahoon, E. B., Lyons, J., Kliebenstein, D. J., Daniels, E., Dorn, K. M., Esfahanian, M., Folstad, N., Frels, K., McGinn, M., Ott, M., Gallaher, C., Altendorf, K., Berroyer, A., Ismail, B., Anderson, J. A., Wyse, D. L., Marks, M. D. (2020). Identification and stacking of crucial traits required for the domestication of pennycress. *Nature Food*, 1(1), 84-91. DOI:10.1038/s43016-019-0007-z
- Debeaujon, B. P., Stupar, R. M., Gingerich, D. J., Vierstra, R. D. (2003). Proanthocyanidin-accumulating cells in *Arabidopsis* testa: regulation of differentiation and role in seed development. *Plant Cell*, 15, 2514-2531.

Gonzalez, A., Mendenhall, J., Huo, Y., Lloyd, M. (2009). TTG1 complex MYBs, MYB5 and TT2, control outer seed coat differentiation. *Dev. Biol.*, 325, 412-421.

Gordon, M. H. (1990). *Food Antioxidants. Elsevier Applied Food Science Series* (ed. Hudson, B.J.F.) Ch. 1.

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Koirala, N., Barker, D. J., Gesch, R. W., Heller, N. J., Hard, A. W., Wells, S. S., Phippen, W. B., Lindsey, A. J. (2023). Seed pelleting and storage effects on germination of Pennycress (*Thlaspi arvense* L.). *Crop Science*, 1–12. DOI:10.1002/csc2.21077

Lepiniec, L., Debeaujon, I., Routaboul, J., Baudry, A., Pourcel, L., Nesi, N., Caboche, M. (2006). Genetics and biochemistry of seed flavonoids. *Annu. Rev. Plant Biol.*, 57, 405-430.

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McGinn, M., Phippen, W. B., Chopra, R., Bansal, S., Jarvis, B. A., Phippen, M. E., Dorn, K. M., Esfahanian, M., Nazarenus, T. J., Cahoon, E. B., Durrett, T. P., Marks, M. D., Sedbrook, J. C. (2019). Molecular tools enabling pennycress (*Thlaspi arvense*) as a model plant and oilseed cash cover crop. *Plant Biotechnol J.*, Apr 17(4), 776-788. DOI:10.1111/pbi.13014. Epub 2018 Oct 25. PMID: 30230695; PMCID: PMC6419581

Moser, B. R., Shah, S. N., Winkler-Moser, J. K., Vaughn, S. F., Evangelista, R. L. (2009). Composition and physical properties of cress (*Lepidium sativum* L.) and field pennycress (*Thlaspi arvense* L.) oils. *Industrial Crops and products*, Sep 30 (2), 199-205. DOI:10.1016/j.indcrop.2009.03.007

Ott, M. A., Gardner, G., Rai, K. M., Wyse, D. L., Marks, M. D., Chopra, R. (2021). TRANSPARENT TESTA 2 allele confers major reduction in pennycress (*Thlaspi arvense* L.) seed dormancy. *Industrial Crops and Products*, Volume 174.

Perng, W., Villamor, E., Mora-Plazas, M., Marin, C. & Baylin, A. (2014). Alphalinolenic acid (ALA) is inversely related to development of adiposity in school-age children. *Eur. J. Clin. Nutr.*, 69, 167–172.

Prieto, M. A., López, C. J., Simal-Gandara, J. (2019). Glucosinolates: Molecular structure, breakdown, genetic, bioavailability, properties and healthy and adverse effects. *Adv Food Nutr Res.*, 90, 305-350. DOI: 10.1016/bs.afnr.2019.02.008. Epub Mar 25. PMID: 31445598

Qiu, D., Morgan, C., Shi, J., Long, Y., Liu, J., Li, R., Zhuang, X., Wang, Y., Tan, X., Dietrich, E., Weihmann, T., Everett, C., Vanstraelen, S., Beckett, P., Fraser, F., Trick, M., Barnes, S., Wilmer, J., Schmidt, R., Li, J., Bancroft, I. (2006). A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. *Theoretical and applied genetics*, 114(1), 67–80. DOI:10.1007/s00122-006-0411-2

Rajaram, S. (2014). Health benefits of plant-derived α -linolenic acid. *Am. J. Clin. Nutr.*, 100, 443S–448S.

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Sedbrook, J. C., Phippen, W. B., Marks, M. D. (2014). New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: example pennycress (*Thlaspi arvense* L.). *Plant Sci.* 227, 122–132.

Simbaya, J., Slominski, B. A., Rakow, G., Campbell, L. D., Downey, R. K., Bell, J. M. (1995). Quality Characteristics of Yellow-Seeded Brassica Seed Meals: Protein, Carbohydrate, and Dietary Fiber Components. *Journal of Agricultural and Food Chemistry*, 43 (8), 2062-2066.

Slominski, B. A., Simbaya, J., Campbell, L. D., Guenter, W. (1994). Carbohydrates and dietary fiber components of yellow and brown-seeded canola. *J. Agric. Food Chem.*, 42, 704–707.

Slominski, B. A., Simbaya, J., Campbell, L. D., Rakow, G., Guenter, W. (1999). Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. *Animal Feed Science and Technology*, 78, 249-262.

Sønderby I. E., Geu-Flores, F., Halkier, B. A. (2010). Biosynthesis of glucosinolates – gene discovery and beyond, *Trends in Plant Science*, 15(5), 283-290, DOI:10.1016/j.tplants.2010.02.005



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Ulmasov, T., Hartnell, G., Sedbrook, J. C., Marks, M. D., Chopra, R., Esfahanian, M. (2020). Low fiber pennycress meal and methods of making (U.S. Patent No. 10,709,151). U.S. Patent and Trademark Office.

Warwick, S. I., Francis, A., & Susko, D. J. (2002). The biology of Canadian weeds. 9. *Thlaspi arvense* L. (updated). *Canadian journal of plant science*, 82(4), 803-823.

Appendix 1 – Full Sequences and Sequence Comparisons

Unmodified and Modified Sequences of *FAE1*, *TT8*, []

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

ATGACGTCCGTTAACGTTAACGCTCCTTACCATTACGTACCATCACCAACTTTAACCTTGCTTCTCCCG
TTAGCGCGATCGTTGCCGGAAAAGCCTCTCGGCTTACCAACAAACGATCTTCACCACTTCTACTATTCCA
TCTCCAACACAACCTAATAACCATACTCTACTCTTGCCTCACCGTTTGGTT **TGGCTCTACATCGT**
AACCCGGCCCAAACCGTTACCTCGTTGACCATTCCGCTACCTTCCACCATCGCATTTAGAAGCAGTA
TCTCTAAGGTATGGATATCTTCTATCAAGTAAGATTAGCCGATCCTTACGGAACGCGGAAGCGATGA
TTCGTCTGGCTGATTCTTGAGGAAGATTAGGGAGCGGGCTGGTCTAGGCGATGAAACCCACGGCC
CGAGGGACTGCTTCAGGTCCTCCACGGAAGACTTTGCCGCGCGTGAAGAAACAGAGCAAGTGA
TCATCGGTGCGCTCGAAAAACTATTGAGAACACCAAAGTTAACCTAAAGAGATTGGTATACTTGTGG
TGAACACTCAAGCATGTTAACCGACTCCTCGCTCGGCGATGGTTGTTAACACTTCAAGCTCCGAAGC
AACATCAGAAGCTTAATCTGGAGGAATGGGTTGAGTGCCTGGCGTTAGCCATTGATCTGGCTAAG
GACTTGTGCATGTCCATAAAACACTTATGCTCTGTGGTAGCAGAGAACATCACTAACACATT
ATGCTGGTATAACAGATCCATGATGGTTGAATTGCTTCCGTTGGTGGGGCCGATTGCT
CTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCGACTTCACACGGTCGGACGCATACCG
GAGCTGACGACAAGTCTTCCGATGTGTGCAACAAAGAACGACGAGAGCGTAAACCGGGGTGTG
TTGTCAGGACATAACCGGTGTTGCCGGAGAACTGTTAGAAAACATAAACACATTGGTCCGTTG
GTTCTCCTTTAGCGAGAAATTCTTCTCGTTACCTCATGCCAACAAACTCTTAAAGACAAGATC
AAACATTACTACGTCCCGATTCAAGCTGCTATCGACCATTGTTATTGATGCCGGAGGCAGAGCCG
TGATCGATGTGCTACAGAAGAACTTAGGTCTATTGCCATCGATGTGGAGGCATCTAGGTCAACGTTAC
ATAGATTGGAACACTCGCTAGCTAATTGGTATGAATTGGCGTACATAGAGGAAAAGGAAGGA
TGAAGAGAGGGAACAAAGTTGGCAGATTGCTTAGGGTTAACGTGAAATAGTGCAGGTTGG
GTGGCTCTACGCAATGTCAAGGCTCGACAAATAGTCCTGGAACATTGCATTGATAGATATCCAGAT
GCAATTGATTCTGATTGGTAAGTCAGAGACTCGTCCAAAACGGTCGGTCTAA

FAE1 Modified Sequence, Representative Sequence*Ta fae1* Mutant1 CDS:

ATGACGTCCGTTAACGTTAACGCTCCTTACCATTACGTACCATCACCAACTTTAACCTTGCTTCTCCCG
TTAGCGCGATCGTTGCCGGAAAAGCCTCTCGGCTTACCAACAAACGATCTTCACCACTTCTACTATTCCA
TCTCCAACACAACCTAATAACCATACTCTACTCTTGCCTCACCGTTTGGTT **TGGCTCTACATCGT**
AA(A)CCCGCCCAAACCGTTACCTCGTTGACCATTCCGCTACCTTCCACCATCGCATTTAGAAGCA
GTATCTCTAAGGTATGGATATCTTCTATCAAGTAAGATTAGCCGATCCTTACGGAACGCGGAAGCGA
TGATTGTCCTGGCTGATTCTTGAGGAAGATTAGGGAGCGGTCTGGTCTAGGCGATGAAACCCACGG
CCCCGAGGGACTGCTTCAGGTCCTCCACGGAAGACTTTGCCGCGCGTGAAGAAACAGAGCAAG
TGATCATCGGTGCGCTCGAAAAACTATTGAGAACACCAAAGTTAACCTAAAGAGATTGGTATACTTGT
GGTGAACCTCAAGCATGTTAACCGACTCCTCGCTCGGCGATGGTTAACACTTCAAGCTCCGA
AGCAACATCAGAAGCTTAATCTGGAGGAATGGGTTGAGTGCCTGGCGTTAGCCATTGATCTGGCT
AAGGACTTGGTGCATGTCCATAAAACACTTATGCTCTGTGGTAGCAGAGAACATCACTAACACA
TTATGCTGGTATAACAGATCCATGATGGTTGAAATTGCTTGGTGGTGGGGCCGCGATT



GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCTCAGCTACTTCACACGGTTCGGACGCATAC
CGGAGCTGACGACAAGTCTTCCGATGTGTGCAACAAGAAGACGACGAGAGCGGTAACCCGGGGTGT
GTTTGTCCAAGGACATAACC GGTTGCCGGGAGAACTGTTAGAAAAACATAACAAACATTGGGTCCGT
TGGTTCTCCTTTAGCGAGAAATTCTTTTGTTGTTACCTTCATGCCAAGAAACTCTTAAAGACAAGA
TCAAACATTACTACGTCCCGGATTCAAGCTTGCTATGCCACATTGTATTGATGCCGGAGGCAGAGC
CGTGATCGATGTGCTACAGAAGAACCTAGGTCTATTGCCGATCGATGTGGAGGCATCTAGGTCAACGTT
ACATAGATTGGGAACACTCGTCTAGCTCAATTGGTATGAATTGGCGTACATAGAGGCAAAAGGAAG
GATGAAGAGAGGGAACAAAGTTGGCAGATTGCTTAGGGTCAGGGTTAAGTGTAAATAGTGCGGTT
GGGTGGCTTACGCAATGTCAAGGCTCGACAAATAGTCCTGGAACATTGCATTGATAGATATCCAG
ATGCAATTGATTCTGATTGGGTAAGTCAGAGACTCGTGTCCAAAACGGTCGGTCCTAA

TT8 Wild Type, Unmodified Sequence

Ta TT8 WT CDS:

CACTAAAGAAAAGAGGCTGCCCGAGAAGAGCTTAATCACGTGGTGCAGAGCGCCGAGGAGAGAG
AAGCTGAATGAGAGATTATAACACTGAGATCATTGGTCCCTTGACCAAGATGGATAAAAGTCTCA
ATTCTGGAGACACCATCAACTACGTAACCCTTCGAATAGGGCCAAGAGCTGGAGACTAACATC
ACGAACAAAAACATAAGCGGATGCGTAGCTGAAGGGAAAACGTGGGAAGAGGTCGTTGAGGTTCC
ATCATAGAGAGTGATGTTTGTAGAGATGAGATGCGAGTACCGAGATGGCTATTGCTGACATCCTC
AGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTACCGCGGTGAACGAGCGTGATTGAGG
CCGAGATAAGGGCTATGGTGAGAGGGAAAGAACCAAGCATTGCTGAGGTCAAAAGAGGCCATCCATCAA
ACTATATCCAATATTAAACTATAG

Tt8 Modified Sequence, Representative Sequence

Ta tt8 Mutant1 CDS:

ATGGATGAATCAAGTATTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTCAAGGGCT
GCTTAAGGCGGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTTCTGGCAACTTGTCTCAACAAAGg
ttcttttttttaataaaattcatcgatctcacaataaaaaccctaaatttatcatattattatattatgttaactacataattatcg
tattttaccgtccatgtgcattttgttcattctgtcatatttacttgaggctcagactccggagcacatctctcggtgtctcgaat
ctgtgagactttcgattttggacttctgtcaattgagttactgaagaattatatgtttaatgaatttagGGTTTGCTGTG
GGAGAATGGATACTACAACGGTGCAATAAAG(G)ACGAGGAAGACAACCTAGCCGGCGGAAGTGACGG
CGGAAGAGGGCTGCGTTAGAGAGGGAGTCAGCAGCTAAGGGAACCTTACGAGGCCCTTGGCCGGAGA
GTCCTCATCGGAAGCTAGGGCATGCACGGCATTATGCCGGAGGACTGACGGAGACTGAATGGTTTA
TCTAATGTGTGTCCTCTTCTCTTCCCTCCTCCGGtaccaactctctctctctctctctctctctct
tctctctctctctttgtctatactgaagttctaatttatctttatcatctctactgaagacaaaaatagtattgtgttaatgcga
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ccattttgtgaacataattggaccgttggagattcttattttgtctgatttctaaatgtttagcataaaatagataataatcataatg
cataacaaattgttttagttatggatagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttag
tttagGATGCCAGGAAAGCGTATGCGAGGAGGAAACACGTATGGCTATGTTGCAATGAGGTTGA
CAGTAAAATCTTCTAGGGCTATTCTGCAAAGGtctatttttttttcttaccactactctatgcatttttttct
atttatatatctatcttcaaattaattttctgtcttattttctggatgtcttctacatcggtcggttcttaccactactctatgcatttttttct
CAAAATCCAGttaacgtgtcttattgtttagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttag
aaaaaaagCAGACAGTGGTTGCATTCCATGCTTGATGGCGTTGGAACTAGGCACAACGAACAAAGgt
acggcgtagttatctttatattgtcataaccatggtaagaaaaaggttagaagagaaaaatagatcatgctttagttatgtttagttatgtttag
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tcataatataaaaattgtttggagatgtacataattctcacaataaaaaataacaaaggatgattaaggaaaggatgtttag
acatgtttagttctgtgtgtgaagGTAAGAAGATATGCGTTGAGCTATAAGAGTTTCCATAA
CCACCCCAAGTCAAACCCAAAGCTGCTTCTGAACACTCCATCAACGAAGAGCACGAAGAACAGCA
AGAACAAAGAAGAAGAAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTT
GGCTCTCCTGATGATGACGTCTCCAATCAAAACCTACTCTGATTCCATGTAGAATCAACCCACAC
TTTAGgtataacttatacattaaattgtttagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttagttatgtttag
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GTGGAAACTATTCTCAGACAGTATCAACACTTCTTATGTACAACACCACGAGTCTTTTCAGATTGAGTT
TCCACATCTTCTTACATCCAATCATCATTGCCACATGGAAGGCTGATAATTAAAGAGCATCAGCGAGT
GGAAACTAAATCGACGTCGTCGCAATGGATGCTAAACACATAATCTTGAGAGTTCCCTTACTCCAC
GACCACACTAAAGAAAAGAGGCTGCCCGAGAAGAGCTTAATCACGTGGTGGCAGAGCGCCGAGGA
GAGAGAAGCTGAATGAGAGATTATAACACTGAGATCATTGGTCCCTTGTGACCAAGATGGATAAAG



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TCTCAATTCTGGAGACACCATCAACTACGTAAACCATCTCGAAATAGGGTCCAAGAGCTGGAGACTAA
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GTTTCATCATAGAGAGTGATGTTAGAGATGAGATGCGAGTACCGAGATGGTCTATTGCTCGAC
ATCCTTCAGGTTCTAAGGAACATGGTATAGAGACTACTGCAGTTCATACCGCGGTGAACGAGCGTGAT
TTCGAGGCCGAGATAAGGGCTATGGTGAGAGGAAAGAACCAAGCATTGCTGAGGTCAAAGAGCCA
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[] Wild Type, Unmodified Sequence

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Ta [] WT CDS:

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[

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]

[] Modified Sequence, Representative Sequence

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Ta [] Mutant CDS:

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[

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[] Wild Type, Unmodified Sequence

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Ta [] WT CDS:

CBI-Deleted

[

CBI-Deleted

]

[] Modified Sequence, Representative Sequence

CBI-Deleted

Ta [] Mutant1 CDS:

CBI-Deleted

[

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[] Wild Type, Unmodified Sequence

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Ta [] WT CDS:

CBI-Deleted

[

CBI-Deleted

]

[] Modified Sequence, Representative Sequence

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Ta [] Mutant1 CDS:

CBI-Deleted

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Sequence Comparisons of the unmodified and modified genes
FAE1

The top lines of the following sequence comparison are the unmodified sequence of *FAE1*. One representative modified gene sequence of *FAE1* is included for comparison (*Ta_fae1_Mut1*). One site in the gene is edited and includes a single A insertion, highlighted in red.

Ta_FAE1	ATGACGTCCGTTAACGTTAACGTTAACGTCATCACCAACTTTCAACCTT
Ta_fae1_Mut1	ATGACGTCCGTTAACGTTAACGTCATCACCAACTTTCAACCTT *****
Ta_FAE1	TGCTCTTCCCGTTAGCGCGATCGTGCAGAAAAGCCTCTCGGCTTACACAAACGAT
Ta_fae1_Mut1	TGCTCTTCCCGTTAGCGCGATCGTGCAGAAAAGCCTCTCGGCTTACACAAACGAT *****
Ta_FAE1	CTTCACCACCTCTACTATCCATCTCAACACAACCTAATAACCATACTCTACTCTT
Ta_fae1_Mut1	CTTCACCACCTCTACTATCCATCTCAACACAACCTAATAACCATACTCTACTCTT *****
Ta_FAE1	GCCTTCACCGTTTCGGTTGGCTCTACATCGTAA-CCCGGCCAAACCGGTTACCT
Ta_fae1_Mut1	GCCTTCACCGTTTCGGTTGGCTCTACATCGTAA <ins>A</ins> CCGGGCCAAACCGGTTACCT *****
Ta_FAE1	CGTTGACCATTCCCTGCTACCTTCCACCATCGCATCTAGAAGCAGTATCTCTAAGGTCA
Ta_fae1_Mut1	CGTTGACCATTCCCTGCTACCTTCCACCATCGCATCTAGAAGCAGTATCTCTAAGGTCA *****
Ta_FAE1	GGATATCTTCTATCAAGTAAGATTAGCCGATCCTTACGGAACCGGGCAAGCGATGATT
Ta_fae1_Mut1	GGATATCTTCTATCAAGTAAGATTAGCCGATCCTTACGGAACCGGGCAAGCGATGATT *****
Ta_FAE1	GTCCTGGCTTGATTCTGAGGAAGATTCAGGAGCGGTCTGGCTAGGCGATGAAACCCA
Ta_fae1_Mut1	GTCCTGGCTTGATTCTGAGGAAGATTCAGGAGCGGTCTGGCTAGGCGATGAAACCCA *****
Ta_FAE1	CGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAAGACTTTGCCGGCGCGTGAAGA
Ta_fae1_Mut1	CGGCCCCGAGGGACTGCTTCAGGTCCCTCCACGGAAAGACTTTGCCGGCGCGTGAAGA *****
Ta_FAE1	AACAGAGCAAGTGATCATCGGTGCGCTCGAAAAACTATTCGAGAACACCAAAGTTAACCC
Ta_fae1_Mut1	AACAGAGCAAGTGATCATCGGTGCGCTCGAAAAACTATTCGAGAACACCAAAGTTAACCC *****
Ta_FAE1	TAAAGAGAGTTGGTATACTTGTGGTGAACCTAAGCATGTTAATCCGACTCCTTCGCTCTC
Ta_fae1_Mut1	TAAAGAGAGTTGGTATACTTGTGGTGAACCTAAGCATGTTAATCCGACTCCTTCGCTCTC *****
Ta_FAE1	GGCGATGGTTGTTAACTTCAAGCTCCGAAGCAACATCAGAAGCTTAAATCTTGGAGG
Ta_fae1_Mut1	GGCGATGGTTGTTAACTTCAAGCTCCGAAGCAACATCAGAAGCTTAAATCTTGGAGG *****
Ta_FAE1	AATGGGTTGTAGTGCCGGCGTTATAGCCATTGATCTGGCTAAGGACTTGTGCATGTCCA
Ta_fae1_Mut1	AATGGGTTGTAGTGCCGGCGTTATAGCCATTGATCTGGCTAAGGACTTGTGCATGTCCA *****



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Ta_FAE1
Ta_fae1_Mut1

TAAAAAACACTTATGCTCTTGTGGTGAGCACAGAGAACATCACTTACAACATTATGCTGG
TAAAAAACACTTATGCTCTTGTGGTGAGCACAGAGAACATCACTTACAACATTATGCTGG

Ta_FAE1
Ta_fae1_Mut1

TGATAACAGATCCATGATGGTTCGAATTGCTTGTCCGTGTTGGTGGGGCCGCGATTT
TGATAACAGATCCATGATGGTTCGAATTGCTTGTCCGTGTTGGTGGGGCCGCGATTT

Ta_FAE1
Ta_fae1_Mut1

GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCACTTACACCGGTTCG
GCTCTCCAACAAGCCGAGGGACCGGAGACGGTCCAAGTACCACTTACACCGGTTCG

Ta_FAE1
Ta_fae1_Mut1

GACGCATACCGGAGCTGACGACAAGTCTTCCGATGTGTGCAACAAGAACGACGAGAG
GACGCATACCGGAGCTGACGACAAGTCTTCCGATGTGTGCAACAAGAACGACGAGAG

Ta_FAE1
Ta_fae1_Mut1

CGGTAAAACGGGGTGTGTTGTCCAAGGACATAACCGGTGTTGCCGGAGAACTGTTCA
CGGTAAAACGGGGTGTGTTGTCCAAGGACATAACCGGTGTTGCCGGAGAACTGTTCA

Ta_FAE1
Ta_fae1_Mut1

GAAAAACATAAACACATTGGTCCGTTGGTCTTCCTTTAGCGAGAAATTCTTTTT
GAAAAACATAAACACATTGGTCCGTTGGTCTTCCTTTAGCGAGAAATTCTTTTT

Ta_FAE1
Ta_fae1_Mut1

CGTTACCTTCATCGCCAAGAAACTCTTAAAGACAAGATCAAACATTACTACGTCCCAGGA
CGTTACCTTCATCGCCAAGAAACTCTTAAAGACAAGATCAAACATTACTACGTCCCAGGA

Ta_FAE1
Ta_fae1_Mut1

TTTCAGAGCTTGTATCGACCATTGGTATTGATTGATCGATGCCGGAGGCAGAGCCGTGATCGATGT
TTTCAGAGCTTGTATCGACCATTGGTATTGATTGATCGATGCCGGAGGCAGAGCCGTGATCGATGT

Ta_FAE1
Ta_fae1_Mut1

GCTACAGAAGAACTTAGGCTATTGCCATCGATGTGGAGGCATCTAGGTCAACGTTACA
GCTACAGAAGAACTTAGGCTATTGCCATCGATGTGGAGGCATCTAGGTCAACGTTACA

Ta_FAE1
Ta_fae1_Mut1

TAGATTGGAAACACTCGCTAGCTCAATTGGTATGAATTGGCGTACATAGAGGCAA
TAGATTGGAAACACTCGCTAGCTCAATTGGTATGAATTGGCGTACATAGAGGCAA

Ta_FAE1
Ta_fae1_Mut1

AGGAAGGATGAAGAGAGGAAACAAAGTTGGCAGATTGCTTAGGGTCAGGGTTAACGT
AGGAAGGATGAAGAGAGGAAACAAAGTTGGCAGATTGCTTAGGGTCAGGGTTAACGT

Ta_FAE1
Ta_fae1_Mut1

TAATAGTCGGTTGGTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGCCTTGGGA
TAATAGTCGGTTGGTGGCTCTACGCAATGTCAAGGCTTCGACAAATAGCCTTGGGA

Ta_FAE1
Ta_fae1_Mut1

ACATTGCATTGATAGATATCCAGATGCAATTGATTGATTGATTCGGTAAGTCAGAGACTCG
ACATTGCATTGATAGATATCCAGATGCAATTGATTGATTGATTCGGTAAGTCAGAGACTCG

Ta_FAE1
Ta_fae1_Mut1

TGTCCAAAACGGTCGGTCCTAA
TGTCCAAAACGGTCGGTCCTAA

TT8

The top lines of the following sequence comparison are 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 (Ta_tt8_Mut1). The edits are highlighted in red.

Ta_TT8_Wt	ATGGATGAATCAAGTATTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT
Ta_tt8_Mut1	ATGGATGAATCAAGTATTTTACGGCAGAGAAAGTGATCGGAGCTGAGAAAAGAGAGCTT *****
Ta_TT8_Wt	CAAGGGCTGCTTAAGGC GGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTGGCAA
Ta_tt8_Mut1	CAAGGGCTGCTTAAGGC GGCGGTGCAATCTGTGGAGTGGACTTATAGTCTCTGGCAA *****
Ta_TT8_Wt	CTTTG C CTCAACAAAGGTTCTTTTTTTAATAAAATTTCATCGATCTCACAATA
Ta_tt8_Mut1	CTTTG C CTCAACAAAGGTTCTTTTTTTAATAAAATTTCATCGATCTCACAATA *****
Ta_TT8_Wt	AAAAC CCTAA TTTATATCATTTATTATATGTTAAC TACATAATTATCAGTATTT
Ta_tt8_Mut1	AAAAC CCTAA TTTATATCATTTATTATATGTTAAC TACATAATTATCAGTATTT *****
Ta_TT8_Wt	TAACCGTCCATGTGCTTATTGGTCCATTCTGCTCATATTTACTTGAGGTTCAGA
Ta_tt8_Mut1	TAACCGTCCATGTGCTTATTGGTCCATTCTGCTCATATTTACTTGAGGTTCAGA *****
Ta_TT8_Wt	CTGCCGAGCACATCTCTCGTTGTCTCGAACATCTGTGAGACTTTCGTTATTGGCACTT
Ta_tt8_Mut1	CTGCCGAGCACATCTCTCGTTGTCTCGAACATCTGTGAGACTTTCGTTATTGGCACTT *****
Ta_TT8_Wt	CTGTGTCAATTGAGTTACTGAAGTAATTATATGTTAAATGAATTAGGGTTTGCTGT
Ta_tt8_Mut1	CTGTGTCAATTGAGTTACTGAAGTAATTATATGTTAAATGAATTAGGGTTTGCTGT *****
Ta_TT8_Wt	GGGAGAATGGATACTACAACGGTGC AATAAAG-ACGAGGAAGACAACTCAGCCGGCGGA
Ta_tt8_Mut1	GGGAGAATGGATACTACAACGGTGC AATAAAG CAC GAGGAAGACAACTCAGCCGGCGGA *****
Ta_TT8_Wt	GTGACGGCGGAAGAGGCTCGTTAGAGAGGGAGTCAGCAGCTAAGGGACTTTACGAGGCC
Ta_tt8_Mut1	GTGACGGCGGAAGAGGCTCGTTAGAGAGGGAGTCAGCAGCTAAGGGACTTTACGAGGCC *****
Ta_TT8_Wt	CTTTGGCCGGAGAGTCCTCATCGAACGCTAGGGCATGCACGGCATTATGCCGGAGGAT
Ta_tt8_Mut1	CTTTGGCCGGAGAGTCCTCATCGAACGCTAGGGCATGCACGGCATTATGCCGGAGGAT *****
Ta_TT8_Wt	CTGACGGAGACTGAATGGTTTATCTAATGTGTCTTTCTCTTCCCTCCTCCTCC
Ta_tt8_Mut1	CTGACGGAGACTGAATGGTTTATCTAATGTGTCTTTCTCTTCCCTCCTCCTCC *****
Ta_TT8_Wt	GGGTACCCA ACT
Ta_tt8_Mut1	GGGTACCCA ACT *****
Ta_TT8_Wt	CTCTTTGTCTACTGAAGTTCTTAATTATCTTTATCATCTCCTACTGAAGACAA
Ta_tt8_Mut1	CTCTTTGTCTACTGAAGTTCTTAATTATCTTTATCATCTCCTACTGAAGACAA *****



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Ta_TT8_Wt	AAATAGTATTGTGTTAAATGCGAATCACGAATATTGTGGAAGCATTAAAAACAAACTG
Ta_tt8_Mut1	*****
Ta_TT8_Wt	AGGAGGTTGAGTTACTGAAAGAAGAAATGTATTGGAGTTGATGAAACGTACACTCCATT
Ta_tt8_Mut1	AGGAGGTTGAGTTACTGAAAGAAGAAATGTATTGGAGTTGATGAAACGTACACTCCATT
Ta_TT8_Wt	*****
Ta_tt8_Mut1	TAGTGAAACATAATTGGACC GTT GAG ATT CT TATT TTTT GCT GATT GATT CT AA AG TA
Ta_TT8_Wt	TAGTGAAACATAATTGGACC GTT GAG ATT CT TATT TTTT GCT GATT GATT CT AA AG TA
Ta_tt8_Mut1	*****
Ta_TT8_Wt	GAAGCATAAAATAGATAAACATAAAATGCATAACAAATTGTTAGTTATGGGTATAGTTA
Ta_tt8_Mut1	GAAGCATAAAATAGATAAACATAAAATGCATAACAAATTGTTAGTTATGGGTATAGTTA
Ta_TT8_Wt	*****
Ta_tt8_Mut1	ATGCTTTCTCATGAGAGAAAAAAAAAAATATATAAATGTGGAAGTAATAATT TT
Ta_TT8_Wt	ATGCTTTCTCATGAGAGAAAAAAAAAAATATATAAATGTGGAAGTAATAATT TT
Ta_tt8_Mut1	*****
Ta_TT8_Wt	GTAGGATGCCAGGAAAGGCGTATCGGAGGAGGAAACACGTATGGCTATGTGGTCAAATG
Ta_tt8_Mut1	GTAGGATGCCAGGAAAGGCGTATCGGAGGAGGAAACACGTATGGCTATGTGGTCAAATG
Ta_TT8_Wt	*****
Ta_tt8_Mut1	AGGTTGACAGTAAAATCTTTCTAGGGCTATTCTCGAAAGGTCTATTCCCTTTTCATT
Ta_TT8_Wt	AGGTTGACAGTAAAATCTTTCTAGGGCTATTCTCGAAAGGTCTATTCCCTTTTCATT
Ta_tt8_Mut1	*****
Ta_TT8_Wt	TACCACTACTCTATGCATCTACTTCTCACCTATTATATATCTCATCTTCAAATTAAAT
Ta_tt8_Mut1	TACCACTACTCTATGCATCTACTTCTCACCTATTATATATCTCATCTTCAAATTAAAT
Ta_TT8_Wt	*****
Ta_tt8_Mut1	TAATTTCTGTCTTATTCTGGATGCTCCTCTACATCGTCGGTTCTTAATGGTT
Ta_TT8_Wt	TAATTTCTGTCTTATTCTGGATGCTCCTCTACATCGTCGGTTCTTAATGGTT
Ta_tt8_Mut1	*****
Ta_TT8_Wt	AGAGTGCCAAATCCAGGTAAACGTTCTTATTGATTAATTCTAATTGGAGTAATAT
Ta_tt8_Mut1	AGAGTGCCAAATCCAGGTAAACGTTCTTATTGATTAATTCTAATTGGAGTAATAT
Ta_TT8_Wt	*****
Ta_tt8_Mut1	TTTACATTTATTTACATGTTGAAATTGTTGTGATAAAAAAAAGCAGACAGTGGT
Ta_TT8_Wt	TTTACATTTATTTACATGTTGAAATTGTTGTGATAAAAAAAAGCAGACAGTGGT
Ta_tt8_Mut1	*****
Ta_TT8_Wt	TTGCATCCCCATGCTGATGGCGTTGTGGA ACTAGGCACAACGAACAAGGTACGGCGTAG
Ta_tt8_Mut1	TTGCATCCCCATGCTGATGGCGTTGTGGA ACTAGGCACAACGAACAAGGTACGGCGTAG
Ta_TT8_Wt	*****
Ta_tt8_Mut1	TTATCTTTATATATGCATAACCAAATGGTAAGAAAAAGGTTAGAAGAGAAATAGATC
Ta_TT8_Wt	TTATCTTTATATATGCATAACCAAATGGTAAGAAAAAGGTTAGAAGAGAAATAGATC
Ta_tt8_Mut1	*****
Ta_TT8_Wt	ATGCTTAAGTTTATCAGTTAAATTAAAAATGTAAAAATAAGATATTATGTTCATTAATA
Ta_tt8_Mut1	ATGCTTAAGTTTATCAGTTAAATTAAAAATGTAAAAATAAGATATTATGTTCATTAATA
Ta_TT8_Wt	*****
Ta_tt8_Mut1	ATGTATAGTCCCTGTTAGTAAAAAAAAGAATAAAATTTAACCATTTGAAGTCATAAT
Ta_TT8_Wt	ATGTATAGTCCCTGTTAGTAAAAAAAAGAATAAAATTTAACCATTTGAAGTCATAAT
Ta_tt8_Mut1	*****

Ta_TT8_Wt	ATAAAAAATATTGTTTGGAGATAGTACATAATTCTCACAAATAAAAAAAATAACAAAGGG
Ta_tt8_Mut1	ATAAAAAATATTGTTTGGAGATAGTACATAATTCTCACAAATAAAAAAAATAACAAAGGG *****
Ta_TT8_Wt	ATGATTAAGGGAGGAGTTGGATACATGTTGTTGTCTGTGTGAAGGTAAGAAG
Ta_tt8_Mut1	ATGATTAAGGGAGGAGTTGGATACATGTTGTTGTCTGTGTGAAGGTAAGAAG *****
Ta_TT8_Wt	ATATAGCGTTGTTGAGCTCATAAAGAGTTTCCATAACCACCCCAAGTCACACCAA
Ta_tt8_Mut1	ATATAGCGTTGTTGAGCTCATAAAGAGTTTCCATAACCACCCCAAGTCACACCAA *****
Ta_TT8_Wt	AAGCTGCTCTTCTGAACACTCCATCAACGAAGAGCACGAAGAACGAAAGACAAG
Ta_tt8_Mut1	AAGCTGCTCTTCTGAACACTCCATCAACGAAGAGCACGAAGAACGAAAGACAAG *****
Ta_TT8_Wt	AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTGGCT
Ta_tt8_Mut1	AAGAAGAAGAAGAAGTAGAAGAAGAAATGACAATGTCAGAGGAGATAAGGCTGGCT *****
Ta_TT8_Wt	CTCCTGATGATGATGACGTCTCCAATCAAACCTACTCTGATTCCATGTAGAATCAA
Ta_tt8_Mut1	CTCCTGATGATGATGACGTCTCCAATCAAACCTACTCTGATTCCATGTAGAATCAA *****
Ta_TT8_Wt	CCCACACTTAGGTATACTTACATTAATTAGTTAACGATATCATTACACGTATCT
Ta_tt8_Mut1	CCCACACTTAGGTATACTTACATTAATTAGTTAACGATATCATTACACGTATCT *****
Ta_TT8_Wt	ATTTATTTGTTAACAGAAATTAAAATATTGCCATTCTTGTTATGCTAAAGAA
Ta_tt8_Mut1	ATTTATTTGTTAACAGAAATTAAAATATTGCCATTCTTGTTATGCTAAAGAA *****
Ta_TT8_Wt	AATCTATAAAATTATGAAATAGACACACATGGACATGATGAATCTAATGGAGGAGGG
Ta_tt8_Mut1	AATCTATAAAATTATGAAATAGACACACATGGACATGATGAATCTAATGGAGGAGGG *****
Ta_TT8_Wt	TGGAAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTC
Ta_tt8_Mut1	TGGAAACTATTCTCAGACAGTATCAACACTTCTTATGTCACAACCCACGAGTCTTTTC *****
Ta_TT8_Wt	AGATTCACTTCCACATCTTCTACATCCAATCATCATTGCCACATGGAAGGCTGATAA
Ta_tt8_Mut1	AGATTCACTTCCACATCTTCTACATCCAATCATCATTGCCACATGGAAGGCTGATAA *****
Ta_TT8_Wt	TTTAAAGAGCATCAGCGAGTGGAAACTAAATGACGTCGTCGCAATGGATGCTCAA
Ta_tt8_Mut1	TTTAAAGAGCATCAGCGAGTGGAAACTAAATGACGTCGTCGCAATGGATGCTCAA *****
Ta_TT8_Wt	ACACATAATCTGAGAGTTCTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG
Ta_tt8_Mut1	ACACATAATCTGAGAGTTCTTACTCCACGACCACACTAAAGAAAAGAGGCTGCCTCG *****
Ta_TT8_Wt	AGAAGAGCTTAATCACGTGGCAGAGGCCGCAGGGAGAGAGAAGCTGAATGAGAGATT
Ta_tt8_Mut1	AGAAGAGCTTAATCACGTGGCAGAGGCCGCAGGGAGAGAGAAGCTGAATGAGAGATT *****
Ta_TT8_Wt	CATAACACTGAGATCATTGGTCCCTTGTGACCAAGATGGATAAGTCTCAATTCTGG
Ta_tt8_Mut1	CATAACACTGAGATCATTGGTCCCTTGTGACCAAGATGGATAAGTCTCAATTCTGG *****



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Ta_TT8_Wt
Ta_tt8_Mut1

AGACACCATACTACGTAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA
AGACACCATACTACGTAACCATCTTCGAAATAGGGTCCAAGAGCTGGAGACTAATCA

Ta_TT8_Wt
Ta_tt8_Mut1

TCACGAACAAAACATAAGCGGATGCGTAGCTGAAGGGAAAAACGTGGGAAGAGGTCGT
TCACGAACAAAACATAAGCGGATGCGTAGCTGAAGGGAAAAACGTGGGAAGAGGTCGT

Ta_TT8_Wt
Ta_tt8_Mut1

TGAGGTTCCATCATAGAGAGTGTAGTTGTTAGAGATGAGATGCGAGTACCGAGATGG
TGAGGTTCCATCATAGAGAGTGTAGTTGTTAGAGATGAGATGCGAGTACCGAGATGG

Ta_TT8_Wt
Ta_tt8_Mut1

TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA
TCTATTGCTCGACATCCTTCAGGTTCTTAAGGAACATGGTATAGAGACTACTGCAGTTCA

Ta_TT8_Wt
Ta_tt8_Mut1

TACCGCGGTGAACGAGCGTGTAGTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAAGAA
TACCGCGGTGAACGAGCGTGTAGTTCGAGGCCGAGATAAGGGCTATGGTGAGAGGGAAAGAA

Ta_TT8_Wt
Ta_tt8_Mut1

ACCAAGCATTGCTGAGGTAAAAGAGCCATCCATCAAAC TATATCCAATATTAACTATA
ACCAAGCATTGCTGAGGTAAAAGAGCCATCCATCAAAC TATATCCAATATTAACTATA

Ta_TT8_Wt
Ta_tt8_Mut1

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

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One representative modified gene sequence of [] is included for CBI-Deleted
comparison. One site in the gene is edited; the modified sequence includes a single A
insertion. The edit is in red.

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