Biosafety of Genome Editing Applications

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In the European Union plants developed by novel genomic techniques for directed mutagenesis are have to undergo an Environmental Risk Assessment (ERA) prior to release or placing on the market. However, specific guidance for such an ERA is still lacking. In this review we discuss the limited suitability of general denominators of risk/safety to predict the risks associated with individual genome edited (GE) plants and argue that there is no safety by default for whole groups of GE applications encompassing different individual GE organisms. We suggest integrating the following two sets of considerations into the ERA to address particular characteristics of GE plants: considerations related to the traits developed by GE and considerations addressing the assessment of method-related unintended effects, e.g. due to off-target modifications. In conclusion, we recommend that further specific guidance for the ERA and monitoring should be developed to facilitate a focused assessment approach for GE plants.

novel genomic techniques | genome editing | CRISPR/Cas | plant modification | GMO | environmental risk assessment | biosafety regulation

1. Overview

An intensely debated question is whether or how a mandatory environmental risk assessment (ERA) should be conducted for plants obtained through novel genomic techniques, including genome editing (GE). Some countries have already exempted certain types of GE applications from their regulations addressing genetically modified organisms (GMOs). In the European Union, the European Court of Justice confirmed in 2018 that plants developed by novel genomic techniques for directed mutagenesis are regulated as GMOs. Thus, they have to undergo an ERA prior to deliberate release or being placed on the market. Recently, the European Food Safety Authority (EFSA) published two opinions on the relevance of the current EU ERA framework for GM plants obtained through novel genomic techniques (NGTs). Regarding GE plants, the opinions confirmed that the existing ERA framework is suitable in general and that the current ERA requirements need to be applied in a case specific manner. Since EFSA did not provide further guidance, this review addresses a couple of issues relevant for the case-specific assessment of GE plants. We discuss the suitability of general denominators of risk/safety and address characteristics of GE plants which require particular assessment approaches. We suggest integrating the following two sets of considerations into the ERA: considerations related to the traits developed by GE and considerations addressing the assessment of method-related unintended effects, e.g., due to off-target modifications. In conclusion, we recommend that further specific guidance for the ERA and monitoring should be developed to facilitate a focused assessment approach for GE plants.

2. Genome Editing

The ruling of the European Court of Justice (ECJ) in the case C-528/16 delivered in July 2018 clarified that plants developed by novel genomic techniques for directed mutagenesis are considered genetically modified organisms (GMOs) in the EU in accordance with Directive 2001/18/EC on the deliberate release and placing on the market of GMOs. The ruling also confirmed they are not exempt from regulations according to Article 3 in conjunction with Annex IB of the Directive (i.e., the “mutagenesis exemption”) [1]. In a broader sense, the decision established that organisms which are developed by methods of directed mutagenesis such as GE are subject to the current EU regulatory framework for biotechnology products. The EU biosafety framework was introduced in 1990 and underwent major amendments. In 2001 and 2003 the Directive 2001/18/EC and Regulation (EC) No. 1829/2003 on GM food and feed were introduced. In and 2013 and 2015 the Implementing Regulation (EU) No 513/2013 on requirements for the authorization of genetically modified (GM) food and feed and Directive 2015/412/EU providing EU Member States with the possibility to implement restricting measures on the cultivation of GMOs in their territories were adopted [2]. The decision of the ECJ was a major step in the long and heated debate in Europe concerning the regulation of organisms developed by novel genomic techniques such as genome editing (GE), but did not resolve all uncertainties regarding the regulation of such applications [3]. First, the ruling does not apply to all types of NGTs, which cover a diverse range of methods including cisgenesis, transgrafting and epigenetic engineering by methods of RNA-directed DNA methylation alongside GE [4]. Secondly, it was argued that the ruling does not resolve all pending questions regarding the practical implementation of the EU regulatory framework for GE organisms [5]. Subsequently to the ECJ ruling, the European Commission conducted a stakeholder survey in the framework of a study regarding NGTs including GE to address some of these issues. The recently published study, however, does not provide concrete policy recommendations for further discussion [6].

GE is mostly done through introducing DNA single- or double-strand breaks at specific loci of a target genome by a range of site-directed nucleases (SDN), with CRISPR-Cas-type nucleases being them most prominent among them [7]. Mutations are then introduced at these genomic sites by cellular DNA repair systems. The outcome of the genetic modification may be directed by template DNA sequences supplied in trans or by modifications of the used SDN [8]. SDN-based GE has quickly become a standard tool in molecular biology for a variety of uses, including fast–track plant breeding [9]. The discovery of the CRISPR-Cas system as a genome editing tool was awarded the 2020 Nobel Prize in Chemistry [10]. Due to its simplicity and accessibility, GE has been used at an increasing pace and scale for the development of genetically modified plants in recent years [11]. GE is believed to be of high importance for future plant breeding by certain stakeholders [12][13]. The regulatory uncertainties surrounding GE organisms, particularly the question of whether GE organisms are GMOs according to many existing biosafety frameworks, led to policy considerations and debates in most countries of the world and at the level of international organizations. The increasing use of GE in plant breeding at the global level made this debate more urgent [14][15][16][17]. Against the background of the different national systems for the regulation of GMOs, some countries, including a number of Latin American countries, have already introduced supplementary legislation to facilitate the determination of the regulatory status of individual GE applications with regard to the existing biosafety laws [18]. Some countries, such as Australia, have decided to exclude some types of GE applications from their regulatory framework for GMOs [19]. Other legislations such as the EU and New Zealand have sought decisions of their

supreme courts to decide whether GE organisms are subject to their existing regulatory system for GMOs. In both cases, the court rulings have positively answered this question [15]. Canada is operating a regulatory system that is based on the novelty of the newly developed traits and the plausibility of hazards that may be associated with the use of modified plants as regulatory triggers. Canadian regulations for plants with novel traits accommodate GMOs as well as plants with novel traits established by GE or conventional breeding within the existing regulatory framework [14][15].

In all countries, the decision to regulate GE plants according to the existing GMO regulations is crucially relevant for the level of regulatory oversight for GE plants. These decisions are thus highly important for the particular risk assessment requirements applied for GE plants [15]. Thus, the current debate in the EU as well as in other countries focuses on two issues: the practical applicability of the current regulatory system for products of novel genomic techniques such as GE and the development of appropriate approaches for the assessment of food safety and the environmental risk assessment (ERA) of organisms developed by GE. This review is focusing on the latter question. Specifically, we discuss considerations regarding an appropriate risk assessment of the traits developed by GE approaches as well as any unintended effects of GE plants. We suggest that further specific guidance for the ERA and monitoring of GE plants should be developed. We note that considerations regarding the risk assessment for GE plants will inform the debate on options for further regulation [20].

3. Conclusions

Our review indicates the challenges faced by policy makers, regulators and risk assessors to provide an appropriate framework for the risk assessment of GE plants. The risk associated with individual GE applications will be highly variable. While the effects of some GE applications may be well known from conventional varieties with similar traits, other GE applications could be associated with plausible risk issues and may be more challenging to assess and monitor. The latter group will likely be comprised of GE plants with complex and novel modifications as indicated by EFSA [21] and other authors [20][22].

Considering the wide ranges of plant species and the GE methods and traits that need to be considered, there is no safety by default for whole groups of GE applications encompassing different individual GE organisms. Biosafety considerations should instead be based on an appropriate ERA prior to the release of GE plants into the environment.

The case-specific approach incorporated in the EU regulatory framework is a viable way forward provided that further guidance for the risk assessment of GE applications is developed. The existing guidance developed by EFSA and their initial work on GE applications is not sufficient to address these challenges, but rather a starting point for further efforts. In this review, **we argue that general considerations concerning risk/safety of all GE applications or of different classes of GE applications are insufficient to address the challenges at hands. Instead, we suggest that a focused case-specific approach is followed to provide a robust risk assessment of individual GE plants**. This ERA approach should focus on risks that may plausibly manifest themselves in the phenotype or the interaction with the environment of a particular GE plant. To this end, we suggest that two sets of

considerations are considered: (1) trait related-considerations to assess the effects associated with the newly developed trait(s); and (2) method-related considerations to assess unintended changes associated with the intended trait(s) or with other modifications in the GE plant. Important aspects concerning both sets of considerations are outlined in Box 1.

Based on these considerations, further guidance should be developed to ensure the high safety standards provided by the current regulatory framework for GMOs in the EU for GE plants in an adequate and efficient way, taking into account the existing knowledge and experience in a case-specific manner. This guidance should thus strengthen the case-specific approach that is recommended by numerous EU and Member States institutions. The precautionary approach of the existing EU GMO regulations should not be weakened by excluding whole groups of GE applications from their scope without having regard to the characteristics of the individual GE plants.

Box 1: Crucial aspects for a two-pronged assessment strategy to address trait-related effects and method-related modifications, respectively.

(1) The assessment of effects associated with the newly developed trait(s) in GE plants should consider, among others:

- The level of knowledge and familiarity with the particular crop and trait combination needs to be considered. As indicated in Section 4.1, only limited scientific knowledge is available for some GE applications.
- Some applications may lead to changes in agricultural management; possible indirect effects resulting from their use need to be addressed during the ERA.
- Complex GE modifications should be thoroughly scrutinized regarding adverse environmental effects resulting from these changes. A robust assessment should be provided for physiological effects of multiple simultaneous changes (multiplexed GE) and for regulatory effects of the introduced modifications on morphology, development and reproduction of the GE plant.
- The ERA conducted for GE plants should also address secondary effects associated with the intended trait(s). This should encompass pleiotropic effects of the intended trait(s).

(2) The assessment of method-related unintended changes associated with the intended trait(s) or with other modifications in the GE plant should take into account the following aspects:

- The available body of evidence with regard to off-target-effects, their occurrence and their identification as indicated in Section 4.2.

- The likelihood that off-target modifications are still present in the final breeding product. This likelihood may be higher with fast-tracked breeding applications, i.e., aimed at modification of elite lines, modification of vegetatively propagated crops, and modification of plant species with longer generation cycles such as trees.
- The available information on unintended secondary modifications introduced by GE systems in the vicinity of the intended genomic target site. Such modifications are tightly linked to the intended traits and are not easily lost during subsequent breeding steps.
- The available recommendations on how an assessment of unintended and off-target effects may be conducted and which kind of aspects should be considered in the framework of the assessment.

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