Evaluation of the scientific publication

"A Real-Time Quantitative PCR Method Specific for Detection and Quantification of the First Commercialized Genome-Edited Plant"

P. Chhalliyil *et al*. in: Foods (2020) 9, 1245

by the European Network of GMO Laboratories (ENGL)

This document has been discussed at the ENGL Plenary meeting held on 30 September 2020 and was endorsed by the ENGL members.

BACKGROUND

In 2019 the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) elaborated, in collaboration with the European Network of GMO Laboratories (ENGL), a report on the detection of food and feed plant products obtained by new mutagenesis techniques[1]. This report acknowledges that there are challenges for developing detection methods in line with the authorisation requirements for GMOs in the EU for some genome-edited plant products, in particular for products that do not contain any inserted recombinant DNA in the final plant. The report concluded that the "*validation of an event-specific detection method and its implementation for market control will only be feasible for genome-edited plant products carrying a known DNA alteration that has been shown to be unique*" and that "*in case a DNA alteration has been detected, there are currently no procedures established that facilitate an unambiguous conclusion that genome editing has created the alteration".*

On September 7th 2020, an article was published in the journal *Foods* (Chhalliyil *et al*., 2020, 9, 1245) presenting a quantitative detection method for a sulfonylurea (SU) herbicide-tolerant oilseed rape (OSR) line from the company Cibus. The method targets single nucleotide variants (SNVs) in the acetolactate synthase gene (AHAS) of this specific OSR line. The authors claim that the method "*meets all legal requirements for GMO analytical methods in jurisdictions such as the EU*", and that "*qPCR-based method development may be applicable to virtually any genome-edited organism*".

[1] European Network of GMO Laboratories (ENGL). Detection of food and feed plant products obtained by new mutagenesis techniques, 26 March 2019 (JRC116289) https://gmo-crl.jrc.ec.europa.eu/ENGL/ENGL.html

EVALUATION

It is the ENGL view that the analytical method published by Chhalliyil *et al*. is able to detect and quantify the targeted SNV in the AHAS1C gene, however this does not provide evidence that an analysed sample contains a product of genome editing. This is further underscored by the ongoing discussion regarding the test material used in this study, namely on the actual origin of the mutation in the Cibus OSR, i.e. whether it originates from somaclonal variation or oligonucleotide-directed mutagenesis (a technique of genome editing). As the method thus does not allow to distinguish SNVs generated by genome editing from those obtained with classical breeding techniques or by natural mutation, it cannot be applied for unequivocal detection, identification and quantification and does not meet the mandatory criteria required by Regulation (EU) No 503/2013 (Annex III). The latter imposes, among others, that the event-specific method "*shall only be functional with the genetically modified organism or genetically modified based product considered*".

The method development approach described by Chhalliyil *et al*. is based on prior knowledge about (a) the SNV present in the acetolactate synthase gene AHAS3A in the A-subgenome of the OSR variety of interest (derived from chemical mutagenesis) and (b) the new SNV in the acetolactate synthase gene AHAS1C in the C-subgenome of the OSR line developed by the Cibus company and obtained by somaclonal variation or oligonucleotide-directed mutagenesis. The detection method developed for the OSR line from Cibus derives its level of specificity from the combined presence of two SNVs that are located in relative vicinity. In other cases, only a single SNV may be available as target for a detection method. The ability of the same methodological approach to detect another genome-edited crop with high specificity would therefore be very much dependent upon the exact DNA sequence being targeted.

According to the information provided in the publication of Chhalliyil *et al*., the method complies in many aspects with the performance requirements described in the ENGL document 'Definition of minimum performance requirements for analytical methods of GMO testing' (MPR guidelines)[2]. However, it was not validated for all criteria according to the MPR guidelines and additional validation work would be required to evaluate further the specificity, sensitivity and applicability of the method.

For instance, the applicability of the method should be discussed including limitations for processed food/feed materials that may contain OSR. This is particularly important for the presented detection method because the amplicon size targeted by the SNV method is unusually large (334 basepairs), which may affect its amplification in highly processed products. For instance, a reduced amplification of the SNV DNA template compared to the taxon-specific DNA template (101 basepairs) would decrease the final measurement result, which is expressed as a ratio between both targets.

Moreover, the specificity of the method needs to be tested on all EU authorised GMOs, as well as on closely related plant species showing similarity to the primer and probe sequences based on an *in silico* analysis and, in addition for this particular line, on other SU-resistant crops and SU-resistant weeds that may occur in OSR samples. This is important for market surveillance applications. The specificity of the method could be compromised because there are more than 160 species of weeds in which mutations in the conserved acetolactate synthase gene resulted in SU resistance and the

[2] https://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm

single nucleotide variation in the Cibus OSR is one of the most widely described mutations in this gene[3].

In conclusion, Chhalliyil *et al.* have described a method for the detection of a known SNV in the Cibus OSR. However, the method cannot identify the origin of the mutation in the particular OSR line targeted and can therefore not prove that the detected mutation is caused by genome editing. In addition, the article does not provide any strategy on how to detect a (unknown) genome-edited based mutation, if e.g. the developer or breeder has not supplied any information.

The statements and conclusions published in the ENGL report on the detection of food and feed plant products obtained by new mutagenesis techniques[1] remain all valid and do not require any revision as a consequence of this publication.

[3] Nandula VK, Giacomini DA, Ray JD (2020) Resistance to acetolactate synthase inhibitors is due to a W574 to L amino acid substitution in the *ALS* gene of redroot pigweed and tall waterhemp. PLoS ONE 15(6): e0235394 (doi.org/10.1371/journal.pone.0235394)