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RISK ASSESSMENT OF NGT PLANTS: DIFFERENTIATED CONSIDERATIONS INSTEAD OF SIMPLE COMPARISONS

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Personal Background

- Senior Biosafety Expert (2005 - 2022)
  Team Landuse & Biosafety,
  Environment Agency Austria, Vienna
- Tasks Biosafety Unit
  - Environmental risk assessment and monitoring of GMOs (Directive 2001/18/EC & Reg. (EU) 1929/2003)
  - Studies addressing New Genomic Techniques (NGTs) e.g. Genome Editing since 2014:
    risk assessment, monitoring, detection/identification, considerations regarding sustainability, etc.

Recent publications on NGT-assessment

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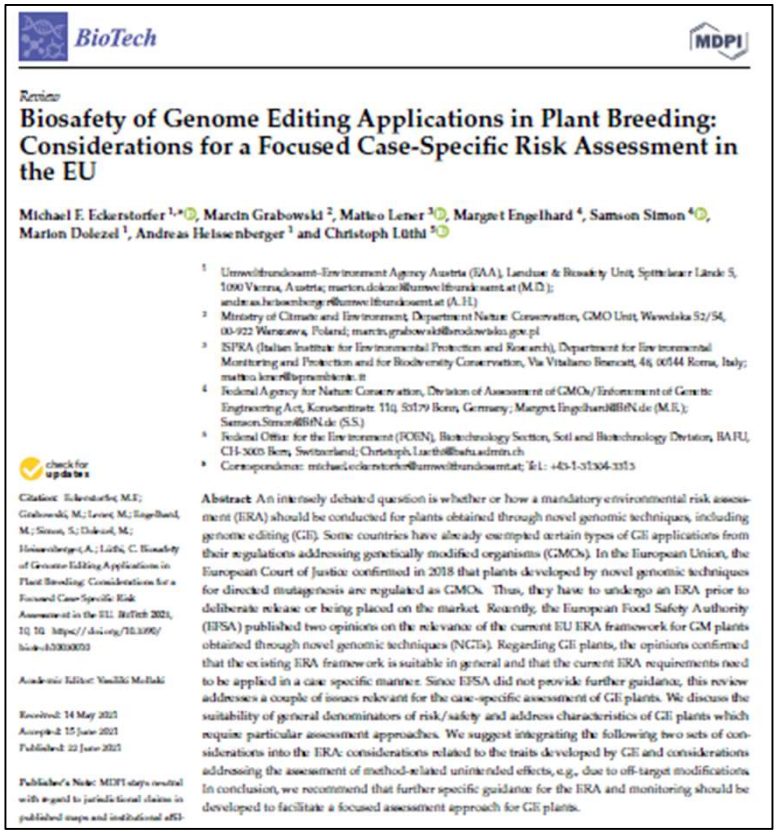
https://doi.org/10.3390/biotech10030010

http://doi.org/10.3389/fbioe.2019.00031



BioTech

MDPI

Review

Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU

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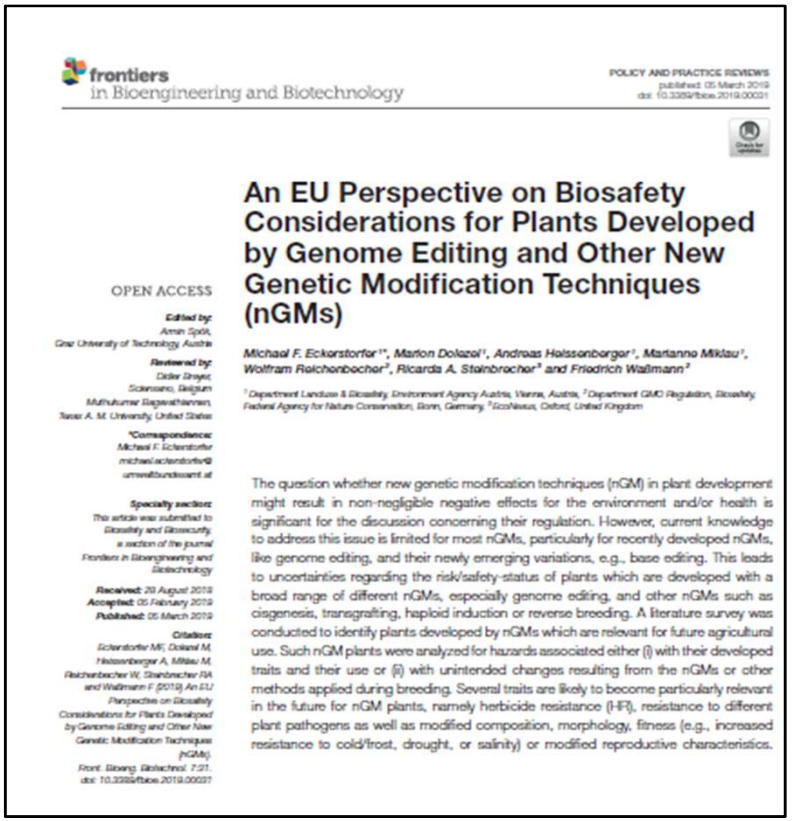
Citation: Eckerstorfer, M.F.; Grabowski, M.; Lener, M.; Engelhard, M.; Simon, S.; Dolezel, M.; Heissenberger, A.; Lüthi, C. Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU. BioTech 2021, 10, 10. https://doi.org/10.3390/biotech10030010

Academic Editor: Vasiliki Morikis

Received: 14 May 2021
Accepted: 15 June 2021
Published: 22 June 2021

Abstract: 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.



frontiers in Bioengineering and Biotechnology

POLICY AND PRACTICE REVIEWS

An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs)

OPEN ACCESS

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Specialty section:
This article was submitted to Biosafety and Biosecurity, a section of the journal Frontiers in Bioengineering and Biotechnology

Received: 28 August 2019
Accepted: 05 February 2019
Published: 05 March 2019

Citation:
Eckerstorfer MF, Dolezel M, Heissenberger A, Miklau M, Reichenbecher W, Steinbrecher RA and Waßmann F (2019) An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs). Front. Bioeng. Biotechnol. 7:31. doi: 10.3389/fbioe.2019.00031

The question whether new genetic modification techniques (nGM) in plant development might result in non-negligible negative effects for the environment and/or health is significant for the discussion concerning their regulation. However, current knowledge to address this issue is limited for most nGMs, particularly for recently developed nGMs, like genome editing, and their newly emerging variations, e.g., base editing. This leads to uncertainties regarding the risk/safety-status of plants which are developed with a broad range of different nGMs, especially genome editing, and other nGMs such as cisgenesis, transgrafting, haploid induction or reverse breeding. A literature survey was conducted to identify plants developed by nGMs which are relevant for future agricultural use. Such nGM plants were analyzed for hazards associated either (i) with their developed traits and their use or (ii) with unintended changes resulting from the nGMs or other methods applied during breeding. Several traits are likely to become particularly relevant in the future for nGM plants, namely herbicide resistance (HR), resistance to different plant pathogens as well as modified composition, morphology, fitness (e.g., increased resistance to cold/frost, drought, or salinity) or modified reproductive characteristics.

Differences NGT-Applications vs. Conventional Breeding

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KEY QUESTIONS for RISK ASSESSMENT & REGULATION

- " …Identify the potential risks that NGT plants could pose for humans, animals and the environment …" (EFSA, 2020/2022)
- " …Compare the risks of NGT plants with those associated with plants obtained by conventional plant breeding techniques …" (EFSA, 2020/2022)
- "… For certain NGTs, EFSA has not identified new hazards compared to both conventional breeding and established genomic techniques (GM Technology) …" (EC-study, 2020)
  - Site-directed nuclease type 1 and type 2, ODM, cisgenesis

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REGULATORY DIFFERENCES MATTER

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GMOs (NGT products)

- EU-wide authorisation framework for individual GMOs (GM products)
  - Comprehensive requirements – incl. **assessment of risks for health and the environment**
  - Labeling / traceability requirements (incl. provision of a detection method)
- For authorised GM plants: separate registration of GM plant varieties

Conventionally bred plants

- No assessment of health and environmental risks (according to GMO-laws)
  - Legal exemptions in GMO laws: Products developed e.g. via classical untargeted mutagenesis
- However: Registration of plant varieties required
  (assessment of stability, uniformity, distinctiveness, value; not focused on safety issues)

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GENERAL VS. SPECIFIC COMPARISONS

- General conclusions vs. a truly case-by-case approach
  - Generalised conclusions in EFSA studies (addressing whole groups of applications)
  - Conclusion based on the general safety of conventionally bred plants
- EFSA opinion SDN1/2 & Impact Assessment consultation questionnaire don´t promote a differentiated case-specific assessment of individual applications according to their characteristics
  - Selective reference to certain EFSA publications (SDN1/2 Opinion vs. SynBio plants Opinion/complex SDN1)
  - Generalised question introduction in IA consultation questionnaire
- General assumption that SDN1/2 applications cannot be identified
  - By analytical detection or else

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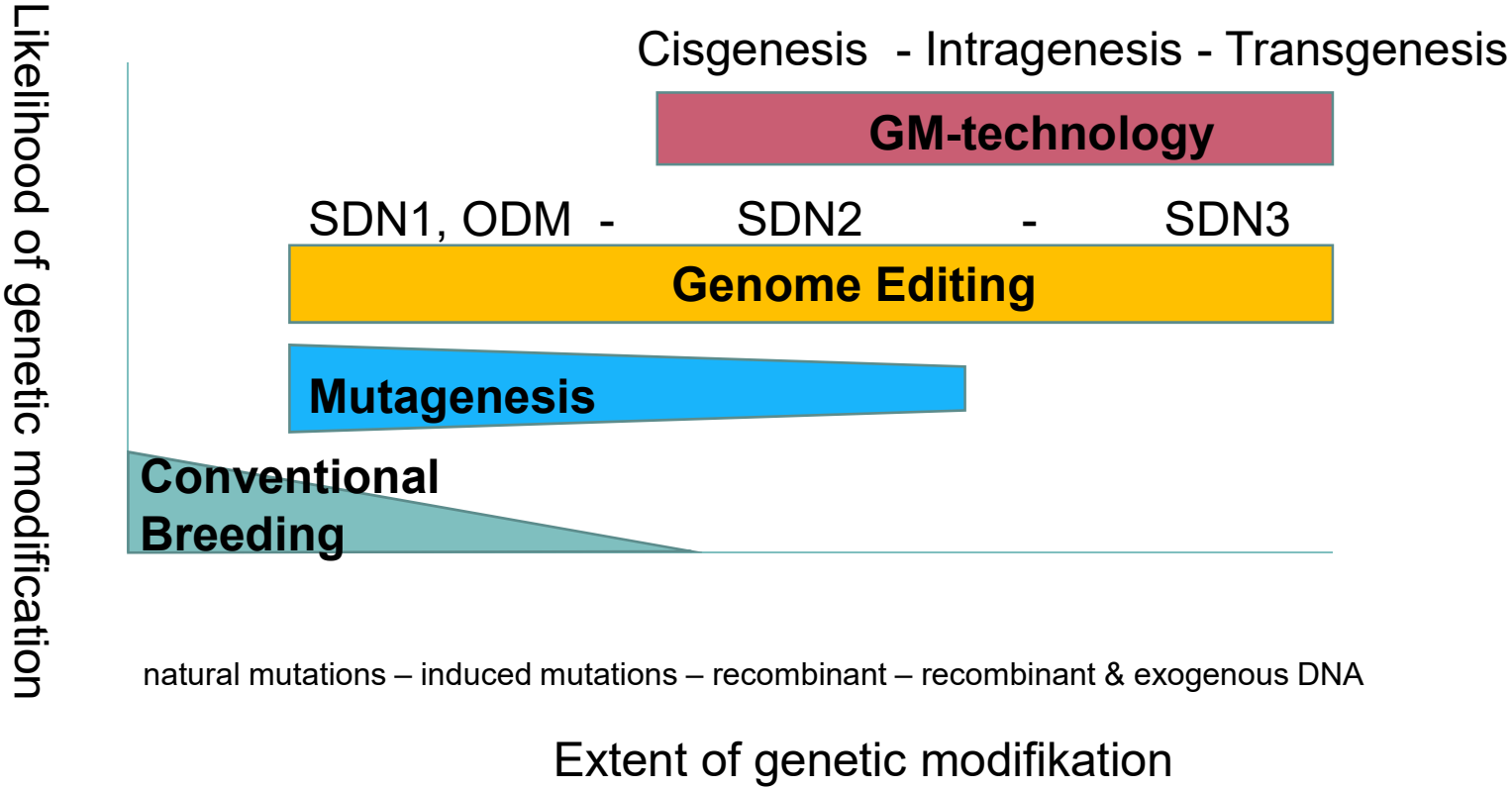

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COMPARABILITY OF NGT & CONVENTIONAL PLANTS?

- Higher speed of molecular development resulting in a limited length of phenotypical observation?
  - Direct modification of elite crop lines, vegetatively cultivated crops, trees & perennials (Gao, Cell 2021)
- Assumed “Likeness” of targeted mutation vs. conventional approaches?
  - Mutations? - Which? – How much? – Where? (Monroe et al., Nature 2022)
- Increased “Depth of intervention” – complexity of NGT plant phenotypes?
  - Simple vs. Complex NGT plants (Kawall, Plants 2021)
- Novelty of traits – possibility of unknown & unfamiliar effects?
  - Comparability to certain GM traits - HR, Disease resistance (Eckerstorfer et al., BioTech 2021)
- Occurrence of unintended genetic modifications?
  - Assumed precision of genome editing / targeted mutagenesis (Eckerstorfer et al., FIBB 2019)

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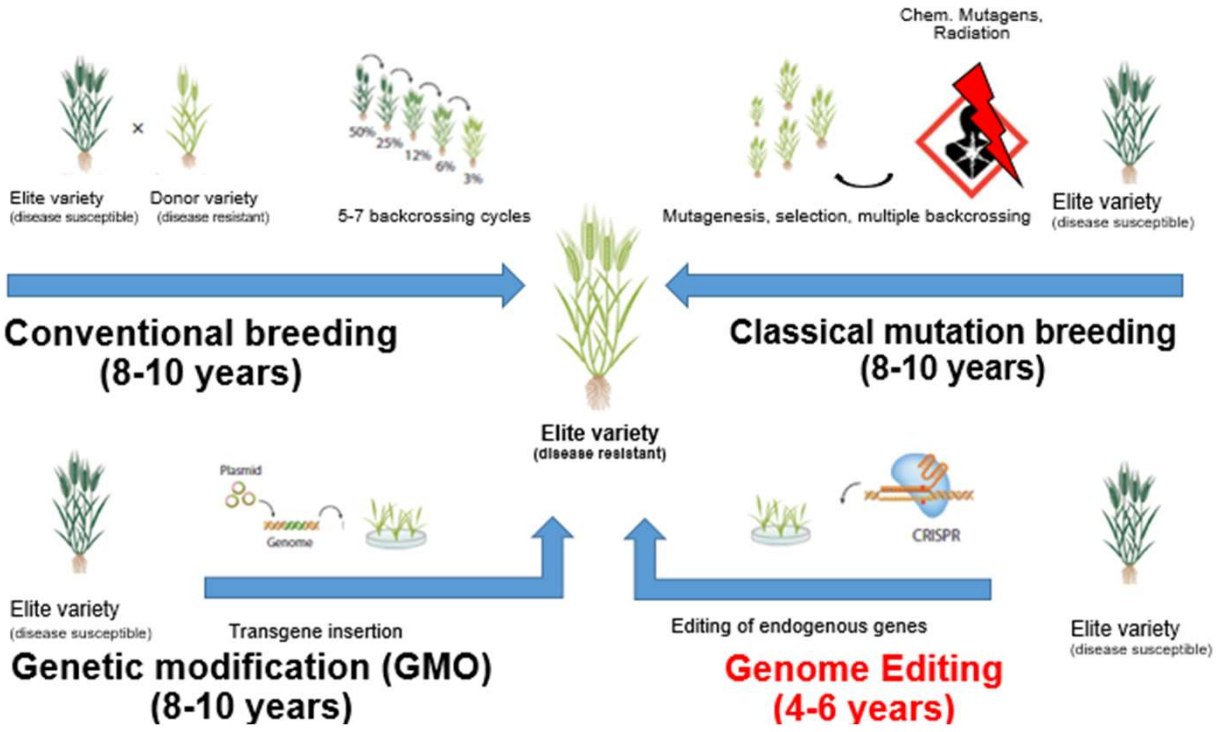
HIGHER SPEED OF MOLECULAR DESIGN



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- Genome Editing facilitates rapid molecular development
  - Simple & fast process
  - Applicable for many plant species
  - Direct modification of elite lines & vegetatively propagated plants
  - Fewer backcrossing required
- Fast development limits the time for phenotypical observation
- How fast is market introduction in practice?
  - Developers assume faster overall development (e.g. Chen et al. 2019, Gao 2021) –
    Such claims are not supported by evidence yet!
  - The development is expected to be much slower for NGT plants with complex modifications & traits

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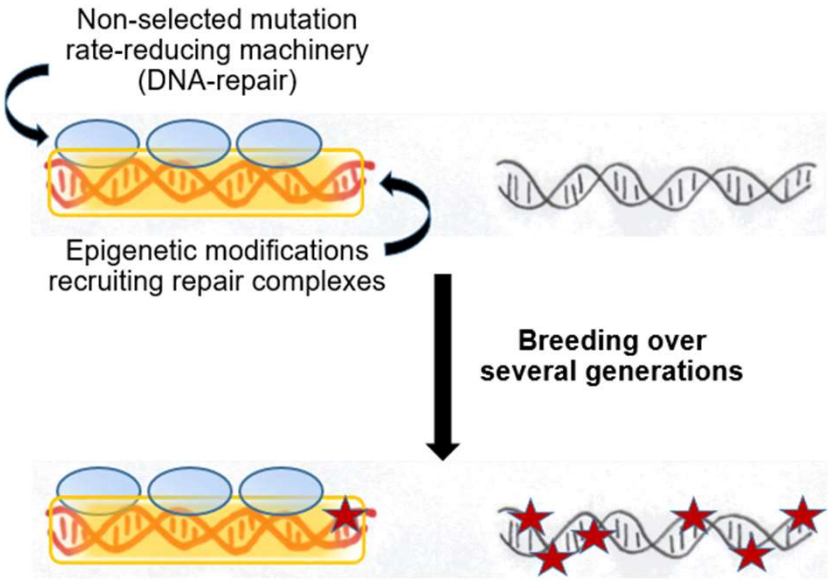
NATURAL MUTATIONS: NOT RANDOM AT ALL!

- Bias in distribution of mutations across genomes: **Assumption of "random" occurrence of spontaneous mutations is wrong!**
- Experimental data by Monroe et al. (Nature 2022)
  - 58% less mutations in gene bodies vs. intergenic regions
  - 37% less mutations in essential vs. non essential genes
  - Epigenetic features associated with a lower mutation load
- Results of literature review by Kawall (FPS 2019)
- **Targeted mutation not prone to such biases!**
  - Recurring action at non-mutated target sequences



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SIMPLE VS. COMPLEX NGT PLANTS

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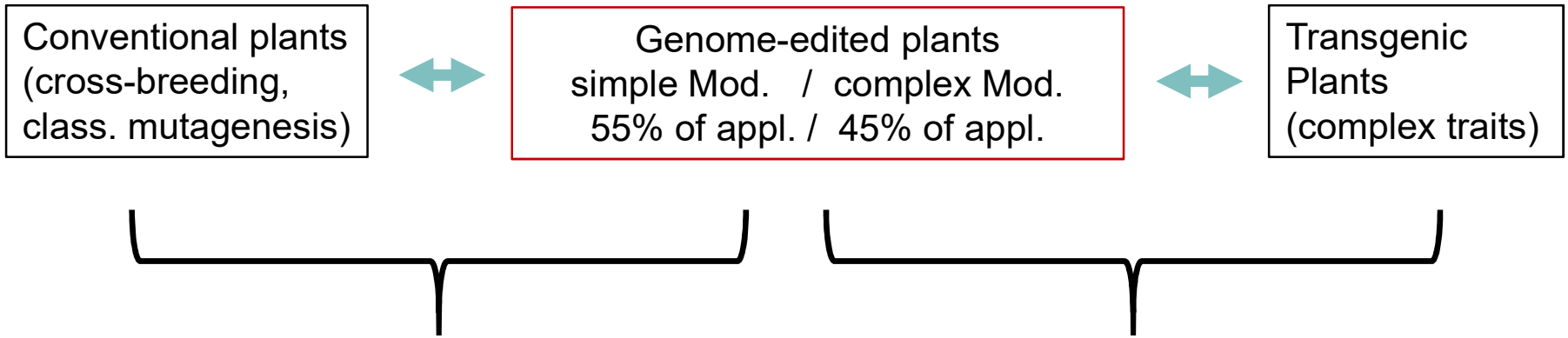

- EFSA-SDN1/2 & ODM Opinion
- EFSA summary RA of NGTs
- Study of the European Commission
- Conclusion: No specific risks

- EFSA-SynBio plants Opinion
- Eckerstorfer et al. 2019, 2021
- Kawall 2019, 2021; Kawall et al. 2020
- Adequate case-specific risk assessment is necessary / may be challenging to conduct!

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OFF-TARGET COMPARISONS

- Overall considerations on unintended modifications
  - Total removal of GM insertions? (Berthod, 2022)
  - Generalised comparison of NGT off-target rate with classical mutagenesis
- Correlation between efficiency of on-target editing & occurrence off-target modification
  - Off-target activity is connected with typical methodic approaches (Park-Yoon, 2022)
- Is there a robust body of evidence for quantifying and assessing off-targets?
  - No - only 5/107 studies used untargeted whole genome sequencing (Sturme et al., 2022)
- An appropriate assessment approach for off-target changes and their effects is required
  - 10-step workflow for a focused assessment (Eckerstorfer et al., 2019 & 2021)

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CONCLUSIONS & CHALLENGES

- Focus on recommendations for a case-specific risk assessment approach
  - **Trait x species x environment** considerations – direct and indirect effects of individual NGT plants
  - Assessment of unintended changes (Eckerstorfer et al. 2019b, Sturme et al. 2022)
- Steps for an improved guidance for risk assessment
  - What are appropriate criteria for an proposed risk assessment approach? (cf. EFSA 2022)
  - How to devise proportional data requirements for risk assessment?
    (discussion of uncertainties of risk profile concept)
- Transparency of assessors on case-specific risk assessment requirements
  - Further guidance by EFSA?
- Other regulatory consequences
  - Detection/Identification methods for NGT plants with complex vs. simple genetic modifications

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