United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

4700 River Road
Riverdale, MD
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Yianni Lagos
Soil Culture Solutions, LLC (d/b/a Soilcea)
3802 Spectrum BLVD, Suite 142H
Tampa, FL 33612

Re: Confirmation of the regulatory status of citrus trees genome-edited with CRISPR/Cas9 to confer improved tolerance to citrus canker

Dear Yianni Lagos,

Thank you for your letter dated September 16, 2019, inquiring whether the sweet orange (*Citrus sinensis*) product described in your letter is a regulated article under 7 CFR part 340. Your letter describes using CRISPR/Cas9 via PEG mediated transient transformation, resulting in the genome-edited citrus trees with improved tolerance to citrus canker.

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.

USDA 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 September 16, 2019 letter, you describe your citrus trees with improved tolerance to citrus canker. You state that this was achieved by small deletions in the CsLob1 gene which are produced by the endogenous DNA repair machinery after Cas9 cleaves the DNA target. A plasmid containing sequences encoding the Cas9 protein and the sgRNA was delivered into protoplasts using PEG-mediated transformation. The plasmid contained bacterial, animal and plant donors as well as a SV40 nuclear localization signal and an artificial peptide tag on Cas9. The plasmid did not contain sequences for genome integration; therefore, the final plant does not contain any foreign DNA sequences. Some plant pest donors were included in the plasmid used for transformation; however, after transformation, the plant cells were cultured and through that process, the cell degraded the plasmid DNA, Cas9 protein, and sgRNA before trees were regenerated from the cell culture.

Based on the information you provided in your September 16, 2019 letter, USDA has determined that the LOB1 gene-edited citrus trees described in your letter do not contain any introduced genetic material. Although the protoplasts isolated from a sweet orange embryogenic callus cultures contained introduced plant pest sequences from the transient transformation with the plasmid and would be regulated pursuant to 7 CFR part 340, the selected plants were cultured using standard plant tissue culture methods. Through this process, the cell degraded the plasmid DNA, Cas9 protein, and sgRNA before the tree regenerated from the cell culture and therefore lacks any introduced DNA sequences. Therefore, consistent with previous responses to similar letters of inquiry, APHIS does not consider LOB1 gene edited citrus trees as described in your September 16, 2019 letter to be regulated pursuant to 7 CFR part 340. Additionally, sweet orange is not listed as a Federal noxious weed pursuant to 7 CFR part 360, and APHIS has no reason to believe that the improved tolerance to citrus canker phenotype in your LOB1 gene-edited citrus would increase the weediness of sweet orange.

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

Please be advised that your LOB1 gene-edited citrus, while not regulated by APHIS under 7 CFR part 340 may still be subject to other regulatory authorities such as the U.S. Environmental Protection Agency (EPA) or the U.S. Food and Drug Administration (FDA). To inquire about the regulatory status of your product with the EPA, please contact Alan Reynolds at 703-605-0515. To inquire about the regulatory status of your product with the FDA, please contact Robert Merker at 240-402-1226.

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,

1/21/2020

Bernadette Juarez
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date