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Swedish Board of Agriculture

Plant and Environment Department
Staffan Eklöf

CRISPR/Cas9 mutated Arabidopsis

This letter is to inform you that the Swedish Board of Agriculture (SBA) has made interpretations whether field trials with certain CRISPR/Cas9-mutated Arabidopsis have to be applied for as genetically modified organisms.

The SBA makes the interpretation that those plants in the description mutated by CRISPR / Cas9 and which do not contain any foreign DNA are exempted from the GM legislation.

CRISPR / Cas9 can be used in many different ways. We have made an assessment only of the specific plants described. The assessment does not necessarily apply to other plants developed with CRISPR / Cas9.

We have informed the universities that SBA's interpretation may be subject to change if there will be a common interpretation of the definitions and exemptions at the EU level. We have also urged for caution during cultivation, as the plant's legal status might change rapidly.

Background

The SBA has received questions from two separate universities if they have to apply for field release in accordance with the GM legislation for thale cress modified with the CRISPR/Cas9 technique.

The plants are described to be transformed with Agrobacterium with a T-DNA encoding nuclease Cas9 and a sequence encoding a (single guide) sgRNA. This CRISPR / Cas9 system causes a site-specific double-strand break in the plant's DNA, which is repaired by the cell's own building blocks. During this repair certain mutations can occur. A template oligonucleotide, or a so-called gene targeting (GT) cassette in the T-DNA for mutation will not be used. Therefore, modification results in a double-strand break in the plant DNA with the re-ligation of chromosomal ends using the cell's own system without a foreign DNA sequence incorporated. The target sequence in the plant's DNA can only be mutated using the cell's own repair system, via so-called non-homologous end-joining. One or a few bases may be lost or added. In some of the plants they are to use two double-strand breaks, which leads to that the intervening DNA sequence is lost.

There are two types of plants. Plants in which mutations as described above have been made and the T-DNA is removed by crossing and plants with the same mutation but carrying T-DNA. Either as a result of where the plants' properties have been restored by transformation using Agrobacterium, so-called complementation, or because the original T-DNA was never removed by

crossing. Both categories meet the definition of GMO since the plant's DNA has been altered in a way that does not occur naturally by mating or natural recombination. This is because the change has been induced with the help of a foreign nuclease and the latter plants also carry foreign DNA.

Our assessment

Definitions of what is a genetically modified organism (GMO) are found in Article 2.2 of Directive 2001/18/EC on the deliberate release of GMOs into the environment. Article 3.1 of the Directive stipulates that GMOs produced by certain methods are exempted from permit requirements and other regulations. To assess if a given plant is covered by the permit one must first determine whether the plant is a GMO or not, as determined above. If it is a GMO it has to be determined if it is exempted from regulation.

The plants carrying T-DNA

The plants carrying the original foreign DNA-sequence or a new DNA sequence (T-DNA) inserted in their genome are developed with one of the techniques of genetic modification as described in Annex 1A, Part 1 of Directive 2001/18 / EC. SBA thinks that they therefore fall within the remit for regulation by the directive. Plants with introduced foreign DNA is the most common category of GM plants and have always been considered to be within the scope of the legislation.

This means that permission must be obtained from the SBA for carrying out field trials of the plants where the T-DNA is still in the plant.

The plants carrying only mutation

The second type of plants contain only the mutation that the CRISPR / Cas9 system induced because the inserted T-DNA sequence has been crossed out.

The plants are exempted regulation if they are produced by mutagenesis according to Annex 1B. The exemption does not apply if the technique involves the use of recombinant nucleic acid molecules or GMOs.

There is no definition of mutagenesis in the legislation. Recital 17 of the Directive says that the Directive should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record. This is the reason for exempting mutagenesis in Annex 1B. SBA believes that it should be interpreted as meaning that the exemption applies to the molecular processes that occurs in traditional mutagenesis and not the method of obtaining the mutations, such as radiation. It should also have been the legislator's intention, because otherwise all the methodological development that has occurred after the directive was written automatically lead to a regulated GMO.

With this interpretation the introduction of foreign DNA is a new molecular process, while changes involving only the cell's own building blocks are not. No

foreign DNA was inserted at the site of mutation in the first category of plants. The changes induced by CRISPR/ Cas9 in the plants are changes in the plant's genome with the building blocks of their own cells. We therefore believe that those plants are produced by mutagenesis.

Further, we consider that the methods yielding these specific mutations do not involve the use of recombinant nucleic acid molecules. We interpret the concept as follows. If the used technique corresponds to techniques described in Annex 1A, part 1, point 1, the technique involves the use of recombinant nucleic acid molecules. The used technique does not correspond to the description.

Previous use of GMO

Since plants have been transformed with T-DNA at an earlier time, recombinant nucleic acids and GMO have been used for the preparation of the mutations. In our interpretation the plants where the T-DNA has been crossed out are not subject to regulation. Occasional or former presence of DNA should not determine whether a plant is a GMO to be regulated or not. It is the current plant genetic material that should be decisive, not that of previous generations. It is also impossible to see or analyse whether a mutation is natural or if an earlier generation has been genetically modified to induce the modification. The same change in the plant's DNA may be obtained by injecting nuclease and sgRNA (the same components as encoded in the actual T-DNA). It would not be equal treatment to distinguish between plants with the same mutation but prepared in different ways.