cellectis
plant sciences

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Mr. Mike Firko
Deputy Administrator
Biotechnology Regulatory Service
Animal and Plant Health Inspection Service
United States Department of Agriculture
4700 River Road, Unit 98
Riverdale, MD 20737

Mr. Firko:

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Re: Confirmation that FAD3KO Soybean is not a regulated article

Cellectis Plant Sciences (CPS) is developing technology that enables precise genome modification in economically important crops used for food and feed. One of the products that CPS is focused on is a soybean product (*Glycine max* (L.) Merr.), FAD3KO Soybean, defined as soybean lines created by the transient expression of the construct described below. FAD3KO Soybean has improved consumer safety and processing attributes attributable to the knockout of two highly related genes achieved through transient expression of a Transcription Activator-Like Effector Nuclease (TALEN™). The FAD3KO Soybean was developed using an identical method as described in the Regulated Letter of Inquiry for the development of the FAD2KO Soybean, which was submitted by CPS to USDA/APHIS on November 17, 2014.

Because soybean is not a plant pest or an invasive species, the genetic elements used to generate FAD3KO Soybean are sourced from fully classified organisms, and the genomic modification process does not introduce any plant pest DNA components, there is no scientifically valid basis for concluding that FAD3KO Soybean is, or will become, a plant pest within the meaning of the Plant Protection Act. CPS therefore asserts that under current regulations, FAD3KO Soybean is not a regulated article within the meaning of 7 CFR §340.1 because it does not satisfy the criteria that would subject it to oversight of the USDA's Animal and Plant Health Inspection Service (APHIS).

Before proceeding with further product development, CPS requests that APHIS confirm that FAD3KO Soybean, modified without incorporating any plant pest elements (as described more fully in Table 1 below), should not be considered a regulated article within the meaning of the current regulations. If the agency does not concur with CPS' interpretation of the current regulations, CPS requests that the Agency provide us with its scientific rationale for concluding that FAD3KO Soybean is or will become a plant pest.

I. Transformation Background

To further assist APHIS in understanding the origin of FAD3KO Soybean, a summary of information on the recipient plant, genetic elements, and process used to modify the recipient plant's genomic DNA, is provided below.

a. FAD3KO Soybean (*Glycine max* (L.) Merr.)

FAD3KO Soybean contains a two-gene knockout achieved through expression of a specially designed TALEN™. TALEN™ technology is a relatively new method of targeted mutagenesis that is functionally equivalent to other methods of achieving targeted deletions such as meganucleases and zinc-finger nucleases. These methods rely on customizable DNA recognition sequences coupled to site-specific nucleases that create double stranded breaks in genomic DNA. Following the introduction of double stranded breaks, the cells' natural DNA repair mechanism completes the repair by non-homologous end-joining (NHEJ) or homologous recombination with or without a DNA template. DNA repair via NHEJ occasionally produces small deletions in the targeted gene that lead to frameshift mutations and disruption of the targeted protein function. In this way, TALEN™ technology can achieve a targeted gene knockout that produces a desirable phenotype.

In FAD3KO Soybean, a specially designed TALEN™ expression cassette was introduced by [ ] transformation of soybean cotyledons. A selectable marker system was used to preferentially identify or concentrate soybean explants with the desired integration of the TALEN™ expression cassette. The TALEN™ reagent was expressed in the soybean cells to achieve the targeted gene knockouts. Standard tissue culture techniques were used to regenerate callus and subsequently whole plants. Polymerase Chain Reaction (PCR) techniques were then used to screen regenerated soybean plants to identify transgenic plants that also contained target-site deletions of the soybean FAD3A and FAD3B genes. Table 1 describes the genetic elements used to produce the intended product quality phenotype in FAD3KO Soybean.

b. Recipient Soybean (*Glycine max* (L.) Merr.)

Soybean is not a federal noxious weed. It is a leguminous crop in the Fabaceae family that is grown worldwide, mainly as a source of oil for human consumption and as an important source of protein for animal nutrition. It is the world's largest source of animal protein feed and the second largest source of vegetable oil behind oil palm, accounting for nearly 1/3 of the worldwide edible oil production. The center of origin of soybean has been reported as eastern Asia, specifically what is present-day northeastern China. In the United States, soybeans can be grown in all 50 states, although commercial production is concentrated in 10 states mostly in the upper Midwest. Soybean is a diploid with 20 chromosomes; the complete genome sequence was published in 2010.

Table 1. Genetic elements used for targeted gene knockout in FAD3KO Soybean

Genetic Element	Source	Function
[ ]	[ ]	Promoter to regulate transcription of the TALEN™ reagent.
TAL effector	Xanthomonas spp.	An array of 34-amino acid DNA-binding motifs that specifically recognize target sequences within the first exon of the Fatty Acid Desaturase 3 (FAD3) genes. The TAL effector binding domain is exclusive of all native sequences responsible for cell infection and pathogenicity: nuclear localization signal peptide (NLS) and acidic transcription activation domain (AAD).
FokI	Flavobacterium okeanokoites	A bacterial type IIS restriction endonuclease consisting of an N-terminal DNA-binding domain and a non-specific DNA cleavage domain at the C-terminal that cleaves soybean genomic DNA downstream of the TALEN™ binding domain.
Nos terminator	Agrobacterium tumefaciens	Sequence to regulate production of an mRNA of the TALEN™ reagent.
[ ]	[ ]	Promoter to regulate transcription of the [ ] selectable marker.
[ ]	[ ]	Selectable marker to confer resistance to the plant herbicide [ ]
Nos terminator	*Agrobacterium tumefaciens*	Sequence to regulate production of an mRNA of the [ ] selectable marker.

II. APHIS' Interpretation of Its 7 CFR §340 Regulations Dictates a Finding that FAD3KO Soybean is Not a Regulated Article

a. APHIS Has Been Clear That Not All Genetically Modified Plants Are Subject to Regulatory Oversight

APHIS defines a "regulated article" as:

> Any organism which has been altered or produced through genetic engineering, if the donor organism, recipient organism, or vector or vector agent belongs to any genera or taxa designated in §340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator, determines is a

> plant pest or has reason to believe is a plant pest. Excluded are recipient microorganisms which are not plant pests and which have resulted from the addition of genetic material from a donor organism where the material is well characterized and contains only non-coding regulatory regions.

Consistent with the PPA's definition of a plant pest, APHIS further defines a "plant pest" as:

> Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants.

APHIS further claims that its regulations are consistent with the Coordinated Framework, because they apply "only [to] genetically engineered organisms or products which are plant pests or for which there is reason to believe are plant pest, and not to... an organism or product merely because of the process by which it was produced. APHIS has further stated that its concern arises only "when an organism or product is altered or produced by genetic engineering and one or more of its constituents (donor, vector/vector agent or recipient) comes from a family or genus of organisms know to contain plant pests... This is because... there is a risk that certain undesirable traits may be transferred to the new organism and may survive when the organism is released into the environments."

b. FAD3KO Soybean Does Not Fall Within the Regulatory Definition of a "Regulated Article"

Under APHIS regulations, an organism is considered a "regulated article" "if the donor organism, recipient organism, or vector or vector agent belongs to a genera or taxa designated in 7 CFR §340.2, *and* the organism meets the definition of a plant pest." The language of the regulation requires that both criteria must be met to satisfy the definition of a regulated article.

The TALEN™ reagent used for targeted mutagenesis of FAD3KO Soybean contains a highly derivatized DNA-binding domain from Xanthamonas, a taxa designated in 7 CFR §340.2. The DNA-binding domain consists of an array of a 34-amino acid highly conserved sequence except for the hypervariable amino acid residues at positions 12 and 13 called repeat-variable di-residues (RVDs). Unlike the TALEs found in Xanthamonas, which are capable of infecting plants, the coding sequences necessary for infection and pathogenicity, the nuclear localization signal peptide (NLS) and the acidic transcription activation domain (AAD), are removed from TALEN™ reagents leaving only the DNA-binding domain. The TALEN™ is customized to recognize the DNA target sequence of the recipient plant, in this case, the second exon of the Fatty Acid Desaturase 3 (FAD3) genes of soybean.

Another definition of a "regulated article" includes "any product which contains such an organism [i.e., an organism that is or contains DNA sequences from a plant pest]." FAD3KO Soybean does not meet that definition because it no longer contains DNA

sequences from a plant pest or pathogen. FAD3KO Soybean is a null segregant of a soybean transgenic producer plant in which the TALEN™ reagent was expressed from a stably integrated expression cassette. In the transgenic producer plant, molecular analyses confirmed the presence of the TALEN™ expression cassette as well as the targeted deletions at the FAD3 genes. Subsequent self-pollination of the transgenic producer plant resulted in the FAD3KO Soybean line, which no longer contained the TALEN™ expression cassette but retained the targeted deletions at the FAD3 genes. Furthermore, PCR analysis confirms the absence of TALEN™-derived DNA or retention of any components of the expression cassette into the genome of FAD3KO Soybean. Therefore, FAD3KO Soybean does not satisfy this criterion to qualify as a "regulated article."

Another definition of a "regulated article" includes organisms that are unclassified or whose classification is unknown. The introduced trait improves the oil profile of soybean and eliminates the need for hydrogenation, thus eliminating the creation of trans fats in processed soybean oil. The consumer health attributes of the FAD3KO Soybean is the result of functional deletion of the two native genes responsible for the conversion of oleic acid precursors to linoleic acid precursors during oil accumulation in developing soybean seeds. High linoleic acid content, as found in commodity soybean oil, is responsible for decreased shelf life and oil stability when heated. High-oleic soybean oil, like that produced by FAD3KO Soybean, has increased shelf life and does not require hydrogenation and the associated production of trans fats. It does not change the soybean's basic biology or produce a plant that would directly feed on, infect, parasitize, or contaminate plants, or adversely affect other organisms that are beneficial to plants.

III. Finding that FAD3KO Soybean is Not a Regulated Article is Consistent With Previous APHIS Determinations

APHIS has made a number of determinations that genetically modified plants are not "regulated articles," including certain plants containing a targeted gene knock-out by zinc-finger nucleases or meganucleases. For example, APHIS determined that "GE plants containing targeted deletions, caused by naturally-occurring DNA repair after the targeted break is made by zinc-finger nuclease, and *in which no genetic material is inserted into the plant genome*, are not regulated articles under CFR part 340 [provided that] the nucleases used are not from a plant pest and no plant pest sequences are inserted into the plant genome" (Gregoire to Dow AgroSciences, Mar 8, 2012). APHIS also determined that certain plants containing "targeted gene deletions, caused by naturally-occurring DNA repair after the break is made by the I-CreI meganuclease... [wherein] no genetic material is inserted into the plant genome... will not, in most cases, be regulated articles under 7 CFR part 340" (Gregoire to Cellectis Plant Sciences, Dec 16, 2011).

APHIS also determined that null segregant plants derived from genetically engineered plants are not "regulated articles." For example, APHIS determined that null segregants derived from a stably transformed sorghum species in which an RNAi construct containing plant pest sequences introduced by *Agrobacterium tumefaciens*-mediated transformation, are not regulated articles, whereas "the GE parent plants are regulated articles because a plant pest

vector was used to introduce DNA that contains plant pest sequences" (Gregoire to University of Nebraska-Lincoln, Jun 6, 2012). APHIS also determined that null segregants derived from stably transformed tobacco species in which a gene expression construct containing plant pest sequences introduced by *Agrobacterium tumefaciens*-mediated transformation, are not regulated articles (Gregoire to North Carolina State University, Oct 27, 2011). In these examples and in the case of the FAD3KO Soybean, null segregant plants contain no inserted DNA, which is confirmed by sensitivity to herbicide application and molecular analyses. Other examples are also posted on USDA's website.

IV. Summary of Conclusions

In summary, soybean is not itself a plant pest, no plant pest elements are contained in FAD3KO Soybean, and all organisms involved in targeted mutagenesis of soybean are fully classified. Therefore, there is no scientifically valid basis to determine that FAD3KO Soybean is or will become a plant pest within the meaning of the Plant Protection Act.

Thank you for your consideration and prompt confirmation of CPS' position that FAD3KO Soybean is not a "regulated article" for the reasons stated above. We look forward to receiving your response.

Sincerely,

Luc Mathis, PhD
Chief Executive Officer