Biotechnology Notification File No. 000189
CFSAN Note to the File

Date: December 4, 2023

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To: Administrative Record, BNF No. 000189

Subject: JA36 Bintje potato

"Information that Simplot considers to be confidential business information is enclosed in brackets."

Keywords: Potato, *Solanum tuberosum*, Bintje, high tuber set, CRISPR/Cas9, genome editing, [redacted] gene, *Gn2* gene, JA36 potato, J.R. Simplot Company

Summary

J.R. Simplot Company (Simplot) has completed a consultation with the Food and Drug Administration (FDA) on food derived from JA36 potato. JA36 potato was developed by knocking out the [redacted] gene (hereafter referred to as *Gn2*)[1] through CRISPR/Cas9-mediated genome editing technology to produce a plant with more tubers. Simplot refers to the resulting trait as high tuber set. This document summarizes Simplot's conclusions and supporting data and information that FDA's Center for Food Safety and Applied Nutrition (CFSAN, we) evaluated pertaining to human food uses. FDA's Center for Veterinary Medicine summarizes its evaluation pertaining to animal food uses in a separate document.

Based on the safety and nutritional assessment Simplot has conducted, it is our understanding that Simplot concludes:

- it has not introduced into human food a new protein or other substance that would require premarket approval as a food additive, and
- human food from JA36 potato is comparable to and as safe as human food from other potato varieties.

[1] In its submission, Simplot uses "*Gn2*" when referring to the [redacted] gene, the genome editing target, designated by Simplot as confidential business information. The [redacted] gene encodes a [redacted]. By knocking out expression of this gene, [redacted], leading to [redacted] and more tubers in JA36 potato. Reference: [redacted]

CFSAN evaluated data and information supporting these conclusions and considered whether JA36 potato raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act. We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from JA36 potato.

The U.S. Environmental Protection Agency (EPA) evaluates and authorizes the use of plant incorporated protectants (PIPs) under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A PIP is defined in 40 CFR 174.3 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." 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; any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant; and any nitrogen stabilizer."

Subject of the Consultation

Crop	Potato
Designation	JA36
Intended trait	High tuber set
Developer	J.R. Simplot Company
Submission received	January 18, 2022[2]
Amendment received	March 4 and 22, 2022; June 27, 2023; September 8, 2023
Intended use	General use in human food.
Intended genetic change	Deletion mutations in the *Gn2* gene
Method for conferring genetic change	A plasmid was designed to deliver CRISPR/Cas9 tools, including two guide RNAs (Gn2g7 and Gn2g8), to potato cells to target *Gn2* gene for editing. The plasmid was transiently introduced into potato (Bintje variety) cells through *Agrobacterium tumefaciens* mediated transformation.

Molecular Characterization

Confirmation of intended genetic change

Simplot selected JA36 potato using two rounds of selection. During the first round, transformed potato cells that showed resistance to kanamycin were selected and plants were regenerated.[3]

[2] Simplot provided some minor corrections in their original submission on January 21, 2022.
[3] The plasmid used to transform potato cells contains an *nptII* gene that confers kanamycin resistance to transformed cells, permitting these cells to grow on media containing kanamycin.

Next, regenerated potato plantlets that exhibited normal phenotype[4] were identified and grown to the maturity. Simplot confirmed mutations at the targeted editing sites by Polymerase Chain Reaction (PCR) and Sanger sequencing. The potato genome is tetraploid and therefore has four alleles of the target gene *Gn2*. Simplot confirmed edits in all four alleles by sequencing. One allele consisted of 110 base pair (bp) deletion between the Gn2g7 and Gn2g8 target sites; the other three alleles consisted of 1-2 bp deletions at the Gn2g8 target site. The mutations in each allele result in frame shifts and premature stop codons, which are predicted to truncate and inactivate the Gn2 protein in JA36 potato.[5]

Simplot conducted *in silico* analysis of the Bintje potato genome sequence and identified a single potential off-target site with a 2-base difference to Gn2g8 guide RNA sequence. However, Simplot did not identify any mutations at the potential off-target site by nested PCR and DNA sequencing. In addition, Simplot noted that compositional analysis of JA36 potato did not raise food safety or nutrition concerns related to off-target edits. Simplot explained these results are not unexpected as the scientific literature shows that the frequency of off-target edits is low and that off-target effects from gene editing, like mutations that occur during conventional breeding, do not necessarily lead to food safety concerns. Simplot concluded that no off-target mutations were present in the JA36 potato genome.

Inheritance and stability

Simplot evaluated the stability of *Gn2* allele edits in JA36 potato by PCR and Sanger sequencing and showed that the mutations in *Gn2* alleles were stable and maintained after three rounds of vegetative propagation.

Open reading frame analysis

Simplot carried out bioinformatics analysis to assess whether any new open reading frame (ORF) were generated as a result of the targeted genome edits in the JA36 potato and, if so, whether the putative translation of any new ORFs raised allergenicity or toxicity concerns relevant to human food safety. Simplot assessed possible ORFs spanning the targeted genome edits in the JA36 potato genome by comparing the deduced peptides sequences (at least 30 amino acids long) of these hypothetical ORFs to the sequences of known allergen and toxins.[6] Simplot stated that deduced peptides that showed identical sequences between native and

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[4] The entire plasmid was inserted into the cells during transformation. Transformation can lead to either transient or stable transformants; however, due to the presence of only a Right Border sequence, transient transformation is favored. Simplot used two marker genes, *IPT* (Isopentenyl transferase) and *Gn1* [redacted], to screen stable and transient transformants. Stable transformants showed abnormal plant growth phenotype due to the expression of the marker genes. In contrast, transiently transformed plants that did not contain the two marker genes showed normal plant growth phenotype. Reference: Smigocki, AC, Owens, LD, 1988. Cytokinin gene fused with a strong promoter enhances shoot organogenesis and zeatin levels in transformed plant cells. Proc. Natl. Acad. Sci. 85, 5131–5135.

[5] [redacted]

[6] Simplot's allergenicity assessment included sequence-based alignment queries of the Food Allergy Research and Resource Program AllergenOnline database (version 21) and the Health and Environmental Science Institute COMPARE database (version 2021); Simplot's toxicity assessment included a sequence-based alignment query of the UniProtKB database (database generated on June 14, 2021) filtered with the keyword "toxin."

edited alleles were not included in the assessment as these were present in the conventional variety Bintje and therefore not new. Simplot reported that none of the deduced peptides of the hypothetical new ORFs from JA36 potato showed significant similarity with the sequences of known allergens using selection criteria, including a full-length sequence homology search (>50% identity, E-value cutoff 10^{-4}), sliding window of 80 amino acid window alignment search (percent identity >35%), and 100% identity over 8 contiguous amino acids. Additionally, Simplot reported that none of the deduced peptides of the hypothetical new ORFs from JA36 potato showed significant similarity with the sequences of known toxins (E-score cutoff 10^{-2}). Based on the weight of evidence, Simplot concluded that it did not identify any food safety concerns for the deduced peptides of the ORFs spanning the *Gn2* deletion sites in the JA36 potato.

Absence of plasmid DNA

Simplot used a combination of PCR, capture-based target enrichment, and next-generation sequencing approaches to confirm the absence of plasmid DNA in JA36 potato. Simplot concluded from the results of its analysis that no plasmid DNA was present in the JA36 potato genome.

Human Food Nutritional Assessment

Analysis of key nutrients, anti-nutrients, and toxicants

The intended trait in JA36 potato is not expected to alter levels of key nutrients, anti-nutrients, or toxicants. To assess potential unintended changes in composition relevant to safety or nutrition, Simplot compared the composition of unpeeled tubers from JA36 potato and the parental potato variety, Bintje, grown in three locations in the United States in 2021.

Simplot analyzed unpeeled tubers for the levels of nutrients including proximates (ash, carbohydrate (by calculation), fat, protein, moisture), total dietary fiber, calories, vitamins (vitamin B3, vitamin B6, vitamin C), and minerals (copper, magnesium, potassium). Unpeeled tubers were also analyzed for the levels of anti-nutrients (α-solanine, α-chaconine, total glycoalkaloids). Simplot selected these components based on the recommendations in the Organisation for Economic Co-operation and Development (OECD) Revised Consensus Document on Compositional Considerations for New Varieties of Potatoes (*Solanum tuberosum*) (2021). Simplot observed statistical differences in the mean levels for carbohydrate, calories, moisture, and magnesium between JA36 potato and Bintje. However, Simplot states that the mean values of these four components were within the ranges observed for the control variety and within the ranges reported in the Agriculture & Food Systems Institute Crop Composition Database (version 8) and in the published literature [7]. Simplot reported that the mean concentration of glycoalkaloids was not statistically different between JA36 potato and Bintje, which has a history of safe use. Simplot concludes that JA36 potato is compositionally

[7] Published literature included the OECD potato consensus document (2021) and Kozukue et al., 2008 Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated *Solanum* species. J. Agric. Food Chem. 56, 11920–8.

comparable to Bintje and other conventional potato varieties and is as safe and nutritious as potatoes that have a long history of safe consumption.

Conclusion

Based on the information provided by Simplot and other information available to CFSAN, we have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from JA36 potato. We consider the consultation with Simplot on JA36 potato to be complete.

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