National Organic Standards Board
Materials Subcommittee Discussion Document
Induced Mutagenesis
August 13, 2024

Introduction and background

At the November 18, 2016 in-person National Organic Standards Board (NOSB) meeting, the NOSB recommended that the National Organic Program (NOP) develop a formal guidance document for the determination and listing of excluded methods. In addition to the 2016 recommendation, a discussion document provided a "To Be Determined (TBD) list" of technologies needing further review to determine if they should be classified as excluded methods or not. The 2016 TBD list included TILLING, Induced Mutagenesis (IM), Haploid Doubling Technology, Transposons, and Cell Fusion. In several comment opportunities since 2016, organic stakeholders, including seed breeders, have urged the NOSB to resolve the status of methods on the TBD list.

Induced Mutagenesis, Transposons, and Cell Fusion using in vitro nucleic acid techniques were determined to be excluded methods in 2019, but those techniques, in the absence of recombinant DNA techniques, remained on the TBD list. In 2022, cell fusion and protoplast fusion were determined to be excluded methods only when donor and recipient cells are not within the same taxonomic plant families and/or when derived using techniques of recombinant DNA technology. This discussion document addresses Induced Mutagenesis (IM) methods used without in vitro recombinant DNA technology.

Goals of this document

The focus of this document is the production of plant varieties using IM.
The Materials' Subcommittee suggests different decisions and rationales regarding the status of IM, and request that stakeholders respond with their opinions and guidance.

Definitions

Under current National Organic Program regulations, 7 CFR 205.2 Terms defined **excluded methods** is defined as:
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.
It is important to note that this definition refers to ***means*** not possible under natural conditions, not ***results*** not possible under natural conditions.

The NOSB previously recommended the use of the following definition of **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 more accurately assess phenotypes, however the emphasis must be on whole plant selection.

In this document, we will use these additional definitions:
A **mutation** is an alteration in the nucleic acid sequence of the genome of an organism, virus, or extrachromosomal DNA.
Mutagenesis is a process by which the genetic information of an organism is changed by the production of a mutation. Mutagenesis may occur spontaneously in nature, or as a result of exposure to mutagens. In nature mutagenesis can lead to cancer and various heritable diseases, and it is also a driving force of evolution.
A **mutagen** is a chemical or physical mutation-causing agent which results in an increased rate of mutations in an organism's genetic code.
Induced mutagenesis is an increased rate of mutations caused by mutagens used in plant, microbe, or animal breeding.

Discussion
Mutations arise as a result of induced changes in the base sequence of DNA. Spontaneous mutations result from a biological process, or from mutagenic agents present in the environment (i.e. cosmic rays, heat, starvation) that change the structure of DNA. That mutation can be an atypical recombination, an atypical segregation, a removal of an amino group from an amino acid, or serious damage to the DNA caused by the breaking of covalent bonds that release nucleic acid components guanine or adenine from DNA. Induced mutations are the result of human interference and can be accomplished through physical agents, such as ultraviolet light, x-rays, heat, irradiation and/or chemical agents (i.e. mustard gas, ethylene amine, and others).
IM techniques were developed beginning in the 1930's. Before the development of direct manipulation of DNA, the use of mutagens was a source of many new mutations for plant and microbe breeding. Hundreds of plant varieties and microbe strains were developed using IM. "Records maintained by the joint FAO/IAEA Division in Vienna show that 2965 crop cultivars, with one or more useful traits obtained from induced mutations, were released worldwide during the [period from 1971 to 2011]" (Sikora et al 2011). IM plant varieties include rice, wheat, cotton, many flowers, grapefruit, pears, tomatoes, and more.

A Technical Review (TR) on Induced Mutagenesis was requested in 2023 and finalized in 2024. Much of this discussion is based on information in that TR. According to the TR, IM is not considered to be recombinant DNA or GMO technology. However, the criteria for excluded methods goes further. According to the NOP definition of excluded methods, they must meet three criteria. They must:
1. be methods used to genetically modify organisms or influence their growth and development

2. use means that are not possible under natural conditions or processes
3. use means that are not considered compatible with organic production

IM methods produce heritable genetic changes, so #1 is met.
For #2, exposure of seed or tissue to high levels of natural mutagens such as colchicine is possible in nature, but extremely unlikely. Typical mutagens used in IM are various forms of concentrated radiation and highly toxic synthetic chemicals including ethyl methanesulfonate (EMS), sodium azide (Az) and methylnitrosourea (MNU). Thus, IM uses means not possible under natural conditions. IM is also not included in the definition of Traditional or Classical breeding methods.
#3--Concerns about IM plant varieties as a general class involve the level of genetic damage they have sustained and how appropriate they are for use in organic farming systems. IM techniques almost invariably involve highly toxic chemical agents or radiation. "All three chemical mutagens are, as can be expected, strongly carcinogenic and should be handled with extreme care" (Sikora et al 2011). These mutagens create widespread genetic damage in the treated tissue (usually seeds). Poorly understood cellular mechanisms repair some or most of this damage, though many seeds are not viable. Those that are, can be grown out and crossed with healthy plants of existing varieties from the same species to start the process of creating a new, desired variety with a novel trait. In general, because of the destructive nature of the process, these new traits involve the deletion of genes, though sometimes a new trait that was previously suppressed can be expressed such as in red grapefruit.
The highly toxic chemicals or radiation used in IM are not allowed for direct use on organic farms or handling operations. Disposal of the chemicals and sources of radiation involve significant environmental risk. However, somewhat similar chemicals (though not radioactive materials) may be used in the production of organic inputs such as pesticides or processing chemicals. A critical difference here is that in the case of IM, living seed or tissues are exposed to them. One exception is in the de-linting of cotton seeds. Cotton seeds may be treated with strong hydrochloric acid for this purpose and used on organic farms. However, in general living seed or tissues used in organic production may not be treated with toxic chemicals or radiation.
The rates of mutation occurrence in IM are very high. On the order of a thousand times more mutations than normal results from the IM process. Spontaneous mutation rates per cell division across the full genome of plants listed in the TR vary from 4×10^{-3} to 567. In contrast, the range following IM was from 63 to 666,000.
In general, a group of seeds is considered "saturated" with mutations when 25-50% of treated seeds die from the mutagenesis treatment (Ke et al 2000).
Many of the survivors may have impaired function, however, desired mutations may be identified in some, and those genes may be introgressed into fully functional varieties via repeated backcrossing. Many, but not all of the damaged genes are removed this way. Backcrossing over a period of years can get rid of unwanted mutations, however some unknown background mutations can remain in the plant undetected even after many generations of selection (ZKBS, 2018).
According to the TR (lines 1863-1864), there is no specific number of backcrosses used to eliminate unwanted mutations. If an original IM plant had 1000x the normal level of mutations and each backcrossing event "cleaned up" 50% of them (Graham et al. 2020), then after 7 backcrosses the new progeny would still have about 8x the normal level. Hidden or apparently minor genetic damage could

be present in IM-derived varieties, and that these may not be fully able to fulfill their role in a healthy agro ecosystem. Thus, they would not be fully compatible with organic production.
It appears that IM meets the definition of an excluded method.
However, there has been history of the wide/safe use of induced mutagenesis in in the US and Europe in various crops like rice, wheat, tomato, soybean, and barley (Wieczorek and Wright, 2012; European Union, 2018). Furthermore, it has been documented that organic growers in the US currently use cultivars developed using induced mutagenesis (National Research Council, 2004). Researchers claim that gamma ray induced mutagenesis could potentially generate new plant varieties with desirable traits, contributing to crop improvement, agricultural sustainability, global food security while helping to mitigate existing climate change issues.
Although it is true that plant breeding is a continuous process with traits being passed down to subsequent generations through cross pollination, it would be difficult to know the extent to which traits (e.g. color, yields, drought, pest/disease tolerance etc.) altered by induced mutagenesis would be present in progeny varieties. Due to the unpredictable behavior of mutagenetic process, undesirable traits/genes may be expressed with the subsequent application of traditional breeding methods to IM varieties.
The decision to accept or exclude the induced mutagenesis breeding method therefore could, on one hand, limit the range of varieties available to organic farmers and on the other hand, contradict organic standards while compromising the integrity of organic products (Nawaz et al. 2020). We wish to honor organic agriculture's core values (ecology, health, fairness, and care) and request input from affected stakeholders including scientists, policy makers, non-breeders, consumers, and farmers to ensure transparency with information sharing.
The effects of IM varieties on crop yield, quality, the environment, and health need to be considered. If approved, comparative field trials with non-IM varieties under organic management could shed more light on this (Ntsomboh et al. 2023).
If IM is classed as an excluded method, the question arises as to whether and how the many plant varieties produced using it can be identified. The TR and other articles provide starting points for this. We request guidance on how this could be reasonably accomplished. Would it be necessary to make a list of prohibited varieties? Note that other new varieties using CRISPR and other excluded methods may require such a list as well, since they may not be readily identified as GMO's.
Another option would be to focus only on varieties introduced after the decision is made. Perhaps only new varieties produced using IM would be prohibited, since prohibiting older varieties produced using IM could be disruptive to the production and markets of many crops. Would it be advisable to "grandfather" such older varieties for use, perhaps prohibiting them for use in future crosses along with any new IM varieties? For instance, could it be argued that they are acceptable, having been sufficiently "cleaned up" of undesirable hidden mutations via many backcrosses with healthy partners?
These are difficult problems. We welcome input from all stakeholders on how best to move forward.

Questions for stakeholders

1. Should induced mutagenesis be classed as an excluded method? On what basis?
2. If IM is determined to be an excluded method, how should varieties produced using it be handled?

a. Should all varieties with IM heritage be disallowed for organic production? How would this be managed?
b. Should varieties with IM background currently in use be allowed, and IM be prohibited from use in plant breeding going forward?

3. Should varieties with IM be allowed, perhaps on the basis that IM is compatible with organic production because subsequent backcrossing sufficiently reduces any negative features it may introduce?

Subcommittee Vote:

Motion to accept the discussion document on excluded methods/Induced mutagenesis
Motion by: Franklin Quarcoo
Seconded by: Logan Petrey
Yes: 7 No: 0 Abstain: 0 Recuse: 0 Absent: 1

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