E 3410-34-P

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. APHIS-2020-0072]

Movement of Organisms Modified or Produced through Genetic Engineering; Notice of Exemptions

AGENCY: Animal and Plant Health Inspection Service, Agriculture (USDA).

ACTION: Notice.

SUMMARY: We are advising the public that we are proposing to exempt plants with additional modifications that could otherwise be achieved through conventional breeding from the regulations that govern the introduction (importation, interstate movement, or release into the environment) of certain organisms modified or produced through genetic engineering. The exempt plants would have distinct modifications on the paternal and maternal alleles of a single gene resulting from repair of a targeted DNA break; deletions generated using an externally provided repair template; or deletions resulting from repair of two targeted double strand breaks on a chromosome. This action would reduce the regulatory burden for developers of certain plants modified or produced through genetic engineering that are unlikely to pose plant pest risks while enabling the Animal and Plant Health Inspection Service to focus its regulatory resources on risk analyses of unfamiliar products and those more likely to pose a plant pest risk.

DATES: We will consider all comments that we receive on or before [Insert date 30 days after date of publication in the Federal Register].

ADDRESSES: You may submit comments by either of the following methods:

- Federal eRulemaking Portal: Go to www.regulations.gov. Enter APHIS-2020-0072 in the Search field. Select the Documents tab, then select the Comment button in the list of documents.

- Postal Mail/Commercial Delivery: Send your comment to Docket No. APHIS-2020-0072, Regulatory Analysis and Development, PPD, APHIS, Station 3A-03.8, 4700 River Road Unit 118, Riverdale, MD 20737-1238.

Supporting documents and any comments we receive on this docket may be viewed at www.regulations.gov or in our reading room, which is located in room 1620 of the USDA South Building, 14th Street and Independence Avenue SW, Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 799-7039 before coming.

FOR FURTHER INFORMATION CONTACT: Dr. Neil Hoffman, Science Advisor, Biotechnology Regulatory Services, APHIS, 4700 River Road Unit 98, Riverdale, MD 20737-1238; (301) 851-3947.

SUPPLEMENTARY INFORMATION:

The regulations in 7 CFR part 340 govern the introduction (importation, interstate movement, or release into the environment) of certain organisms modified or produced through genetic engineering. The Animal and Plant Health Inspection Service (APHIS) first issued these regulations in 1987 under the authority of the Federal Plant Pest Act of 1957 and the Plant Quarantine Act of 1912, two acts that were subsumed into the Plant Protection Act (PPA, 7 U.S.C. 7701 *et seq.*) in 2000, along with other provisions. Since 1987, APHIS has amended the regulations seven times, in 1988, 1990, 1993, 1994, 1997, 2005, and 2020.

On May 18, 2020, we published in the *Federal Register* (85 FR 29790-29838, Docket No. APHIS-2018-0034) a final rule[1] that marked the first comprehensive revision of the regulations since they were established in 1987. The final rule provided a clear, predictable, and efficient regulatory pathway for innovators, facilitating the development of organisms developed using genetic engineering that are unlikely to pose plant pest risks.

[1] To view the final rule and supporting documents, go to https://www.regulations.gov, and enter APHIS-2018-0034 in the Search field.

The May 2020 final rule included regulatory exemptions for certain categories of plants that have been modified. Specifically, § 340.1(b) exempted plants that contain a single modification of one of the following types, specified in § 340.1(b)(1) through (3):

- The genetic modification is a change resulting from cellular repair of a targeted DNA break in the absence of an externally provided repair template; or
- The genetic modification is a targeted single base pair substitution; or
- The genetic modification introduces a gene known to occur in the plant's gene pool or makes changes in a targeted sequence to correspond to a known allele of such a gene or to a known structural variation present in the gene pool.

In addition to the modifications listed above, § 340.1(b)(4) provides that the Administrator may propose to exempt plants with additional modifications, based on what could be achieved through conventional breeding. Such proposals may either be APHIS-initiated or may be initiated via a request that is accompanied by adequate supporting information and submitted by another party. In either case, APHIS will publish a notice in the *Federal Register* of the proposal, along with the supporting documentation, and will request public comments. After reviewing the comments, APHIS will publish a subsequent notice in the *Federal Register* announcing its final determination. A list specifying modifications a plant can contain and be exempt pursuant to paragraph (b)(4) is available on the APHIS website at https://www.aphis.usda.gov/aphis/ourfocus/biotechnology.

In this document, we are proposing to add three modifications that plants can contain and be exempt from regulation pursuant to § 340.1. These modifications are similar and functionally equivalent to modifications that commonly occur within conventional breeding and to the modification described in § 340.1(b)(1), but enable a developer to more efficiently obtain a complete loss of function of a targeted gene. We are also making available for public review scientific literature that we consulted prior to initiating the proposal. The literature supports exempting plants with these additional modifications.

Under the first additional genetic modification proposed, plants would not be subject to the regulations when cellular repair of a targeted DNA break in the same location on two homologous chromosomes, in the absence of a repair template, results in homozygous or heterozygous biallelic mutations, each of which is a loss of function mutation. A double strand break followed by cellular repair often occurs in both paternal and maternal alleles (biallelic) during genome editing. As a range of DNA indels frequently occur after a double strand break, the mutation in the paternal allele often differs from the mutation in the maternal allele. Biallelic knockout mutations are easily obtained in conventional breeding through self-fertilizing or backcrossing and selection. In this case, the biallelic mutation is usually homozygous. However, in cases where the deletions are not identical but both deletions lead to a loss of function of the allele, the phenotype will be the same as the homozygous biallelic mutation obtained through conventional breeding. If both alleles are modified by indels such that neither allele is functional, the size, position, and sequence of the indels within the gene need not be identical to qualify for the exemption.

The second additional genetic modification proposed is a contiguous deletion of any size resulting from cellular repair of a targeted DNA break in the presence of an externally supplied repair template. The deletion can occur on one or two homologous chromosomes. This modification is similar to the one described in § 340.1(b)(1), except that it allows an externally supplied repair template to be used. When genome editing is used to create a single DNA break, a range of indels result from the cellular repair mechanism. To limit the range of mutations recovered and, therefore, to more efficiently obtain a complete loss of function of the targeted gene(s), some developers also add a template to guide the repair process. To limit this proposed additional modification to what is achievable through conventional breeding, it would only apply to deletions created by the double strand break and externally supplied repair template.

The third additional genetic modification proposed is for a change resulting from cellular repair of two targeted DNA breaks on a single chromosome or at the same location on two

homologous chromosomes, when the repair results in a contiguous deletion of any size in the presence or absence of a repair template, or in a contiguous deletion of any size combined with an insertion of DNA in the absence of a repair template. The insertion cannot result from the insertion of exogenous construct DNA. The modifications on two homologous chromosomes can be heterozygous as long as each results in a loss of function of the targeted gene(s). To qualify for the exemption, the plant must have mutations that are restricted to a pair of homologous chromosomes in diploids and allopolyploids or any two homologous chromosomes in autopolyploids. Radiation mutagenesis, which is commonly used in conventional breeding, can create any size deletion. As mutations are typically detrimental to the organism, what is achievable in practice is limited by the viability and fertility of the organism. Large mutations can be maintained in a heterozygous state but do not tend to undergo homozygous inheritance (Naito, 2005).[2] For example, in Arabidopsis, which has a genome size of 135 Mb (Arabidopsis Genome Initiative, 2000), a radiation-induced deletion of 3.1 Mb was obtained that disrupted 852 genes and was maintainable only as a heterozygote, presumably because genes essential for survival are present in the deleted region (Kazama, *et al*., 2017).[3] Polyploid plants and those with large genomes are better able to accommodate even larger deletions (Men *et al*., 2002).[4] For example, in hexaploid wheat, X-ray mutagenesis was used to create a mutant, *ph1*, widely

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[2] Naito, K., M. Kusaba, N. Shikazono, T. Takano, A. Tanaka, T. Tanisaka, and M. Nishimura (2005). Transmissible and nontransmissible mutations induced by irradiating Arabidopsis thaliana pollen with gamma-rays and carbon ions. Genetics, 169, 881-889.

[3] Kazama, Y., K. Ishii, T. Hirano, T. Wakana, M. Yamada, S. Ohbu, and T. Abe (2017). Different mutational function of low- and high-linear energy transfer heavy-ion irradiation demonstrated by whole-genome resequencing of Arabidopsis mutants. Plant J. 92, 1020-1030.

[4] Men, A.E., T.S. Laniya, I.R. Searle, I. Iturbe-Ormaetxe, I. Gresshoff, Q. Jiang, B.J. Carroll, and P.M. Gresshoff (2002). Fast Neutron Mutagenesis of Soybean (Glycine soja L.) Produces a Supernodulating Mutant Containing a Large Deletion in Linkage Group H. Genome Letters 3: 147-155.

used in breeding programs, that has a 70 Mb deletion (Sears, 1977).[5] To put the size of this wheat deletion in perspective, it is larger than half of the entire genome of Arabidopsis. Based on the use of plants with large deletion mutations in conventional breeding programs, any size contiguous deletion created by two double strand breaks should be exempted because it falls well within what could be achieved through conventional breeding.

After reviewing any comments we receive, we will announce our decision regarding the three new modifications that plants could contain and qualify for exemption in a subsequent notice.

Authority: 7 U.S.C. 7701-7772 and 7781-7786; 31 U.S.C. 9701; 7 CFR 2.22, 2.80, and 371.3.

Done in Washington, DC, this 14th day of July, 2021.

Michael Watson,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 2021-15236 Filed: 7/16/2021 8:45 am; Publication Date: 7/19/2021]

[5] Sears, E. A. (1977). An induced mutant with homoeologous pairing in common wheat. Canadian J of Genetics and Cytology 19: 585-593.