National Organic Standards Board
Materials/GMO Subcommittee Discussion Document
Excluded Methods Determinations
August 12, 2021

Introduction and background
Cell fusion and protoplast fusion have a nuanced history in the context of the USDA's National Organic Program and the work of the National Organic Standards Board. Cell fusion is included under terms defined at §205.2 as an excluded method. In 2013, the NOP clarified its position on both techniques in Policy Memo 13-1 allowing for both techniques to be used solely within taxonomic plant families. As work by the NOSB progressed in this area, cell fusion and protoplast fusion continue to be included as techniques to be evaluated on the excluded methods "TBD list" with notes indicating follow-up work by the NOSB.

Goals of this document
The Materials Subcommittee is seeking feedback on the TBD list terms 'cell fusion' and 'protoplast fusion.' This document will outline the history and explore context towards determining if more discussion is necessary on the issues of cell fusion and protoplast fusion as excluded methods in organic systems.

Definitions and Criteria
Under the NOP organic regulations, methods that employ genetic engineering techniques are excluded from use in organic production. The current regulation defines an excluded method at §205.2 Terms defined:

A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production. Such methods include cell fusion, microencapsulation and macroencapsulation, and recombinant DNA technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by recombinant DNA technology). Such methods do not include the use of traditional breeding, conjugation, fermentation, hybridization, in vitro fertilization, or tissue culture.

The NOSB previously recommended the use of the following definitions to determine whether or not a method should be/is excluded.

Genetic engineering (GE) – A set of techniques from modern biotechnology (such as altered and/or recombinant DNA and RNA) by which the genetic material of plants, animals, organisms, cells, and other biological units are altered and recombined.

Genetically Modified Organism (GMO) – A plant, animal, or organism that is from genetic engineering as defined here. This term will also apply to products and derivatives from genetically engineered sources. (Modified slightly from IFOAM Position)

Modern Biotechnology – (i) in vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles, or (ii) fusion of cells beyond the taxonomic family, that overcomes natural, physiological reproductive or recombination barriers, and that are not techniques used in traditional breeding and selection. (From Codex Alimentarius)

Synthetic Biology – A further development and new dimension of modern biotechnology that combines science, technology, and engineering to facilitate and accelerate the design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems. (Operational Definition developed by the Ad Hoc Technical Expert Group on Synthetic Biology of the UN Convention on Biological Diversity)

Non-GMO – The term used to describe or label a product that was produced without any of the excluded methods defined in the organic regulations and corresponding NOP policy. The term "non-GMO" is consistent with process-based standards of the NOP where preventive practices and procedures are in place to prevent GMO contamination while recognizing the possibility of inadvertent presence.

Classical/Traditional plant breeding – Classical (also known as traditional) plant breeding relies on phenotypic selection, field-based testing, and statistical methods for developing varieties or identifying superior individuals from a population, rather than on techniques of modern biotechnology. The steps to conduct breeding include: generation of genetic variability in plant populations for traits of interest through controlled crossing (or starting with genetically diverse populations), phenotypic selection among genetically distinct individuals for traits of interest, and stabilization of selected individuals to form a unique and recognizable cultivar. Classical plant breeding does not exclude the use of genetic or genomic information to assess phenotypes more accurately, however the emphasis must be on whole plant selection.

Criteria

Below are the criteria listed in the 2016, 2017, 2018, and 2019 NOSB recommendations to determine if methods should be excluded.

1. The genome is respected as an indivisible entity, and technical/physical insertion, deletions, or rearrangements in the genome is refrained from (e.g., through transmission of isolated DNA, RNA, or proteins). *In vitro* nucleic acid techniques are considered to be an invasion into the plant genome.
2. The ability of a variety to reproduce in a species-specific manner has to be maintained, and genetic use restriction technologies are refrained from (e.g., Terminator technology).
3. Novel proteins and other molecules produced from modern biotechnology must be prevented from being introduced into the agro-ecosystem and into the organic food supply.
4. The exchange of genetic resources is encouraged. In order to ensure farmers have a legal avenue to save seed and plant breeders have access to germplasm for research and developing new varieties, the application of restrictive intellectual property protection (e.g., utility patents and licensing agreements that restrict such uses to living organisms, their metabolites, gene sequences, or breeding processes) are refrained from.

The NOSB has voted on the following and determined them to be excluded methods:

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Targeted genetic modification (TagMo) syn. Synthetic gene technologies syn. Genome engineering syn. Gene editing syn. Gene targeting	Sequence-specific nucleases (SSNs) Meganucleases Zinc finger nuclease (ZFN) Mutagenesis via Oligonucleotides CRISPR-Cas system (Clustered regularly interspaced short palindromic repeats) and associated protein genes TALENs (Transcription activator-like effector nucleases) Oligonucleotide directed mutagenesis (ODM) Rapid Trait Development System	YES	1, 3, 4	Most of these new techniques are not regulated by USDA and are currently difficult to determine through testing.
Gene Silencing	RNA-dependent DNA methylation (RdDM) Silencing via RNAi pathway RNAi pesticides	YES	1, 2, 4	
Accelerated plant breeding techniques	Reverse Breeding Genome Elimination FasTrack Fast flowering	YES	1, 2, 4	These may pose an enforcement problem for organics because they are not detectable in tests.
Synthetic Biology	Creating new DNA sequences Synthetic chromosomes Engineered biological functions and systems	YES	1, 3, 4	
Cloned animals and offspring	Somatic nuclear transfer	YES	1, 3	
Plastid transformation		YES	1, 3, 4	
Cisgenesis	The gene modification of a recipient plant with a natural gene from a crossable-sexually compatible-plant. The introduced gene includes its introns and is flanked by its native promoter and terminator in the normal-sense orientation.	YES	1, 3, 4	Even though the genetic manipulation may be within the same species; this method of gene insertion can create characteristics that are not possible within that individual with natural processes and can have unintended consequences.

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Intragenesis	The full or partial coding of DNA sequences of genes originating from the sexually compatible gene pool of the recipient plant and arranged in sense or antisense orientation. In addition, the promoter, spacer, and terminator may originate from a sexually compatible gene pool of the recipient plant.	YES	1, 3, 4	Even though the genetic manipulation may be within the same species, this method of gene rearrangement can create characteristics that are not possible within that individual with natural processes and can have unintended consequences.
Agro-infiltration		YES	1, 3, 4	*In vitro* nucleic acids are introduced to plant leaves to be infiltrated into them. The resulting plants could not have been achieved through natural processes and are a manipulation of the genetic code within the nucleus of the organism.
Transposons-Developed via use of in vitro nucleic acid techniques		YES	1,3,4	Does not include transposons developed through environmental stress such as heat, drought or cold.
Induced Mutagenesis		YES	1	Developed through in vitro nucleic acid techniques does not include mutagenesis developed through exposure to UV light, chemicals, irradiation, or other stress-causing activities.

The following genetic engineering methods were found by the NOSB NOT to be excluded methods:

Method and synonyms	Types	Excluded Methods	Criteria Applied	Notes
Marker Assisted Selection		NO		
Transduction		NO		
Embryo rescue in plants		NO		IFOAM's 2018 position paper on Techniques in Organic Systems considers this technique compatible with organic systems.
Embryo transfer, or embryo rescue, in animals		NO		*use of hormones not allowed in recipient animals.
Transposons		NO		Developed through environmental stress, such as heat, drought, or cold.

The following TBD methods will continue to be researched in future NOSB proposals:

Terminology				
Method and synonyms	**Types**	**Excluded Methods**	**Criteria Used**	**Notes**
Protoplast Fusion		*TBD*		There are many ways to achieve protoplast fusion, and until the criteria about cell wall integrity are discussed and developed, these technologies cannot yet be evaluated.
Cell Fusion within Plant Family		*TBD*		Subject of an NOP memo in 2013. The Crops Subcommittee will continue to explore the issue.
TILLING	Eco-TILLING	*TBD*		Stands for "Targeted Induced Local Lesions in Genomes." It is a type of mutagenesis.

Doubled Haploid Technology (DHT)		*TBD*		There are several ways to make double haploids, and some do not involve genetic engineering while some do. It is difficult or impossible to detect DHT with tests.
Induced Mutagenesis		*TBD*		Induced mutagenesis developed through exposure to UV light, chemicals, irradiation, or other stress.
Transposons		*TBD*		Produced from chemicals, ultraviolet radiation, or other synthetic activities.

Discussion

Under the NOP organic regulation, cell fusion is by definition an excluded method at §205.2. In 2013, NOP Policy Memo 13-1 provided further context for the use of cell fusion in organic systems which included protoplast fusion. Both were deemed to be excluded methods except when either technique was employed within taxonomic plant families. The policy memo defends this assertion that this limited use mimics natural phenomenon and is therefore allowed.

In February 2013, the NOSB discussion document on Excluded Methods Terminology references the policy memo explaining "that cell fusion techniques are considered an 'excluded method' when the donor cells/protoplasts do not fall within the same taxonomic family. Cell fusion is also an 'excluded method' when the donor or recipient organism is derived using techniques of recombinant DNA technology and techniques involving the direct introduction into the organism of hereditary materials prepared outside of the organism."

As the NOSB continued its work around issues of excluded methods, both cell fusion and protoplast fusion were included on a list of techniques that needed consideration for allowance/prohibition (see Appendix for NOSB proposal and discussion document April 2016). This "TBD list" included cell fusion with the note column giving the explanation "[s]ubject of an NOP memo in 2013. The Crops Subcommittee will continue to explore the issue." Protoplast fusion was included in the TBD list with the note "[t]here are many ways to achieve protoplast fusion, and until the criteria about cell wall integrity are discussed and developed, these technologies cannot yet be evaluated." The Materials Subcommittee is exploring whether its work is complete with cell/protoplast fusion, and by extension, the need for additional criteria to approach future TBD list determinations.

Questions for our Stakeholders

1. Should the NOSB prioritize developing additional criteria for excluded methods determinations before continuing to work on the remaining TBD list techniques?

2. Is Policy Memo 13-1 complete and applied consistently in organic systems, i.e., do cell fusion and protoplast fusion need to remain on the TBD list or can they be moved to the excluded method section with the notes that allowance is made for these techniques when employed within taxonomic plant families?

3. As the NOSB makes excluded methods determinations on the remaining TBD list techniques, should this organic system include allowance for historical use and a time frame for phasing out excluded uses?

Appendix

National Organic Program (February 2013).
Policy Memorandum Cell Fusion Techniques used in Seed Production. AMS.USDA.GOV
https://www.ams.usda.gov/sites/default/files/media/NOP-PM-13-1-CellFusion.pdf

National Organic Standards Board. Materials/GMO Proposals. (April 2013).
Discussion Document on Excluded Methods Terminology. AMS.USDA.GOV
https://www.ams.usda.gov/sites/default/files/media/GMOSCTrmnlgyExclddMthdsApril%202013.pdf

National Organic Standards Board. Materials/GMO Proposals. (April 2016).
Excluded Methods Terminology – Third Discussion Document. AMS.USDA.GOV
https://www.ams.usda.gov/sites/default/files/media/MSDDExcludedMethodsApr2016.pdf

National Organic Standards Board Materials/GMO Proposals. (April 2016).
Excluded Methods Terminology – Proposal. AMS.USDA.GOV
https://www.ams.usda.gov/sites/default/files/media/MSPrpslExcldMethTerminologyApr2016.pdf

Motion to accept the Fall 2021 excluded methods discussion document
Motion by: Mindee Jeffery
Seconded by: Brian Caldwell
Yes: 5 No: 0 Abstain: 0 Absent: 1 Recuse: 0

Approved by Wood Turner, Materials Subcommittee Chair, to transmit to NOP August 12, 2021.