FDA U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR VETERINARY MEDICINE

Biotechnology Notification File No. 000197
CVM Note to the File

Date: August 1, 2024

From: Jing Ning, Ph.D.

To: Administrative Record, BNF No. 000197

Subject: Event BG25 Potato

Keywords: Potato, *Solanum tuberosum* subsp. *tuberosum*, RNA interference (RNAi), *Rpi-amr3* gene, *Solanum americanum*, *Rpi-blb2* gene, *Solanum bulbocastanum*, *Rpi-vnt1* gene, *Solanum venturii*, Late blight resistance, PVY-CP, *Potato virus Y*, *VInv* gene, *Solanum tuberosum*, Lower reducing sugars, *Ppo* gene, *Solanum verrucosum*, Reduced black spot, *StmAls*, OECD unique identifier SPS-ØBG25-7, 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 000197. The J.R. Simplot Company (Simplot) submitted a safety and nutritional assessment for a genetically engineered (GE) potato, transformation event BG25 (hereafter referred to as BG25 potato), and additional information afterwards. We evaluated the information in Simplot's submissions to ensure that regulatory and safety issues regarding animal food derived from BG25 potato have been resolved prior to commercial distribution. FDA's Center for Food Safety and Applied Nutrition summarizes its evaluation of uses of BG25 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 first intended effect of the modification in BG25 potato is to confer late blight and *Potato virus Y* (PVY) resistance. Simplot introduced three resistance genes: *Rpi-amr3* gene from *Solanum americanum*, *Rpi-blb2* gene from *Solanum bulbocastanum*, and *Rpi-vnt1* gene from *Solanum venturii*, which confer protection against late blight caused by *Phytophthora infestans*. To confer PVY resistance, Simplot introduced DNA sequences containing inverted repeat segments of PVY coat protein gene, which encodes double-stranded ribonucleic acid (dsRNA) transcripts that trigger an RNA-mediated

silencing mechanism. The second intended effects of the modifications in BG25 potato are to lower levels of reducing sugars and reduce enzymatic darkening (referred to as "black spot"). Simplot introduced DNA sequences containing inverted repeat segments of *vacuolar invertase* gene (*VInv*) and *polyphenol oxidase* gene (*Ppo*), which produce dsRNA to reduce RNA transcript levels of *VInv* and *Ppo,* respectively. *VInv* gene encodes the VINV protein which participates in converting sucrose to its component reducing sugars, while *Ppo* gene encode the Ppo protein which oxidizes phenolic compounds to produce dark pigments. Thirdly, Simplot introduced a modified *acetolactate synthase* gene (*StmAls*) from *Solanum tuberosum* that encodes the StmAls protein which confers tolerance to the acetolactate synthase (ALS) inhibiting herbicides and was used as a selectable marker.

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 regulates PIPs under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the FD&C Act. Under EPA's regulations, the *Rpi-amr3*, *Rpi-blb2*, *Rpi-vnt1* and PVY-CP dsRNA expression cassette and the expression products in BG25 potato are considered pesticidal substances, and the StmAls protein and the genetic material used to express it in BG25 potato are considered inert ingredients. Therefore, the safety assessment of these products falls under the regulatory purview of EPA.

Genetic Modification and Characterization

Introduced DNA and Transformation Method

Simplot transformed internode segments obtained from a conventional potato variety Russet Burbank with plasmid pSIM4363 using disarmed *Agrobacterium tumefaciens* mediated transformation.[1] The transfer-DNA (T-DNA) region within plasmid pSIM4363 contains the following six expression cassettes between left and right border sequences:

- Modified version of the *StmAls* from *S.tuberosum*, which is preceded by the promoter region of the polyubiquitin gene (*ubi7*) and followed by the *Ubiquitin-3* gene terminator (both obtained from *S. tuberosum*).
- *Rpi-vnt1* gene from *S. venturii*, which is preceded and followed by its native promoter and terminator sequences, respectively.
- *Rpi-amr3* gene from *S. americanum*, which is preceded and followed by its native promoter and terminator sequences, respectively.

[1] Richael, C.M., M. Kalyaeva, R.C. Chretien, H. Yan, S. Adimulam, A. Stivison, J.T. Weeks, and C.M. Rommens. 2008. Cytokinin vectors mediate marker-free and backbone-free plant transformation. Transgenic Res. 17: 905-917.

- *Rpi-blb2* gene from *S. bulbocastanum,* which is preceded and followed by its native promoter and terminator sequences, respectively.
- A partial sequence of the *VInv* gene from *S.tuberosum*, a partial sequence of the *Ppo* gene from *Solanum verrucosum*, and a spacer sequence, followed by the complementary partial sequences of *Ppo* and *VInv* in reverse orientation. The cassette is preceded by the *granule-bound starch synthase* promoter and followed by the *ADP glucose pyrophosphorylase* promoter (both promoter sequences were obtained from *S. tuberosum* var. Ranger Russet) in reverse orientation. When transcribed, this cassette generates dsRNAs with partial sequences of *VInv* and *Ppo*.
- A partial sequence of the PVY-CP from *Potato virus Y* and a spacer sequence, followed by the reverse complementary sequence of the partial PVY-CP sequence. The cassette is preceded by the promoter region of the *ubi7* gene and followed by the *Ubiquitin-3* gene terminator (both sequences were obtained from *S. tuberosum*).

Following transformation, explants were grown in selection medium[2] and then grown in fresh medium that induced shoot development. Shoots that displayed cytokinin overproduction phenotype were discarded. Genomic DNA was isolated from leaves of established plants and polymerase chain reaction (PCR) was used to confirm the presence of the T-DNA region of plasmid pSIM4363. Regenerated plantlets were grown to maturity prior to analysis of the transformed lines for insert number, insert integrity, absence of vector backbone sequences, gene silencing efficacy, and agronomic performance.

Simplot used a combination of techniques, including whole genome sequencing (WGS), droplet digital PCR (ddPCR), and Sanger sequencing to characterize the insertion event in BG25 potato. The parental variety, Russet Burbank, was used as the comparator in these analyses. Simplot reports a minimum average read depth of 99-fold in WGS. Simplot also reports that a single copy of the T-DNA sequence was inserted into the BG25 potato genome and that backbone sequences from pSIM4363 were not present in BG25 potato. WGS and Sanger sequencing demonstrated that the T-DNA insert replaced 55 bp of potato genomic DNA, with a minor truncation at the right border and another 330bp truncation at the left border. Simplot stated that the genetic modification does not interrupt any known genes.

The stability of the inserted T-DNA sequences in BG25 potato was assessed using ddPCR and PCR in plants that had undergone successive rounds of vegetative propagation. Six targeted regions were assessed by ddPCR and two event-specific primer pairs were used in PCR to detect the T-DNA insertion site. Simplot states that PCR analysis confirmed the stability of T-DNA insert in BG25 potato across three rounds of vegetative propagation.

Simplot performed bioinformatics analyses using sequences obtained for the T-DNA insert and junction sequences to determine whether insertion of the introduced DNA

[2] The selection media contained imazamox, an ALS-inhibiting herbicide for selection of transformants and timentin for inhibition of *A. tumefaciens* growth.

created any potential open reading frames (ORF) that could encode putative polypeptides. Simplot utilized the UniProtKB database to determine the similarity of the putative polypeptides to known toxins, and subsequently identified several sucrose-degrading proteins with sequence similarity to the partial VINV sequence on pSIM4363. Simplot concludes that these proteins do not raise safety concerns because sucrose degrading proteins are ubiquitously expressed in pathogenic and non-pathogenic bacteria. In addition, the dsRNA sequence is processed into short RNA interference transcripts, thus is unlikely to be translated into a protein. Based on the results of bioinformatics analyses, Simplot concludes that the T-DNA insertion do not lead to the production of putative polypeptides that would raise animal food safety concerns.

Intended Effects: Reduced Levels of Specific RNA Transcripts in Tubers

To characterize the level of the *VInv* transcript targeted by the gene silencing construct, Simplot performed Reverse transcription-quantitative PCR (RT-qPCR) on RNA isolated from tubers obtained from three replicates of field grown BG25 potato and Russet Burbank (control) varieties. Simplot reported reduction in RNA transcript for the *VInv* gene in BG25 potato tubers when compared to control tubers.[3]

Animal Food Use

Simplot states that BG25 potato is expected to be grown for the same uses as Russet Burbank potatoes. The typical uses of potato-derived food and feed are well documented in Organisation for Economic Co-operation and Development (OECD) potato composition consensus document,[4] including use of potato peels, out of specification potatoes, unprocessed raw potatoes, and processed discards from cooked potatoes for animal food. Simplot states that potatoes are commonly used in diets for cattle, sheep and swine.

Composition

Scope of Analysis

Simplot conducted compositional analyses on tubers obtained from BG25 potato and the non-GE control variety, Russet Burbank (control). The components selected for analysis were listed as key nutrients in the OECD potato consensus document.

Study Design

Simplot grew BG25 potato and control at six locations in the United States in 2021. A randomized complete block design with four replicate plots was used at each field site. The cultivation practices were location specific, based on recommendations from regional specialists. Each sample was comprised of randomly selected six tubers from each replicate at each site and, thus, resulted in a total of 48 samples.

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[3] Simplot states that the down regulation of *Ppo* has been previously evaluated in several plant varieties (BNF No. 000141, BNF No. 000146, BNF No. 000152, BNF No. 000153 and BNF No. 000174), and it does not affect the nutritional composition of the BG25 potato. Therefore, this data is not included in the submission.

[4] 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.

Simplot presents mean values, standard deviations, and range of values for each component for BG25 potato and control, and results of the statistical comparison of BG25 potato and control. If analyte values were below the limit of quantitation (LOQ), Simplot replaced values below the LOQ with a value equal to half of the LOQ. If there was a statistically significant difference in a component between BG25 potato and control across locations, then the means for the component were compared to ranges obtained from public databases or scientific literatures (hereafter referred to as combined reference range).[5]

Results of Analyses

Simplot reports values for proximates (moisture, crude protein, total fat, carbohydrates by calculation, and ash), fiber ((acid detergent fiber (ADF), neutral detergent fiber (NDF), and total dietary fiber (TDF)), two vitamins (vitamin B6 and vitamin C), two minerals (magnesium and potassium), and total glycoalkaloids. Simplot reported no statistically significant differences for each of these components between BG25 potato and control, with the exception of total glycoalkaloids. Statistically significant differences between BG25 potato and the control were reported for total glycoalkaloids, 12.7 and 9.84 milligrams/100 grams for BG25 potato and control, respectively. Simplot notes that the mean values for all of these components in BG25 potato and control fell within the combined reference range. Simplot concludes that BG25 potato is compositionally comparable to control potato.

Intended Compositional Change – Reduction in Reducing Sugars

To assess the efficacy of intended effects, Simplot also determined the levels of sucrose and reducing sugars (fructose and glucose) in tubers. Simplot measured concentration of sucrose and reducing sugars in potatoes at harvest and after storage at 7°C for six months. Simplot reports that the concentrations of reducing sugars were statistically lower in stored BG25 potato when compared to control. The concentration of sucrose was statistically higher in stored BG25 potato when compared to control. Simplot notes that the mean values for all of these components in fresh and stored BG25 potato fell within the combined reference range.

Summary of Compositional Analyses

Simplot states that BG25 potato is compositionally equivalent to Russet Burbank, a

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[5] For proximates, minerals, vitamins and starch, Simplot referred to data presented in Agriculture and Food Systems Institute (AFSI) Crop Composition Database at https://www.cropcomposition.org/CCDB/SelectAnalytes, OECD potato consensus document and USDA FoodData Center at https://fdc.nal.usda.gov/fdc-app.html#/food-details/170027/nutrients. For glycoalkaloids, Simplot referred to Kozukue et al 2008. For sugars, Simplot referred to Amrein et al 2003 and Vivanti et al 2006 and AFSI Database. For fructose and glucose, Simplot referred to AFSI Database. Kozukue, N., K.-S. Yoon, G.-I. Byun, S. Misoo, C.E. Levin, and M. Friedman. 2008. Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated *Solanum* species. J. Agric. Food Chem. 56: 11920-11928. Amrein, T.M., S. Bachmann, A. Noti, M. Biedermann, M.F. Barbosa, S. Biedermann-Brem, K. Grob, A. Keiser, P. Realini, F. Escher, R. Amado. 2003. Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. J. Agric. Food Chem. 51:5556-5560; Vivanti, V. E. Finotti, and M. Friedman. 2006. Level of acrylamide precursors asparagine, fructose, glucose, and sucrose in potatoes sold at retail in Italy and in the United States. J. Food Sci. 71:C81-C85.

commercially grown potato variety, and BG25 potato is as safe and nutritious for use in animal food as conventional potato varieties that have a long history of safe use in animal food.

Conclusion

CVM evaluated Simplot's submissions to determine whether BG25 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 BG25 potato and the animal foods derived from it are as safe as and are not materially different in composition 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 BG25 potato for use in animal food to be complete.

Jing Ning -S

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Jing Ning, Ph.D.
Molecular Biology Staff Fellow