



United States  
Department of  
Agriculture

Animal and  
Plant Health  
Inspection  
Service

Biotechnology  
Regulatory  
Services

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Dr. Thomas Brutnell  
Director, Enterprise Institute for Renewable Fuels  
Donald Danforth Plant Science Center  
975 N. Warson Road  
St. Louis, MO 63132

Re: Confirmation of regulatory status of CRISPR-Cas9-mutagenized *Setaria viridis*  
line Cas9\_193-31

Dear Dr. Thomas Brutnell:

Thank you for your letter dated February 29, 2016 inquiring whether the CRISPR-Cas9-mutagenized *Setaria viridis* lines described in your letter are regulated articles under 7 CFR part 340. Your letter described four lines (Cas9\_193-16, Cas9\_193-17, Cas9\_193-24, and Cas9-193-31) of *S. viridis* in which the homolog of the *Zea mays* ID1 gene has been deactivated by a CRISPR-Cas9 induced mutation. In subsequent conversations concerning your request for regulatory status of these four lines of *S. viridis*, you described them as having a delayed flowering phenotype. Based on assessment of the deletion, this is consistent with our interpretation.

In a follow-up letter dated March 15, 2017, you stated that except for the line Cas9\_193-31 you are no longer inquiring the regulatory status of other three lines (Cas9\_193-16, Cas9\_193-17, and Cas9\_193-24) that were part of your original inquiry dated February 29, 2016. Therefore, only *S. viridis* line Cas9\_193-31 is the subject of this response.

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 Plant Protection Act, 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 February 29, 2016 letter, you described a CRISPR-Cas9-mutagenized *S. viridis* line with a targeted mutation in the *S. viridis* homolog of the *Z. mays* ID1 gene resulting in a delayed flowering phenotype. As described in your letter, the parent plant of the CRISPR-Cas9-mutagenized line was genetically engineered with a plant pest vector (*Agrobacterium tumefaciens*) and contained DNA from plant pests (*A. tumefaciens* and cauliflower mosaic caulimovirus). Subsequently, the inserted DNA was segregated from the targeted deletion through conventional breeding, thus producing progeny without the inserted DNA.

APHIS has reviewed the information in your February 29, 2016 and March 15, 2017 letters and has determined that *S. viridis* itself is not a plant pest. Additionally, APHIS agrees that the CRISPR-Cas9-mutagenized *S. viridis* line described in your two letters does not contain any introduced genetic material from plant pests. APHIS also has no reason to believe that the CRISPR-Cas9-mutagenized line is a plant pest. Furthermore, similar changes in the genome, in this case a deletion, can occur naturally, therefore, consistent with previous responses to similar letters of inquiry, APHIS does not consider your CRISPR-Cas9-mutagenized *S. viridis* line Cas9\_193-31, as described in your two letters, to be regulated under 7 CFR part 340.

APHIS is also authorized to protect American agriculture from damage caused by noxious weeds. If APHIS determines that a plant poses a noxious weed risk, APHIS would consider regulating the plant under the noxious weed regulation, 7 CFR part 360. APHIS has the option to regulate plants under 7 CFR part 360 regardless of whether or not they meet the definition of regulated article under 7 CFR part 340. APHIS has determined that *S. viridis* is not currently listed as a Federal Noxious Weed or listed in any U.S. State as a noxious weed. However, APHIS has concluded after reviewing the relevant literature and a weed risk assessment conducted by APHIS, that *S. viridis* has the potential to be a problematic weed in certain environments, including agriculture. Moreover, *S. viridis* is known to produce fertile hybrids with the cultivated type *S. italica* (foxtail millet) that is grown for hay in the United States. As it is unknown whether producing fertile crosses would have an impact on the quality of *S. italica*, we recommend appropriate isolation distances be maintained to avoid this potential as a Best Management Practice (BMP).

Please be advised like all *S. viridis*, the importation of CRISPR-Cas9-mutagenized *S. viridis* will be subject to APHIS Plant Protection and Quarantine (PPQ), permit and/or quarantine requirements. For further information, on the importation of *S. viridis*, you may contact Shailaja Rabindran at 301-851-2167 or contact PPQ general number for such inquiries at (877) 770-5990.



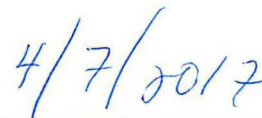
Please be advised that your CRISPR-Cas9-mutagenized *S. viridis* line may still be subject to other regulatory authorities such as FDA or EPA.

Should you become aware at any time of any issues or additional information 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 you appreciate our commitment to plant health and support for the responsible stewardship for the introduction of GE plants.

Sincerely,



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



Date

