Biotechnology Notification File No. 000189
CVM Note to the File

Date: December 4, 2023

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To: Administrative Record, BNF No. 000189

Subject: Event JA36 Potato

Simplot considers information that is enclosed within square brackets to be confidential business information.

Keywords: Potato, *Solanum tuberosum*, Bintje variety, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 nuclease, Genome editing, Gene knock out, [redacted] gene, *Gn2* gene, high tuber set, event JA36, J.R. Simplot Company

Purpose

This document summarizes the Food and Drug Administration (FDA) Center for Veterinary Medicine's (CVM, we) evaluation of biotechnology notification file (BNF) number 000189. J.R. Simplot Company (Simplot) submitted a safety and nutritional assessment for a new potato variety, transformation event JA36 (hereafter referred to as JA36 potato) and additional information afterwards. CVM evaluated the information in Simplot's submissions to ensure that regulatory and safety issues regarding animal food derived from JA36 potato have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of uses of JA36 potato in human food in a separate document.

In CVM's evaluation, we considered all of the information provided by Simplot as well as publicly available information and information in the agency's files. Here we discuss the outcome of the consultation for animal food use, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effects

The intended effect of the modification in JA36 potato is [redacted] and, thereby, [redacted]. The modification results in many tubers produced by a single plant, also known as high tuber set. With the resources of the plant nourishing more tubers, smaller tubers are the natural outcome. Simplot accomplished this intended effect by deletion of nucleotide

sequences within the alleles of the *Gn2*[1] gene, which results in early termination of the Gn2 protein.

Regulatory Considerations

The purposes of this evaluation are (1) to assess whether Simplot has introduced into animal food a substance requiring premarket approval as a food additive and (2) to determine whether use of the new plant variety in animal food raises other regulatory issues with respect to the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) defines a plant-incorporated protectant (PIP) as "a pesticidal substance that is intended to be produced and used in a living plant, or the produce thereof, and the genetic material necessary for the production of such a pesticidal substance," including "any inert ingredient contained in the plant , or produce thereof" (40 CFR 174.3) EPA regulated PIPs under the Federal Insecticide, Fungicide, and Rodenticide Act and the FD&C Act. The term pesticide, as defined in FIFRA section 2(u) includes "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest; and substances or mixture of substances intended for use as a plant regulator, defoliant, or desiccant; and any nitrogen stabilizer."

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Simplot states that JA36 potato was developed through disarmed *Agrobacterium tumefaciens* mediated transformation of parental variety Bintje cells with plasmid pSIM4706. The plasmid contained a single right border, a short nucleotide sequence that enhances cleavage at the right border site, and gene sequences to enable replication of the plasmid in *Escherichia coli* and *A. tumefaciens*. This plasmid also contained single copies of opposing guide ribonucleic acids (RNA)[2] and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated protein 9 (Cas9 endonuclease) coding sequence from *Streptococcus pyogenes*. It also contained the *aminoglycoside phosphotransferase II* gene from *Staphylococcus aureus*, the *isopentenyl transferase* gene from *A. tumefaciens* Ti-plasmid, and the *Gn1*[3] gene, which were used for selection. The aminoglycoside phosphotransferase II protein was used to select for transformed cells, while the other selection marker genes were used to select against stable transformants, i.e., those that contained pSIM4706 plasmid DNA. Each of these genes were under the control of well characterized promoter and terminator sequences.

Following transformation, cells were cultivated on medium containing kanamycin to remove non-transformed cells and timentin to inhibit *A. tumefaciens* growth. Regenerated plantlets displaying normal development were then grown to maturity.

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[1] The [redacted] gene is called *Gn2* gene because Simplot considers the gene and its mechanism of action to be confidential business information.

[2] Each guide RNA contains a unique CRISPR RNA (20 nucleotides) and a trans-acting CRISPR RNA (approximately 80 nucleotides).

[3] The [redacted] gene is called the *Gn1* gene because Simplot considers the gene and its mechanism of action to be confidential business information.

Simplot used quantitative polymerase chain reaction (qPCR) to identify alterations in the nucleotide sequence of the four alleles of the targeted gene in these plantlets. The tips from these plantlets were excised and grown in media that induce root growth and contained timentin, to select against *A. tumefaciens*.

Simplot used a combination of techniques to confirm the absence of pSIM4706 vector sequences in JA36 potato. This included PCR using primers for twelve regions of the plasmid and further assessment using DNA capture-based target enrichment and sequencing of captured sequences on an Illumina NovaSeq 6000 sequencer. A bioinformatics approach was used to align the captured sequences to pSIM4706 plasmid and the reference potato genome (PGSC DM v4.03). Simplot concludes that JA36 potato does not contain pSIM4706 plasmid DNA.

The stability of the modified alleles in JA36 potato was assessed using PCR and Sanger sequencing in plants that had undergone successive rounds of vegetative propagation.[4] Simplot states that sequence analysis confirmed the stability of edited alleles in JA36 potato across three rounds of vegetative propagation.

Simplot states that Cas-Designer software was utilized in the design of its guide RNA (gRNA) sequences and that this software identified a single potential off-target sequence in the Bintje genome. Subsequently, analysis of JA36 potato genome sequence demonstrated that there was no modification of the off-target site.

Safety of Gn2 Proteins

Simplot states that the Gn2 protein is a [redacted] and, thus the early termination of the Gn2 protein is predicted to increase the number of tubers and tubers are smaller due to limited nutrients being available for a larger number of tubers per plant.

Simplot states that the nucleotide sequence of the edited alleles, obtained by Sanger sequencing, was used to characterize each of the four targeted alleles in JA36 potato. Simplot reported that one of the alleles contained a 110 base pair (bp) deletion at CRISPR target site, whereas the other three alleles had one or two bp deletions. Simplot states that all of the deletions result in frame-shifts and premature stop codons, which are predicted to truncate and inactivate the proteins. Simplot concludes that premature termination of the Gn2 gene would lead to functional inactivation of the protein.

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[4] Simplot highlights that DNA instability in plants is associated with recombination events that occur during meiosis and that vegetatively propagated potatoes do not undergo meiosis, genetic recombination, or segregation and, thus, the edited DNA is considered to be genetically stable during vegetative reproduction.

[5] [redacted]

Simplot used bioinformatic analysis to identify potential new open reading frames (ORFs) related to the edited sequences and to assess whether the edited proteins could be toxic. Simplot compared the putative protein sequences obtained from open reading frame analysis of the edited Gn2 alleles to sequences in the National Center for Bioinformatics Information non-redundant protein sequence database with a keyword filter for "toxin". No unique matches were recovered when the edited proteins were used as query terms. Simplot concludes that there is no increased safety risk compared to the Bintje variety.

Animal Food Use

Simplot states that operational and ration-balancing constraints often limit the inclusion level of potatoes in animal diets. Simplot notes that inclusion rates of up to 30% of the diet dry matter may occur in cattle that are near potato processing plants. Potatoes are commonly used in diets for sheep and swine, but typically the inclusion rate are lower than for cattle that are near potato processing plants. Simplot notes that JA36 potato will be used in animal food in the same manner as other types of potatoes.

Composition

Scope of Analysis

Simplot conducted compositional analyses on tubers obtained from JA36 and Bintje potato varieties. The components selected for analysis were listed as key nutrients and toxicants in the Organisation for Economic Co-operation and Development (OECD) consensus document[6] and other components considered important in the potato industry.

Study Design

Simplot conducted field trials in the 2021 growing season at three sites within the potato growing regions of the United States. Simplot grew JA36 potato (G1 seed pieces) and Bintje potato at each field site with four replicates per site. The plants were vine killed when the JA36 tubers were the desired small size. Each sample was comprised of randomly selected tubers from each replicate at each site and, thus, resulted in a total of 12 samples. A sample consisted of two pounds of tubers, including the peel. Fresh tubers were transported to a commercial laboratory and were analyzed within three days. The two pounds of tubers for each replicate were processed by grinding all tubers together with liquid nitrogen and homogenizing for a composite sample prior to analysis.

Simplot presents mean values, standard deviations, and range of values for each component for JA36 potato and the Bintje potato and the results of the statistical comparison of JA36 potato and Bintje potato. If there was a statistically significant ($P<0.05$) difference in a component between JA36 potato and Bintje potato across

[6] Organisation for Economic Co-operation and Development. 2021. Revised consensus document on compositional considerations for new varieties of potato (*Solanum tuberosum*): Key food and feed nutrients, toxicants, allergens, anti-nutrients, and other secondary metabolites. Series on the safety of novel foods and feeds No. 33. ENV/JM/MONO. OECD, Paris.

locations, then the means for the component was compared to ranges obtained from public databases.[7]

Results of Analyses

Simplot reports values for proximates (moisture, crude protein, crude fat, carbohydrates by calculation, and ash), calories (calculated), fiber (acid detergent fiber (ADF), neutral detergent fiber (NDF), and total dietary fiber (TDF)), three vitamins (vitamin B3, vitamin B6, and vitamin C), three minerals (copper, magnesium, and potassium), and glycoalkaloids (alpha-chaconine, alpha-solanine, and total). Simplot states that the mean values for each of these components, with the exception of carbohydrates, calories, moisture, and magnesium, in JA36 potato were similar to the mean values for these components in Bintje potato. Simplot notes that the mean values and minimum and maximum values for all these components fall within the range of values observed in the combined databases. Simplot concludes that JA36 potato is compositionally comparable to Bintje potato.

Summary of Compositional Analyses

Simplot states that the levels of nutrients and anti-nutrients did not differ significantly between JA36 and Bintje potato varieties. Therefore, Simplot concludes that JA36 potato is compositionally comparable to Bintje potato and other conventional potato varieties that have a long history of safe consumption in animal food.

Conclusion

CVM evaluated Simplot's submissions to determine whether JA36 potato raises any safety or regulatory issues with respect to its use in animal food. Based on the information provided by Simplot and other information available to the agency, CVM did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

Simplot concludes that JA36 potato and the animal foods derived from it are as safe as and are not materially different in composition, safety, or any other relevant parameter from other potato varieties now grown, marketed, and consumed. At this time, based on Simplot's data and information, CVM considers Simplot's consultation on JA36 potato for use in animal food to be complete.

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[7] The combined literature ranges were obtained from Agriculture and Food Systems Institute Crop Composition Database at https://www.cropcomposition.org/CCDB/SelectAnalytes (accessed 12/6/2021) and OECD, 2021.