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May 18, 2021

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

Contains Confidential Business Information

RE: Regulatory Status Review (RSR) of transgenic soybean with increased oil and protein content, RSR number 21-117-01rsr.

Dear Ms. Juarez,

ZeaKal, Inc. respectfully requests a Regulatory Status Review (RSR) of our transgenic soybean line that [**CBI-Deleted**] results in a soybean plant with increased oil and protein content in the seed. As described below, this soybean line is unlikely to pose a plant pest risk and we request confirmation that it is not regulated under 7 CFR part 340[1].

In order to provide you with necessary details to evaluate this GE plant and its plant-trait-mechanism of action (MOA), we will describe this plant and plant-trait-MOA by following the guidelines presented in part 340.4(a)(4)(i-iii) as well as on pages 29808-29809 of 85 FR 29790-29838, Docket No. APHIS–2018–0034.

Description of the comparator plant(s), to include genus, species, and any relevant subspecies information

Soybean, *Glycine max*, L.

[1] On May 18th, 2020, USDA-APHIS revised its regulations at 7 CFR part 340 (85 FR 29790- 29838, Docket No. APHIS–2018–0034) with an effective date of April 5, 2021 for RSR processes of certain crops, including soybean, under Sections 340.4. Unless otherwise indicated, the references to "Part 340" herein are as of April 5, 2021.

Genotype of the modified plant, including a detailed description of the differences in genotype between the modified and unmodified plant

The modified plant contains [

CBI-Deleted

] (Table 1).

Table 1. Description of genetic elements present in the soybean plant.

Genetic Element	Source Organism	Function
[		
		]

CBI-Deleted

ZeaKal

		[
		]

CBI-Deleted

		[
		]

CBI-Deleted

T-DNA in GenBank format:

```
LOCUS       ZeaKal_T_DNA          [                                        ]
DEFINITION  .
ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
[
```

CBI-Deleted

CBI-Deleted

]

ZeaKal

[

CBI-Deleted

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ZeaKal

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CBI-Deleted

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ZeaKal

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ZeaKal

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CBI-Deleted

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CBI-Deleted

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[

CBI-Deleted

]

Detailed description of the new trait(s) of the modified plant.

ZeaKal's GE soybean has increased oil and protein content in the seed. This phenotype is accomplished through [

CBI-Deleted

]. The production and accumulation of [

CBI-Deleted

]

[ ]. CBI-Deleted

Over the course of the growing season, the elevated photosynthesis results in the fixation of additional carbon which manifests as increased seed oil content. The elevated photosynthesis also supports higher nitrogen fixation by rhizobia which is seen as increased protein content in the seed (Table 2).

The [ ] plant genes co-expressed include [CBI-Deleted
CBI-Deleted

]. In many oil seed species [CBI-Deleted

].

[

CBI-Deleted

]

Table 2. Trait, Phenotype and MOA of ZeaKal's transgenic soybean.

Plant	Scientific Name	Trait	Phenotype	Mechanism of Action
Soybean	*Glycine max*	Altered seed composition	Increased seed oil and protein content	Co-expression of oil synthesizing enzyme and oil encapsulating protein in green tissues resulting in elevated photosynthesis

Additional data

Over the past several years, ZeaKal [ ] have generated several dozen CBI-Deleted
soybean events with different variations of the technology [CBI-Deleted

] a

well as several [ ] events that have been grown in USDA-APHIS regulated field trials at CBI-Deleted
multiple sites. Over 5 years and all 5 sites, we have not observed differences in the overall reproductive cycle or overall soybean plant morphology compared to the non-transgenic control plants. In addition, we have not observed any differences in the susceptibility to pests

nor observed any harm to non-target organisms beneficial to agriculture, nor have we observed any evidence that the technology has increased the plants' ability to be a pest or act as a reservoir for a plant pest.

Thank you for taking the time to review this inquiry. I'm happy to address any questions you may have.

Sincerely,

Amy Curran, Ph.D.
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zeakal.com

References

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No CBI
CBI-Justification Letter

May 18, 2021

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

RE: CBI Justification for Regulatory Status Review (RSR) of transgenic soybean with increased oil and protein content, RSR number 21-117-01rsr

Dear Ms. Juarez,

ZeaKal, Inc. respectfully requests a Regulatory Status Review (RSR) of our transgenic soybean that has increased oil and protein content in the seed. Since this RSR request contains confidential business information (CBI), we are supplying the CBI justification in this letter.

All claims of CBI follow the USDA APHIS/ BRS guidance document[1]. More specifically, the genetic modifications, construct details, and transformation methods as well as references that contain this information represent key information needed to replicate our proprietary technology in soybeans and could result in competitive harm. Other CBI claims include details from collaborator data which are under specific NDAs.

Thank you for taking the time to review our request for RSR.
Please let me know if you have any questions.

Sincerely,

Amy Curran, Ph.D.
Chief Operating Officer
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(619) 218-2013 (mobile)
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[1]Document ID BRS-GD-2020-0004, *Guidance for Claiming Confidential Business Information (CBI) in Submission to USDA APHIS Biotechnology Regulatory Services (BRS),* June 18, 2020)