FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

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CFSAN Note to the File

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From: Charles Kanobe, Ph.D.

To: Administrative Record, BNF No. 000197

Subject: Potato with transformation event BG25 (BG25 potato)

Keywords: *Solanum tuberosum*, potato, late blight resistance, *Phytophthora infestans*, *Rpi-amr3* gene, AMR3 protein, *Solanum americanum*, *Rpi-blb2* gene, BLB2 protein, *Solanum bulbocastanum*, *Rpi-vnt1* gene, VNT1 protein, *Solanum venturii*, *PVY-CP* gene, *Potato virus Y* resistance, lowered reducing sugars, *VInv* gene, reduced polyphenol oxidase, *Ppo* gene, *Solanum verrucosum,* reduced back spot, RNA interference (RNAi), *StmAls* gene, StmAls protein, acetolactate synthase (ALS)-inhibiting herbicides, *Agrobacterium tumefaciens*, J.R. Simplot Company, BG25, OECD unique identifier SPS-ØBG25-7

Summary

J.R. Simplot Company (Simplot) has completed a consultation with the Food and Drug Administration (FDA) on food derived from BG25 potato. The BG25 potato was genetically engineered to express the Resistance to *Phytophthora infestans* (Rpi) proteins AMR3, BLB2, and VNT1 for resistance against potato late blight disease, and the StmAls protein, which confers tolerance to acetolactate synthase (ALS)-inhibiting herbicides. StmAls was used as a selectable marker. The BG25 potato was also genetically engineered to suppress the expression of *Potato virus Y* Coat Protein (PVY-CP) and induce resistance to PVY using RNA interference (RNAi). Lastly, the BG25 potato was engineered to suppress expression of vacuolar invertase (VINV), and polyphenol oxidase (PPO) to lower levels of reducing sugars and reduce enzymatic browning, referred to as "black spot," respectively, using RNAi. 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 of BG25 potato. 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 BG25 potato is comparable to and as safe as human food from other potato varieties.

CFSAN evaluated data and information supporting these conclusions and considered whether BG25 potato raises other regulatory issues involving human food within FDA's authority under the Federal Food, Drug, and Cosmetic Act (FD&C Act). We have no further questions at this time about the safety, nutrition, and regulatory compliance of human food from BG25 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." In BG25 potato, the AMR3, BLB2, and VNT1 proteins, and the PVY-CP double-stranded RNA (ds-RNA) transcripts are PIPs, while the StmAls protein, which was used as a selectable marker, is a PIP inert ingredient.

Simplot reports that EPA has issued an exemption from the requirement of a tolerance for residues of VNT1 protein in potato (40 CFR § 174.534) and StmAls protein in potato when used as a PIP inert ingredient (40 CFR § 174.544). A temporary exemption from the requirement of a tolerance for residues of AMR3 and BLB2 proteins in potato has also been issued by EPA (40 CFR § 174.545). Since PVY protection relies on presence of nucleic acids, the PVY-CP ds-RNA transcripts in BG25 potato fall under the established exemption from the requirement of a tolerance for residues of nucleic acids that are part of a PIP (40 CFR § 174.507). Simplot further explained that permanent tolerance exemption petitions for ARM3 and BLB2 proteins will be part of the Section 3 registration for BG25 potato. The safety of the AMR3, BLB2, VNT1, and StmAls proteins and the PVY-CP ds-RNA transcripts in BG25 potato falls under EPA's purview and is therefore not addressed in this document.

Subject of the Consultation

Crop	Potato
Designation	BG25
Intended trait 1	Disease resistance against potato late blight
Intended trait 2	Disease resistance against PVY
Intended trait 3	Reduced levels of reducing sugars
Intended trait 4	Reduced enzymatic browning, referred to as "black spot"
Developer	J.R. Simplot Company
Submission received	May 25, 2023
Amendment received	August 31, 2023; April 15, 2024
Intended use	General use in human food
Transformation plasmid	Plasmid pSIM4363

Expression cassette 1	The *Rpi-amr3* gene expression cassette encodes the AMR3 protein from *Solanum americanum*. It confers resistance to potato late blight.
Expression cassette 2	The *Rpi-blb2* gene expression cassette encodes the BLB2 protein from *Solanum bulbocastanum*. It confers resistance to potato late blight.
Expression cassette 3	The *Rpi-vnt1* gene expression cassette encodes the VNT1 protein from *Solanum venturii*. It confers resistance to potato late blight.
Expression cassette 4	RNAi gene suppression cassette targeting the *PVY-CP* gene. The downregulation of PVY-CP confers protection against *Potato virus Y*.
Expression cassette 5	RNAi gene suppression cassette targeting the *VInv* and *Ppo* genes. The downregulation of the VINV and PPO proteins results in reduced conversion of sucrose to glucose and fructose during cold storage of tubers and reduced black spots, respectively.
Expression cassette 6	The *StmAls* gene expression cassette encodes a modified StmAls protein conferring tolerance to ALS-inhibiting herbicides.[1] StmAls was used as a selection marker during transformation.
Method for conferring genetic change	*Agrobacterium*-mediated transformation.

Molecular Characterization

Confirmation of intended genetic change

Simplot developed the BG25 potato by *Agrobacterium*-mediated transformation of Russet Burbank potato with plasmid pSIM4363. After transformation, Simplot used ALS-inhibiting herbicide tolerance to select plantlets containing the T-DNA insert.

Simplot then analyzed the T-DNA insert structure, flanking sequences, insert copy number, genomic integration site, and the absence of vector backbone using next generation sequencing (NGS), droplet digital polymerase chain reaction (ddPCR), PCR, and Sanger sequencing. Simplot reported that BG25 potato contains a nearly full-length T-DNA insert, with a 330 bp deletion from the annotated left border and a 34 bp deletion from the annotated right border element. In addition, 55 bp of genomic DNA were deleted upon insertion of the T-DNA. The insertional site and the section deleted by the insertion were not in a region containing any annotated gene. Simplot reported that BG25 potato contains a single insert on chromosome 12. Analysis of the flanking sequences showed that BG25 potato contains only two unique genome-insertion junctions and no unexpected junctions between non-contiguous regions of the intended insertion. This is consistent with the presence of a single insert. Simplot reported no amplification from six assays designed to amplify regions of the vector backbone, thereby confirming absence of any vector backbone sequences in BG25 potato.

[1] Simplot states that herbicide tolerance is not a commercial trait in BG25 potato, and it will not claim tolerance to field applications of ALS-inhibiting herbicides.

Inheritance and stability

Since commercial potatoes are propagated vegetatively, they do not undergo meiotic recombination and therefore are expected to be genetically stable. Simplot assessed genetic stability of the BG25 event using ddPCR and PCR across three clonal generations, that is: G0, G1, and G2. Simplot observed consistent amplification patterns in each of the three generations indicating a stable transmission of the insertion across clonal generations.

Open reading frame analysis

Simplot assessed the insertion site of BG25 potato and its flanking borders for potential open reading frames (ORFs) and compared the protein translations of these sequences with those from known allergens using the Comprehensive Protein Allergen Resource (COMPARE)[2] database. Simplot identified potential translated ORFs with sequence similarity to a minor allergen, vacuolar invertase, from tomato. Simplot explained that these amino acid sequences are a result of the endogenous VINV protein from *S. tuberosum,* since the construct used to develop the *VInv* cassette in BG25 potato was from the endogenous *Vinv* gene. Since the VINV sequences exist naturally in the potato and were not designed to be translated into protein, Simplot concluded that they would not raise concerns of allergenicity.

Simplot also compared the potential translated ORF sequences with those of known toxins in the UniProtKB[3] database filtered with the keyword "toxin." While some proteins were identified with similarity to the partial VINV sequence, they were not toxins. The identified proteins were homologues of sucrose-degrading enzymes ubiquitously expressed in pathogenic and non-pathogenic bacteria. The accession records for the protein matches contain the keyword "toxin" due to pathogenicity of the host bacteria and not the sucrose degrading activity of the proteins. Therefore, Simplot concluded that the potential translated ORFs would not raise concerns of toxicity.

Characterization of Intended Traits (reduced *Ppo* and *VInv* transcripts)

BG25 potato was partly designed to downregulate the expression of *VInv* and *Ppo* genes through RNAi. Simplot asserts that similar to other submissions previously reviewed by FDA,[4] *Ppo* downregulation did not impact nutritional composition of BG25 potato. Therefore, no data for *Ppo* downregulation was presented. Simplot used reverse transcription-quantitative PCR (RT-qPCR) to determine *VInv* RNA transcript levels in BG25 potato tuber samples. Simplot reported that BG25 potato had reduced expression of *VInv* in tubers, with associated reduction in levels of fructose and glucose, compared to Russet Burbank potato, as expected. Simplot also reported that downregulation of *VInv* and *Ppo* genes is dependent on presence of nucleic acids and that nucleic acids are generally recognized as safe.[5]

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[2] http://db.comparedatabase.org/
[3] https://www.uniprot.org/uniprotkb?query=*
[4] Downregulation of *Ppo* using RNAi was the subject of previous consultations including BNFs 141, 152, 146, 153, and 174, while down regulation of *VInv* using RNAi was the subject of previous consultations including BNFs 146, 153, and 174.
[5] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/statement-policy-foods-derived-new-plant-varieties

Human Food Nutritional Assessment

With the exception of lowering levels of reducing sugars, the intended traits in BG25 potato are not expected to alter levels of key nutrients or anti-nutrients. To assess the intended change in reducing sugars as well as potential unintended changes in composition relevant to human safety or nutrition, Simplot analyzed tubers from BG25 potato, and Russet Burbank potato, the non-genetically engineered (non-GE) control, grown in six locations in the United States in 2021. Field trials were conducted in a randomized complete block design with four replicates per site. At harvest, two samples were collected per replicate per site, resulting in two sets of 24 samples each (48 samples total). Each sample was composed of six randomly selected tubers from each replicate per site. The first set of 24 samples was analyzed immediately after harvest while the second set of samples was stored at 7 °C for six months before being analyzed for sugars.

Lower levels of reducing sugars

Simplot analyzed reducing sugars in fresh tubers and tubers stored for six months. Simplot reported the results stating that BG25 potato had significantly lower levels of reducing sugars at harvest and after storage compared to Russet Burbank potato. Simplot concluded that changes to levels of reducing sugars do not affect levels of key nutrients important in potato and are therefore not nutritionally consequential.[6]

Analysis of key nutrients, anti-nutrients, and toxicants

Simplot assessed tuber samples for proximates (protein, total fat, ash, moisture, and carbohydrates by calculation), total dietary fiber, starch, vitamins, minerals, and glycoalkaloids. For analytes whose measured values were below the limit of quantitation (LOQ), half of the LOQ was used for that value when calculating the mean. When statistically significant differences were identified between BG25 potato and Russet Burbank potato analytical values, the mean values of BG25 potato were compared to ranges in published literature and publicly available databases.[7,8,9] Comparison of results to literature ranges provides context for natural variation of plant composition resulting from a combination of genetic diversity and environmental conditions at time of production.

Simplot reported that no statistically significant differences were observed for analytes, except glycoalkaloids, between BG25 potato and Russet Burbank potato. The mean total glycoalkaloid level was significantly higher in BG25 potato compared to Russet Burbank potato. However, for both BG25 potato and Russet Burbank potato, the mean total glycoalkaloids were lower than the

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[6] OECD, 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 Plant Metabolites.

[7] AFSI, 2022. Potato - Solanum tuberosum. https://www.cropcomposition.org/CCDB/SelectAnalytes.

[8] USDA, 2019. USDA FoodData Central - Potatoes, russet, flesh, and skin, raw (Includes foods for USDA's Food Distribution Program). U.S. DEPARTMENT OF AGRICULTURE Agricultural Research Service. https://fdc.nal.usda.gov/fdc-app.html#/food-details/170027/nutrients.

[9] OECD, 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 Plant Metabolites.

safety limit of 20 mg/100 g fresh weight.[10] In addition, the mean values of these components were within the combined reference range. Simplot therefore concluded that the results of the composition assessment demonstrate that BG25 potato is compositionally equivalent to Russet Burbank potato, the non-GE control and is as safe and nutritious for human food as conventional potatoes.

Human Food Labeling Considerations

It is a producer's or distributor's responsibility to ensure that labeling of the food it markets meets applicable legal requirements, including disclosure of any material differences in the food. It is our understanding that BG25 potato may be used in various food applications. Depending on the particular food application, the reduced browning aspect of BG25 potato may be considered material information requiring disclosure to the consumer under Section 201(n) and Section 403(a)(1) of the FD&C Act. Companies marketing BG25 potato or products containing BG25 potato are advised to consult with FDA's Office of Nutrition and Food Labeling, Food Labeling and Standards Staff to discuss any required or voluntary labeling, including statements relating to attributes of this potato or any other type of claim.

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 BG25 potato. We consider the consultation with Simplot on BG25 potato to be complete.

Charles Kanobe -S

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[10] Smith, D.B., Roddick, J.G., Jones, J.L., 1996. Potato glycoalkaloids: Some unanswered questions. Trends Food Sci Technol 7, 126–131. https://doi.org/10.1016/0924-2244(96)10013-3