Advisory Committee on Releases to the Environment

Advice on genome-edited Camelina plants with increased levels of oleic acid

Advice of the Advisory Committee on Releases to the Environment (ACRE) to the Secretary of State under S.124 of the Environmental Protection Act 1990

Advice: ACRE considers that *Camelina sativa* plants produced by CRISPR-Cas9 genome-editing could have been produced through traditional breeding techniques. CRISPR-Cas9 genome-editing is a mutagenesis technique. Recombinant nucleic acid molecules were involved in the development of CRISPR-Cas9 genome-edited Camelina lines. However, DNA from the CRISPR-Cas9 transformation vector is not present in either of the two genome-edited Camelina lines in question i.e. no transgenes are present. Consequently, it would not be possible to determine whether these lines had been produced by genome-editing or by traditional mutagenesis because they would be genetically indistinguishable.

Background

Defra has received a request for advice from Rothamsted Research on whether two lines of genome-edited Camelina plants it plans to grow in a small-scale field trial, are captured by the GMO legislation. These plants have mutations in the delta-12 desaturase (FAD2) loci present in the hexaploid Camelina genome. These mutations result in increased oleic acid content. Oleic acid is a monounsaturated fatty acid that occurs naturally in plants and animals.

In order to reach a conclusion, Defra has asked ACRE to advise on whether these lines could have been produced by traditional breeding methods, including whether they contain any DNA from the CRISPR-Cas 9 transformation vector. Defra also asked whether the CRISPR –Cas 9 genome

editing technique used to produce these plants is a form of mutagenesis and how recombinant nucleic acid molecules were used in the generation of these plants.

The production of these genome-edited Camelina lines is described in a paper by Morineau et al. (2017)[1]. Their first step was to transform Camelina lines (cultivar Celine) by dipping Camelina flowers in a solution of GM *Agrobacterium* containing DNA encoding the Cas9 nuclease and a guide RNA that targets the nuclease to FAD2 loci. In subsequent generations, Camelina plants were selected if they contained mutations in *FAD2* genes but no transformation vector-derived DNA. PCR on DNA extracted from at least two successive generations of selfed genome-edited Camelina lines and the absence of fluorescence from the DsRED marker demonstrated the absence of transgenes in these lines.

The guide RNA sequence used to direct the Cas9 nuclease to the *FAD2* genes was selected based on a number of criteria including the absence of predicted off-target effects. However, ACRE noted that traditional mutagenesis techniques used in plant breeding generate many hundreds of off-target effects. The majority of these are lost when the mutant plants with desired characteristics are 'backcrossed' to lines that have not been mutated.

Conclusions

The Camelina genome is composed of three subgenomes, which makes it challenging to identify recessive *fad2* mutants using traditional mutagenesis. This is because expression of *FAD2* genes that are not mutated masks the mutant phenotype. However, a mutation in one of the *FAD2* genes has been generated using traditional mutagenesis (using ethyl methanesulfonate) and this resulted in Camelina plants with an increased oleic acid content of approximately 27% (Kang *et al*. 2011[2]). To date, the search for naturally

[1] Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid *Camelina sativa*. Plant Biotechnol J. 2017 Jun;15(6):729-739. Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogué F and Faure J.D

[2] Identification of three genes encoding microsomal oleate desaturases (*FAD2*) from the oilseed crop *Camelina sativa* . Plant Physiol. Biochem. (2011) 49: 223–229. Kang J., Snapp A.R. and Lu C.

occurring, triple *FAD2* mutants in different Camelina accessions has been unsuccessful.

Although generating double or triple *FAD2* recessive Camelina mutants (such as the two genome-edited lines in question) is limited by this high genetic redundancy in *Camelina sativa*, ACRE's conclusion is that it would be possible using traditional breeding techniques but it would take considerably longer than using genome-editing.

The use of CRISPR-Cas9 genome-editing generates mutations and is therefore a type of mutagenesis. The process of genome-editing involves recombining nucleic acid molecules both in constructing a transformation vector and also when (part of) this vector is inserted into Camelina plants in the early stages of the breeding process. However, the Camelina plants that will be used in the trial will not contain this recombinant DNA (i.e. they will not contain transgenes). Consequently, it would not be possible to determine whether these lines had been produced by genome-editing or by traditional mutagenesis because they would be genetically indistinguishable.