United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

4700 River Road
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Dr. John Sedbrook
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Normal, IL 61790 USA

Re: Confirmation of the regulatory status of CRISPR mutagenized *Thlaspi arvense* L. (pennycress) lines.

Dear Dr. Sedbrook,

Thank you for your letter dated December 11th, 2018, inquiring whether the pennycress (*Thlaspi arvense* L.) lines described in your letter are regulated articles under 7 CFR part 340. Your letter describes mutant pennycress lines obtained using CRISPR gene editing technology and traditional breeding methods resulting in the desired phenotype.

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 December 11, 2018 letter you describe the production process of your mutant pennycress (*T. arvense*) lines, the intended phenotype and supporting evidence. These pennycress lines were obtained using CRISPR DNA constructs designed for targeted genome editing of two genes, using a disarmed strain of *Agrobacterium tumefaciens* and a standard plant transformation method. The expression cassettes introduced into the wild type pennycress lines contained the DNA elements necessary for gene editing and

antibiotic resistance, and included DNA from plant pests. Of the six independent pennycress lines selected, four had mutations (insertions or deletions) in the first targeted gene, while two lines had mutations (insertions or deletions) in the second targeted gene. Your inquiry stated that these mutations resulted from the plant's error prone endogenous DNA repair mechanism. Mutant lines containing no traces of the CRISPR DNA construct were generated by back-crossing the originally-transformed lines to wild-type plants.

In your December 11, 2018, letter of inquiry you stated that the segregation of inserted transgenic material was possible since the CRISPR construct had inserted into chromosomal locations unlinked to the two targeted genes. The progeny lines were screened for lack of antibiotic resistance and by PCR analysis to confirm the absence of intentionally inserted DNA sequences. You also stated that although the original transgenic lines were generated by deliberately inserting DNA sequences, the resulting six mutant progeny lines do not contain any deliberately inserted DNA sequences and express the desired phenotype.

Based on the information you provided in your letter, USDA has concluded that your mutant pennycress lines are not themselves plant pests. USDA has accepted your attestation that these genome edited pennycress lines do not contain any deliberately inserted DNA sequences. Therefore, consistent with previous responses to similar letters of inquiry, USDA does not consider the CRISPR gene edited lines of pennycress, as described in your December 11, 2018 letter, to be regulated pursuant to 7 CFR part 340. However, please be aware that accidental release of GE pennycress plants that do contain deliberately inserted DNA sequences may be a violation of our regulations. We encourage Illinois State University to continue to perform both phenotypic and molecular analyses to confirm that your genome edited products do not contain deliberately insertd DNA sequences inherited from the GE parents.

Additionally, although pennycress is not listed as a Federal noxious weed pursuant to 7 CFR part 360, it is a troublesome agricultural weed in the U.S. Based on the information provided in your letter of inquiry, USDA has no reason to believe that the genetic modifications described in your inquiry would increase the weediness of the mutant pennycress lines relative to the wild type pennycress comparator.

Please be advised that the importation of CRISPR gene edited pennycress seeds or plants, like all other pennycress, will be subject to Plant Protection and Quarantine (PPQ), permit and/or quarantine requirements. For further information, should you plan to

import these pennycress seeds or plants, you may contact the PPQ general number for such inquiries at (877) 770-5990.

Please be advised that your pennycress lines, 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

4/19/2019

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

Date