QI BIODESIGN
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May 23, 2024
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Subject: Response for the Regulatory Status Review (RSR) of high oleic acid soybean line (24-116-02rsr) under 7 CFR § 340.4

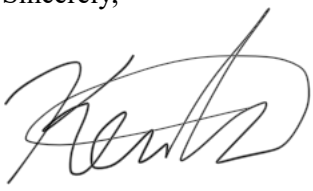
Dear Dr. Srinivasa Chaluvadi,

We thank you for your review and response to our request. Here, we have made changes to address the questions you raised to continue the review for our genome edited soybean product. In detail, we have made the following adjustments:

- We removed any uncited data of fatty acid content in our modified plant and included a new citation from a published reference in section 5 (readjust section).
- We removed the outdated USDA's Legacy Biotechnology Regulations.
- We submitted a copy of revised RSR request with all CBI information redacted in a separate PDF document.
- We now include additional non-sensitive details about the intended trait and phenotype in section 5 so that they may be included in the PTMOA table.

The enclosed information is presented in two copies (one with Confidential Business Information (CBI) bracketed and a second with CBI deleted). We would be pleased to respond to any additional questions you may have, or to provide you with additional information that you may request.

Sincerely,



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1. Information about requestor

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2. Confidential Business Information (CBI) Statement

The Freedom of Information Act (FOIA) exempts federal agencies from releasing trade secrets and commercial or financial information that is privileged or confidential (5 U.S.C.552(b)(4)). Qi Biodesign considers certain information in this RSR request to be confidential business information (CBI). Release of such information would cause harm to Qi Biodesign's and permit other companies to compete unfairly.

The following information are considered CBI within our request:

- Gene sequences, protein sequences
- Details of the genome editing tool
- Variety of the comparator plant
- Select details of the methodology and processes

The above information is kept private in the context of industry practices, which may reveal commercially valuable details and strategies on our product portfolios, and commercial partnerships and suppliers with Qi Biodesign. The release of this information will cause significant harm to Qi Biodesign by making such information available to our competitors and reveal internal business and technical strategy. In some cases, revealing this could also violate the terms of a nondisclosure agreement.

3. Description of the comparator plant

The recipient soybean variety used in this experiment, **[ ]**, was developed by the **[ ]**. It was approved for cultivation by the national variety approval committee and now have been widely cultivated in China. The taxonomy of the recipient soybean is summarized as follows: **CBI-Del** **CBI-Del** **CBI-Del**

Taxonomic rank	Name
Order	Fabales
Family	Fabaceae
Genus	Glycine
Species	*Glycine max* (L.) Merr.

Soybean oil is one of the most consumed vegetable oils worldwide. The content of oleic acid in soybean oil (18~20%) is significantly lower than that of rapeseed oil and olive oil (Fehr, 2007). Edible oils containing high levels of oleic acid and low levels of linoleic acid have higher oxidative stability and improved shelf life, which also prevents the need for hydrogenation treatments that create unhealthy trans-unsaturated fatty acids; therefore, these oils are healthier for human consumption (Ascherio and Willett, 1997).

4. Genotype of the modified plant (No genetic material inserted)

A. Target genes

Fatty Acid Desaturase (Δ12-fatty acid desaturase II, FAD2) is a key enzyme that determine the levels of monounsaturated fats in soybean oil (Schlueter *et al.*, 2007). FAD2 is responsible for the conversion of monounsaturated fatty acid, oleic acid (C18:1), to the polyunsaturated fatty acid, linoleic acid (C18:2), in developing soybean seeds by introducing a carbon-carbon double bond at the Δ12 position of oleic acid (Cahoon and Kinney, 2004). Two seed-specific isoforms of *FAD2*, designated as *FAD2-1A* and *FAD2-1B,* are expressed primarily in developing seeds and likely determine oleic acid levels in resulting soybean oil (Tang *et al.*, 2005).

B. Development of the modified plant

Our modified soybean line was generated using **[ ]** technology. **CBI-Del**

[ ] targeting the **[ ]** of the *GmFAD2-1A* and *GmFAD2-1B* genes, **CBI-Del, CBI-Del**
were cloned into the **[ ]** skeleton vector to generate **[ ]** **CBI-Del, CBI-Del**
expression vector (Figure 1, Table 1), and then introduced into the recipient soybean
cultivar by **[ ]** transformation. **[** **CBI-Del, CBI-Del**
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]. Self-pollination of the selected **CBI-Del**
gene-edited-positive events led to the harvest of T_1 seeds. The expected gene modified
soybean lines that contain **[** **CBI-Del**
] at the desired edit sites were identified in the T_1 population **[** **CBI-Del, CBI-Del**
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Figure 1 Expression vector map

Table 1 Annotation of the genetic elements in the expression vector

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C. Genotype of the modified plant

Our soybean product, was confirmed as a null-segregant line with **[CBI-Del]** in *GmFAD2-1A* gene, and **[ ] in CBI-Del, CBI-Del** *GmFAD2-1B* gene. The two genes in the soybean mutant contain **[ ] CBI-Del** of different lengths, which result in frameshift and pre-mature termination of translation, leading to complete gene knockout. **[ ]** analysis further **CBI-Del** demonstrated no foreign DNA residue in the resulting soybean line and no off-target edits.

D. Sequence of the modification and sequences comparison

The original and edited sequences of the targeted genes are provided in Appendix A and B. The comparison of the edited sequences with the original sequences are provided in Appendix C.

5. Description of the new trait

A. Intended trait

The intended trait is an increased oleic acid content in soybean seeds.

B. Intended phenotype

The fatty acid profile of the seed was dramatically changed in plants homozygous for mutations in both *GmFAD2-1A* and *GmFAD2-1B*. Specifically, there is an increase in oleic acid content and a decrease in linoleic acid content (Haun *et al.*, 2014).

C. Description of the Mechanism of Action (MOA)

The endoplasmic reticulum-associated oleate desaturase FAD2 is the key enzyme responsible for producing linoleic acid in non-photosynthetic tissues of plants. In soybean, two different *FAD2* isoforms have been reported: a constitutively expressed gene designated *FAD2-2*, and a seed-specific gene designated *FAD2-1*. Two seed-specific isoforms of *FAD2-1*, designated *FAD2-1A* and *FAD2-1B*, which differ at only 24 amino acid residues (Tang *et al.*, 2005), play a pivotal role in determining the fatty acid composition of the seed-storage oil (Heppard et al., 1996; Kinney, 1997). Unlike *GmFAD2-1* members, the members of the *GmFAD2-2* exhibited cytoplasmic localization, which may suggest the presence of an alternative fatty acid desaturase pathway in soybean for converting oleic acid content without substantially altering the traditional plastidial/ER fatty acid production (Lakhssassi *et al.*, 2021).

FAD2 has several functional domains, which include ER localization signals, four transmembrane domains, two membrane binding sites and three conserved histidine motifs (Hisbox1, Hisbox2, and Hisbox3) (Schlueter *et al.*, 2007). The histidine-rich motifs form the catalytic active center of fatty acid dehydrogenase, as well as the iron atom binding site, and play a key role in fatty acid dehydrogenase pathway (Guan *et al.*, 2013; Dyer and Mullen, 2001). During the de novo synthesis pathway of fatty acids, 16C and 18C long-chain fatty acids were synthesized by a series of enzymatic reactions in the cytoplasmic space using acetyl-CoA as the substrate. The desaturation of fatty acids occurs in the endoplasmic reticulum and chloroplasts. *FAD2* catalyzes the formation of a carbon-carbon double bond at the Δ12 position of oleic acid to produce linoleic acid. Oleic acid is the precursor of linoleic acid synthesis, and the catalytic effects of *FAD2* can directly determine the content and proportion of monounsaturated fatty acids and polyunsaturated fatty acids in soybean seeds (Schlueter *et al.*, 2007; Tang *et al.*, 2005; Clemente and Cahoon, 2009).

Mutations in the *GmFAD2-1A* and *GmFAD2-1B* genes encoding fatty acid desaturases (omega-6 FAD) in soybean plants were regarded as the best approach to develop soybean varieties with elevated oleic acid content (Lakhssassi *et al.*, 2017; Tang *et al.*, 2005; Wagner *et al.*, 2011). Conventional breeding and genetic engineering tools have been widely used to create new soybean varieties with high oleic acid content of its total oil (Pham *et al.*, 2010, 2011; Hoshino *et al.*, 2010; Haun *et al.*, 2014; Wu *et al.*, 2020; Do *et al.*, 2019). Soybean products such as Vistive Gold (75% oleic acid) (Monsanto, 2013), and Plenish (77% oleic acid) (Pioneer, 2013) are good examples.

6. References

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Appendix A. Original sequences of the targeted genes

Appendix A1: Original *GmFAD2-1A* [ ] genome sequences with a length of 1,331 bp. 2 exons of *GmFAD2-1A* gene are marked by wavy lines. sgRNAs and PAM are identified by red and blue bold font, respectively.

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Appendix A2: Original GmFAD2-1A amino acid sequences with a length of 387 aa.

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Appendix A3: Original *GmFAD2-1B* [ ] genome sequences with a length of 2,310 bp. 2 exons of *GmFAD2-1B* gene are marked by wavy lines. sgRNAs and PAM are identified by red and blue bold font, respectively. **CBI-Del**

CBI-Del

Appendix A4: Original GmFAD2-1B amino acid sequences with a length of 387 aa.

CBI-Del

Appendix B. Edited sequences in soybean mutant

Appendix B1: Edited *GmFAD2-1A* genome sequences. 2 exons of *GmFAD2-1A* gene are marked by wavy lines. sgRNAs and PAM are identified by red and blue bold font, respectively. There are **[** **CBI-Del** **]** of *GmFAD2-1A* gene respectively, which result **CBI-Del** in a frameshift mutation (the new stop codon is highlighted in green) and then pre-mature termination of the translation.

[**CBI-Del**]

Appendix B2: Predicted GmFAD2-1A amino acid sequences after edited, 97 aa.

[**CBI-Del**]

Appendix B3. Edited *GmFAD2-1B* genome sequences. 2 exons of *GmFAD2-1B* gene are marked by wavy lines. sgRNAs and PAM are identified by red and blue bold font, respectively. There are **[** **CBI-Del** **]** of *GmFAD2-1B* gene respectively, which **CBI-Del** results in a frameshift mutation (the new stop codon is highlighted by green) and then pre-mature termination of the translation.

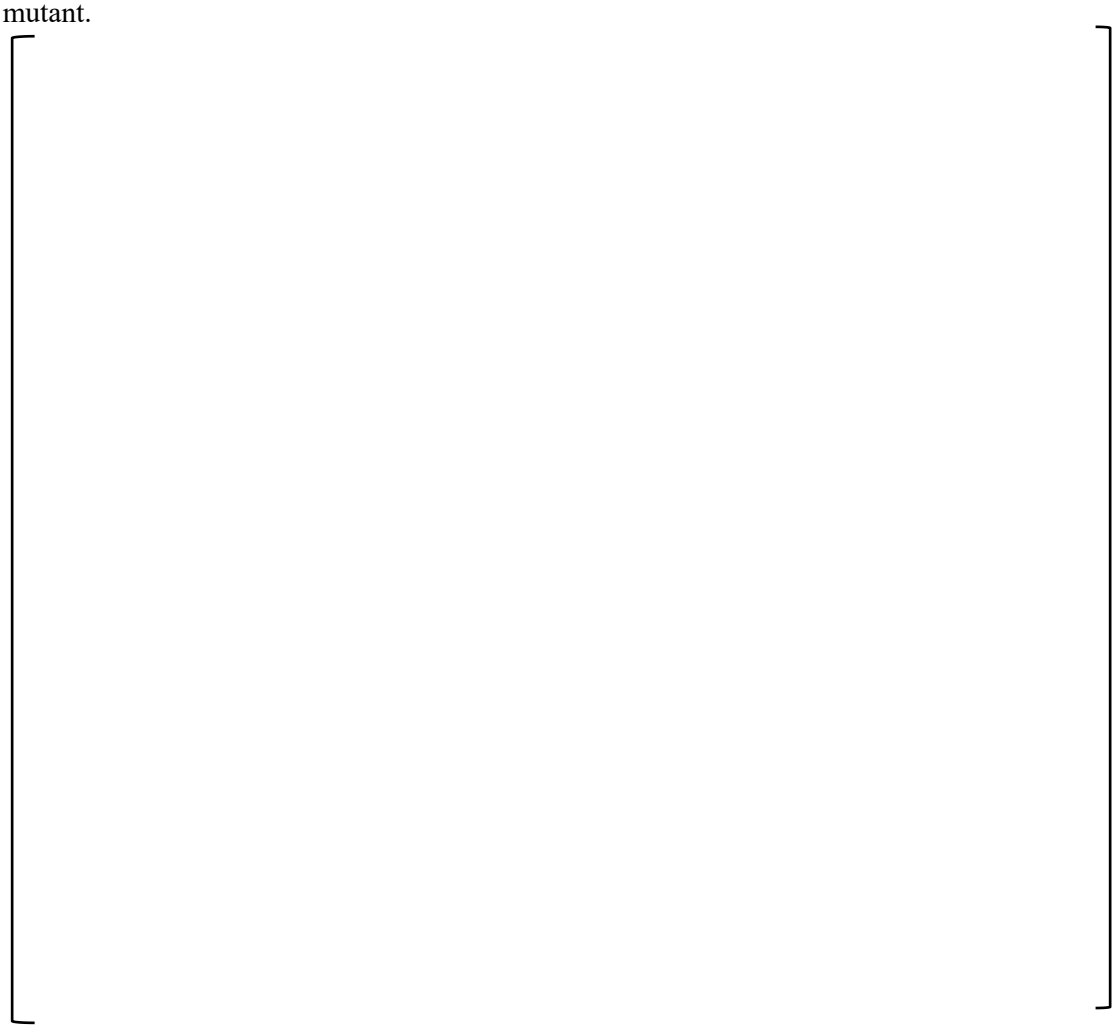
[**CBI-Del**]

Appendix B4. Predicted GmFAD2-1B amino acid sequences after edited, 123 aa.

[**CBI-Del**]

Appendix C. Nucleotide and amino acid sequences alignments

Appendix C1: Alignment of the *GmFAD2-1A* nucleotide sequences between the original and edited sequences. The green highlight shows the different sequences in the soybean mutant.

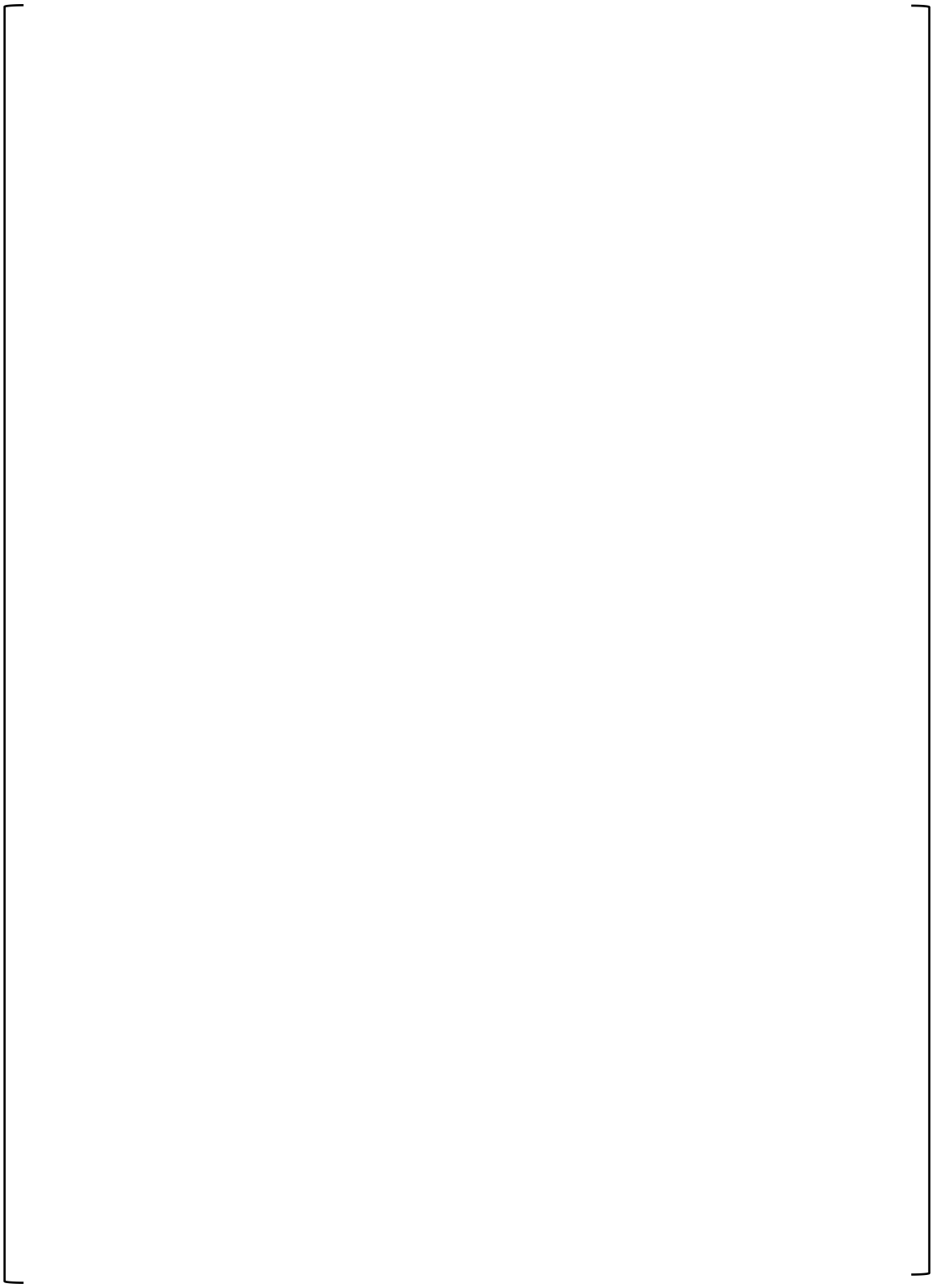


CBI-Del

Appendix C2: Alignment of the GmFAD2-1A amino acid sequences between the original and edited sequences.

CBI-Del

Appendix C3: Alignment of the *GmFAD2-1B* nucleotide sequences between the original and edited sequences. The green highlight shows the different sequences in the soybean mutant.



CBI-Del

Appendix C4: Alignment of the GmFAD2-1B amino acid sequences between the original and edited sequences.

[ ] **CBI-Del**