United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

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Dr. Shaun Curtin
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Re: Confirmation that a *Glycine max* (soybean) line mutagenized using CRISPR-Cas9 is not a regulated article

Dear Dr. Curtin,

Thank you for your letter dated August 7, 2017, inquiring whether the soybean product described in your letter is a regulated article. Your letter describes the *Drb2a-Drb2b* mutant soybean line that will be used to study putative drought and salt stress tolerance. In your letter you requested deregulation by APHIS of your soybean line 590-4-28-5 rather than inquiring about its regulatory status. This line has not been regulated under a notification or permit issued pursuant to 7 CFR part 340 and therefore it cannot be deregulated. Accordingly, we are responding to your letter by informing you of the regulatory status of your soybean line through the "Am I Regulated" (AIR) process.

The Plant Protection Act (PPA) of 2000 gives USDA the authority to oversee the detection, control, eradication, suppression, prevention, or retardation of the spread of plant pests or noxious weeds to protect the agriculture, environment, and economy of the United States. The APHIS mission is to protect the health and value of American agriculture and natural resources.

APHIS regulates the importation, interstate movement and environmental release (field testing) of certain genetically engineered (GE) organisms that are, or have the potential to be, plant pests. Regulations for GE organisms that are or have the potential to be plant pests, under the PPA, are codified at 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests." Under the provisions of these regulations, a GE organism is deemed a regulated article if it has been genetically engineered using a donor organism, recipient organism, or vector or vector agent that is listed in §340.2 and meets the definition of a plant pest, or that is an unclassified organism and/or an organism whose classification is unknown, or if the Administrator determines that the GE organism is a plant pest or has reason to believe it is a plant pest.

In your August 7, 2017 letter, you describe your soybean (*Glycine max* cv. Bert) line 590-4-28-5, which has been modified to have deactivated double-stranded RNA binding protein2 (*Drb2a* and *Drb2b*) genes, which are genomic loci Glyma.12g075700 and Glyma.11g145900. You state that the line was developed by introducing a CRISPR-Cas9 reagent into soybean parent line 590-4, using *Agrobacterium rhizogenes-*

mediated transformation. The integrated CRISPR construct expressed two guide RNAs, targeted to the coding regions of the *Drb2a* and *Drb2b* genes. The action of the CRISPR reagent resulted in frame shift mutations and deactivation of the target genes. The original transformed line 590-4 was self-pollinated, and progeny lines that contained the mutations in *Drb2a* and *Drb2B*, but lacked the CRISPR reagent construct sequence were selected. You state that you used PCR assays based on whole genome sequencing (WGS) data to confirm lines that lacked the CRISPR reagent construct sequence.

Based on the information you provided in your letter, APHIS has determined that soybean line 590-4-28-5 does not contain any introduced genetic material. Although soybean parent line 590-4 contained introduced plant pest sequences and would be regulated pursuant to 7 CFR part 340, the selected soybean *Drb2a- Drb2b* mutant line 590-4-28-5 lacked any introduced sequences. Therefore, consistent with previous responses to similar letters of inquiry, APHIS does not consider soybean line 590-4-28-5 as described in your August 7, 2017 letter to be regulated pursuant to 7 CFR part 340. Additionally, soybean is not listed as a Federal noxious weed pursuant to 7 CFR part 360, and APHIS has no reason to believe that the drought and salt tolerance phenotype in your soybean line 590-4-28-5 would increase the weediness of soybean.

Please be advised that the importation of soybean seeds or plants, like all other soybean, will be subject to APHIS Plant Protection and Quarantine (PPQ), permit and/or quarantine requirements. For further information, should you plan to import these soybean seeds or plants, you may contact Shailaja Rabindran at 301-851-2167 or contact PPQ general number for such inquiries at (877) 770-5990.

Please be advised that your soybean line 590-4-28-5, while not regulated by APHIS under 7 CFR part 340 may still be subject to other regulatory authorities such as FDA or EPA.

Should you become aware at any time of any issues that may affect the Agency's conclusion regarding this inquiry, you must immediately notify the Agency in writing of the nature of the issue. We hope that you appreciate our commitment to plant health and support for the responsible stewardship for the introduction of GE plants.

Sincerely,

M. J. Firko

10/16/2017

Michael J. Firko, Ph.D.
APHIS Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date