Application in COSMOS

In the COSMOS project, the CRISPR/Cas9 tool is used to introduce mutations in specific genes involved in fatty acid and glucosinolate biosynthesis in oil crops. The aim is to improve the seed oil and seed meal quality of the two industrial oil crops camelina and crambe. This entails the creation of high levels of mono-unsaturated fatty acids, optimisation of fatty acid profiles and the reduction of Anti-Nutritional Factors (ANF) such as glucosinolates.

The creation of high mono-unsaturated fatty acid profiles means that the financial yield per hectare can be increased. The reduction of ANF enables dual use of the crops: the seeds can be pressed for the oil while the press cake, which contains the protein, can be used as feed, leading to an increased availability of domestic feed.

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COSMOS
ADDING VALUE TO CAMELINA AND CRAMBE OIL

More information

For more detailed explanation and a more extended brochure please visit the COSMOS website: **http://cosmos-h2020.eu**

What is CRISPR/Cas9 and how is it used in the COSMOS project?

Camelina & crambe Oil crops as Sources for Medium-chain Oils for Specialty oleochemicals

3/2015 – 8/2019
www.cosmos-h2020.eu

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What is CRISPR/Cas9 and how is it used in the COSMOS project?

The CRISPR/Cas system has been making headlines throughout the media in the recent years as a new genome editing tool to speed up the breeding of crops and animals and even as a tool to cure diseases. But what is the CRISPR/Cas system actually and what are the genetic effects? This leaflet explains what the CRISPR/Cas system is and how this tool is used in the COSMOS project.

Description of the technology

In bacteria, the CRISPR/Cas system provides resistance to viral infections. Based on the genetic information of previous infections, the CRISPR/Cas system is able to cut the DNA of the invading virus in a very precise and effective way, thereby inactivating the virus.

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and was identified in bacteria as part of their defence system against viruses, while Cas stands for CRISPR associated system. In the case of Cas9, it refers to CRISPR associated protein 9.

Molecular scientists discovered that this system could also work in plants, animals and even humans. It was found that the CRISPR part of the system (the DNA targeting device) could be modified in such a way that it targets a DNA sequence of choice after which a Cas protein (the most prominent and used one being Cas9) cuts the DNA like a scissor at exactly that position. A specific DNA break is the result of this action. Interestingly, plant and animal cells have a system to repair such breaks, but upon repairing, a little stretch of DNA is either inserted or deleted. In this way, molecular biologists are able to knock out a gene (deletion of a small part of the DNA) or to add a new gene (insertion of a DNA fragment).

Because of its simplicity and precision, the CRISPR/Cas system has become a versatile tool in molecular biology to change the properties of the plant or animal cells.

The COSMOS project focusses on the deletion of a small part of a gene in plants using the CRISPR/Cas9 system. Such a small modification in a gene is called a mutation. Introduction of mutations is a technique widely used in traditional plant breeding – in these cases introduced in the genome by radiation or chemical treatment. In comparison with traditional mutation breeding tools, the CRISPR/Cas9 system provides very high precision in the position of mutations as it targets genes of interest only, while traditional mutation breeding tools cause random mutations not only in the target gene. Consequently, the time needed for creating a new plant with a specific mutation is much shorter when the CRISPR/Cas9 system is used.

The legal issue

The CRISPR/Cas mutation system can be introduced in plant cells in different ways, but most efficiently today using genetic modification (GM) to introduce the CRISPR/Cas system itself in the first phase. The CRISPR/Cas system can then easily be removed using crossing after it has done its job in a second phase. The final plants contain the desired change in DNA (a mutation) but not the CRISPR/Cas system anymore.

The application of CRISPR/Cas in plants has raised a debate on the legal status of these plants: Should these plants be considered being a genetically modified organism (GMO) or not?

The use of genetic modification in every organism including bacteria, fungi, plants etc. is regulated in EU Directive 2001/18/EC. This Directive contains a list of gene modification and breeding techniques (such as transgenesis) of which the resulting organisms are considered GMO and these GMO are subject to strict regulations. According to the Directive, especially traditional breeding including traditional mutation breeding is not subject to the strict GMO regulations because of a history of safe use. CRISPR/Cas mutants made in the way described before do not contain the transgenic CRISPR elements since these were removed by crossing. Therefore, as long as no foreign genes are introduced, CRISPR/Cas mutants do not contain transgenes. However, the EU Directive considers an organism to be genetically modified when genetic modification was part of the *process* of making it, even when the transgenic element is no longer present in the final *product*.

It is clear that if foreign genes have been introduced using CRISPR/Cas, the product is considered a GMO. However, via the CRISPR/Cas system improved plants without foreign genes could be regarded at least as safe as products of traditional mutation breeding. In fact, the CRISPR/Cas system can be used to create the same functional mutation without causing the additional random mutations that occur when using traditional mutation breeding.

On July 25th, 2018, the European Court of Justice decided that organisms (including crops, plants, bacteria etc.) obtained by genome editing techniques, including the CRISPR/Cas system (mutagenesis) are GMOs and are subject to the obligations laid down by the EU Directive 2001/18/EC.