

Gene editing of hexaploid *Camelina sativa*, from the lab to the field

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Plant oil production is continuously increasing (+50% in the last decade, source USDA) due to the high demand for food, feed and new industrial uses. Innovation is required to meet the market needs, but also to facilitate a more sustainable production associated with reduced agricultural inputs, such as chemicals, fertilizers and irrigation. The low diversity of the oil crop portfolio is an issue both at the crop level as well as for agrosystem practices to meet the diverse needs of farmers. *Camelina sativa* has been identified as a promising oilseed crop for its "resilience" with relatively low agricultural input requirements and diseases as well as tolerance to drought and low temperature. Camelina also presents an interesting oil profile for food, feed and biobased uses, with more than 50% polyunsaturated fatty acids (35% n-3 linolenic acid). Finally, camelina is a great translational model to validate new traits, since its genome is very close to that of the model plant *Arabidopsis*. The ease of its genetic modification allows rapid development of new traits by conventional as well as biotechnological modification like gene editing. Obtaining recessive mutants in Camelina is limited by the high genetic redundancy and gene editing appears therefore as a valuable tool to selectively enlarge camelina gene pool.

CRISPR/Cas9 strategy was used to mutagenize $\Delta 12$ desaturase FAD2 to enhance oleic acid content. More than 133 lines were obtained resulting in a collection of alleles in single, double and triple *csfad2* mutants in homozygous, heterozygous and heteroallelic configurations (Morineau et al 2017). A total of 21 different mutant alleles were identified, ranging from insertions or deletions of 1 to a few nucleotides, to larger deletions that would be predicted to impact FAD2 protein structure differently. The different genetic combinations resulted in quantitative variation of oleic acid content in both leaf and seed ranging from 9.8% (wild type Celine) to 62.5% and corresponding to double and triple mutants.

To evaluate whether high oleic content would have an effect in agronomical conditions, transgene-free edited lines were selected for field trial evaluation. Two lines with 3 and 6 times respectively more oleic acid in seed oil than wild type were selected and evaluated in a GM application submitted to UK Department for Environment, Food and Rural affairs (DEFRA). DEFRA concluded that the 2 camelina GE lines were not genetically modified organisms and were included in the GM experimental trial as additional transgene-free controls. The objective is to investigate the influence of fatty composition on plant response to environmental changes in the field.

In conclusion, GE provides a powerful alternative to engineer diversity and enlarge the gene pool in a species like *Camelina sativa*. CRISPR-Cas9 editing is efficient even in an hexaploid species and the close proximity between subgenomes allows simple and rapid identification of even triple homozygous mutants. The high redundancy of CsFAD2 homeologs provided also a tunable quantitative phenotype based on combinatorial assembly of alleles. Gene dosage is a valuable tool for plant breeding since it could help minimizing side-effects of strong traits by finding the best trade-off for an optimum trait value. Nonetheless GE traits have to be thoroughly evaluated in the field to assess their potential agronomical value. The tougher

European regulation as proposed by the European Court of Justice ruling of July 25th 2018 will clearly limit the discovery of potentially new agricultural traits and weaken European plant breeding.