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Risk Assessment and Regulation of Plants Modified by Modern Biotechniques: Current Status and Future Challenges

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risk assessment, modern biotechniques, genetically modified plants, genome editing, synthetic biology, gene drive

Abstract

This review describes the current status and future challenges of risk assessment and regulation of plants modified by modern biotechniques, namely genetic engineering and genome editing. It provides a general overview of the biosafety and regulation of genetically modified plants and details different regulatory frameworks with a focus on the European situation. The environmental risk and safety assessment of genetically modified plants is explained, and aspects of toxicological assessments are discussed, especially the controversial debate in Europe on the added scientific value of untargeted animal feeding studies. Because RNA interference (RNAi) is increasingly explored for commercial applications, the risk and safety assessment of RNAi-based genetically modified plants is also elucidated. The production, detection, and identification of genome-edited plants are described. Recent applications of modern biotechniques, namely synthetic biology and gene drives, are discussed, and a short outlook on the future follows.

Contents

1. INTRODUCTION

Modern biotechniques: genetic engineering and genome editing, including synthetic biology and gene drives

Genome editing: a number of different alterations/mutations in the genomes of animals, fungi, and plants characterized by high precision and efficiency

A variety of techniques are available to select and introduce desirable traits in plants ranging from conventional breeding techniques and genetic engineering to a growing number of modern biotechniques, including genome editing. Each of these techniques to modify plant genomes is expected to remain in use to different extents.

Products of genetic engineering are a reality in our daily lives—whether as industrial and medicinal applications or for animal and human consumption. During the twenty-second year of commercialization of genetically modified (GM) crops in 2017, they were grown in 24 countries on 189.8 million hectares—19 developing and 5 industrial countries. Developing countries grew 53% of the global biotech crop area compared with 47% for industrial countries. An additional 43 countries formally imported biotech crops for food, feed, and processing (67).

The increased precision now possible in plant breeding using genome-editing techniques represents a big change from conventional breeding approaches, which, in large part, rely on random, uncontrolled chemical- or radiation-induced mutagenesis, and from genetic engineering, which relies on unpredictable insertions of isolated genes into the plant genome (10). If conducive regulatory and social conditions are in place, genome editing could substantially increase the positive impacts of plant breeding on human welfare and sustainability (14).

The development and use of modern biotechniques are regulated by different countries and communities of states according to their national laws and governance structures. Generally,

the legal frameworks require submission of comprehensive scientific evidence regarding the biology of the organism, its safety to human and animal health, and its effect upon the environment in which it will be released. The Shakespearian question to be or not to be a genetically modified organism (GMO) has dramatic implications for research and development in different jurisdictions, especially in the European Union (EU). Legal interpretations of the regulatory oversights of biotechnology recently published by Israel, the United States, Canada, Argentina, Brazil, Chile, and Australia tend to exclude most or all genome-edited plants from GMO regulation, although this may be dependent on the trait modified (Canada and others), the absence of pest characteristics (the United States), or the absence of template DNA (Australia). It is important to note that classification as a GMO or not a GMO is not per se a safety-related issue.

In comparing conventional breeding techniques, established techniques of genetic modification, and new breeding techniques, the European Commission (EC)'s Group of Chief Scientific Advisors concluded that (*a*) assessment of safety can only realistically be made on a case-by-case basis and depends on features of the end product, and (*b*) genetically and phenotypically similar products deriving from the use of different techniques are not expected to present significantly different risks (10). In line with these important conclusions, the European Academies Science Advisory Council states in its policy report on genome editing that there should be full transparency in disclosing the process used, but the aim should be to regulate the specific agricultural trait or product rather than the technology by which it is produced (32). Consequently, products of modern biotechniques would be excluded from a specific regulation if the genetic changes they produce are similar to, or indistinguishable from, a product of conventional breeding and if no novel, product-based risk can be identified.

Our review discusses the current status and future challenges of risk assessment and regulation of plants modified by modern biotechniques, namely genetic engineering and genome editing. Section 2 provides a general overview on the biosafety and regulation of GM plants, describing different regulatory frameworks with a focus on Europe. In Section 3, we explain the environmental risk assessment (ERA) of GM plants and discuss aspects of toxicological assessment, especially the controversial debate in Europe on the added scientific value of untargeted animal feeding studies for each novel GM plant and whether such studies reduce uncertainties. Because RNA interference (RNAi) is increasingly explored for commercial applications, the risk and safety assessment of RNAi-based GM plants is additionally described. This is followed by Section 4 on the production, detection, and identification of genome-edited plants and Section 5 on synthetic biology (SynBio) and gene drives. We conclude with a brief outlook.

2. BIOSAFETY AND REGULATION OF GENETICALLY MODIFIED PLANTS

The risk assessment process of GM plants follows an internationally harmonized, multi-step approach to identify and characterize possible hazards and to determine the likelihood of harmful outcomes. Assessments conclude about the possible risks posed by particular GMOs and the need to implement risk management measures (**Figure 1**). Problem formulation is the first step of the risk assessment process, which provides a logical and traceable framing approach to downstream risk assessment steps and which assures that the provided information is relevant for decision making (43, 62, 115). Problem formulation starts with the identification of potential adverse effects (hazards) by considering the characteristics of the GM plant and its closest non-GM counterpart (69). Using this comparative approach, it elucidates possible pathways to harm by which the GM plant may adversely affect human and animal health or the environment. Furthermore, problem

Genetically modified organism (GMO): a nonhuman organism possessing genetic material that has been altered nonnaturally rather than by mating or natural recombination

Environmental risk assessment (ERA): evaluation of the probability and seriousness of harmful/adverse effects to human health and the environment, whether direct/indirect or immediate/delayed

RNA interference (RNAi): a variety of natural sequence homology–dependent gene silencing processes in eukaryotic organisms

Synthetic biology (SynBio): a rapidly developing, diverse collection of modern technologies aiming to transmit the application of standardized engineering techniques to biology

Gene drive: system of biased inheritance to enhance the passage of a genetic element to offspring through sexual reproduction

Problem formulation: first step in the risk assessment process providing a logical and traceable approach to frame further risk assessment steps

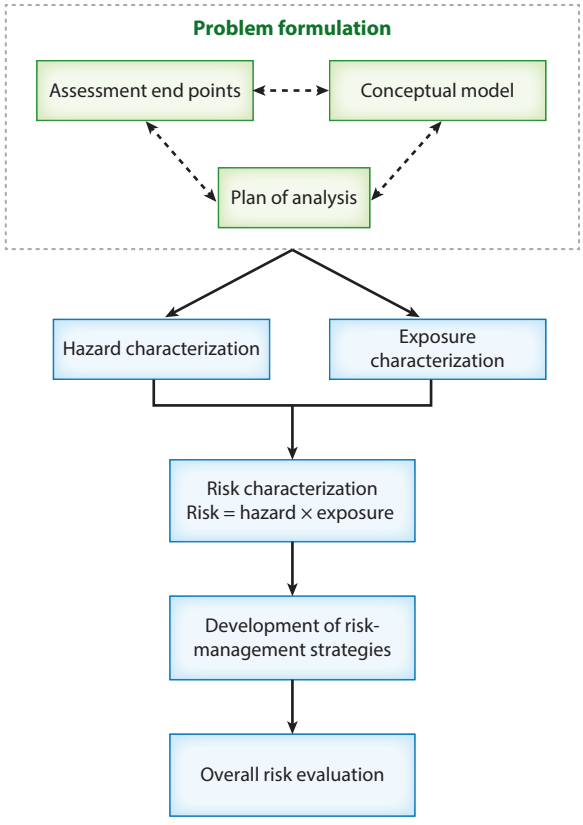

Figure 1

Core steps of the risk assessment process of genetically modified (GM) plants. The risk assessment of a GM plant is based on a multistep approach that aims to identify and characterize possible hazards and determine the likelihood of harmful outcomes in order to draw conclusions about the possible risks posed by a certain GM plant and the need for the implementation of risk management measures. Figure adapted from References 25 and 35.

formulation defines assessment end points using legislation and policy goals to specify valued entities, to develop testable hypotheses, and to guide the generation and evaluation of data in subsequent risk assessment steps (26). Based on one or more conceptual model(s), problem formulation helps to develop a plan of analysis, detailing the measures that will be included and the studies to be conducted (25, 35, 120).

Despite the existence of general principles, GM-plant regulation differs between jurisdictions. One major difference relates to the legislative trigger that determines the need for regulatory oversight (novelty of product versus nature of the applied technique). The diversity of strategies and standards for GM plants might be caused, among other things, by the fact that not all countries (e.g., Argentina, the United States, and Canada) follow the Cartagena Protocol on Biosafety, which was adopted in January 2000 at the Convention on Biological Diversity and entered into force on September 11, 2003 (25). The Cartagena Protocol on Biosafety facilitated the establishment of national biosafety regulatory systems with the objective of contributing "to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology" (25; 103, p. 3).

In the following subsections, the different regulatory systems of the five biggest GM plant–producing countries—namely the United States, Brazil, Argentina, Canada, and India (109)—are briefly described, and the regulatory framework of the EU is illustrated in more detail.

2.1. Regulatory Framework, United States

In the United States, three federal agencies are responsible for the evaluation of plants produced by modern biotechnology, and the legislative focus is placed on the characteristics of the product (59).

The US Department of Agriculture (USDA), through the Biotechnology Regulatory Services of the Animal and Plant Health Inspection Service (APHIS), is responsible for protecting agriculture from pests and diseases. The regulatory authority to determine the potential of a GM plant to pose such a risk is derived from the Plant Protection Act (59, 77). The US Environmental Protection Agency (EPA) is focusing on protecting the environment and human health from pesticides and regulates GM plants with altered pesticide characteristics under the Federal Insecticide, Fungicide, and Rodenticide Act (116). The US Food and Drug Administration (FDA) regulates the safety of food for human consumption and feed for animal consumption under the Federal Food, Drug, and Cosmetic Act. Even though the consultation step occurs on a voluntary basis, the FDA has assessed all GM plant–derived food and feed products currently on the market (77).

2.2. Regulatory Framework, Canada

Regulation of plants and livestock feed with novel traits is the responsibility of the Canadian Food Inspection Agency (CFIA), and the inspection of novel foods for human consumption falls under the responsibility of Health Canada. Thus, novelty is the major trigger for regulatory oversight in Canada. However, novelty is defined differently for plants with novel traits, feed with novel traits, and novel foods (108).

Under the authority of the Seeds Act, Feeds Act, and their respective regulations, CFIA is responsible for assessing the potential impacts resulting from novel plant and feed cultivation or consumption by livestock. Following both Acts, it is the presence of the novel trait that triggers regulatory oversight and not the method used to introduce it (15, 16). Under the Food and Drugs Act, Health Canada is responsible for verifying that novel foods do not pose any safety concern upon human consumption (108).

2.3. Regulatory Framework, Argentina

Under a series of different Argentine laws, resolutions, and directives, the Secretariat of Agriculture, Livestock, Fisheries and Food (SAGPyA) is responsible for GM-plant regulation. Major regulatory agencies within SAGPyA are the National Advisory Commission on Agricultural Biotechnology (CONABIA), the National Service of Agricultural and Food Health and Quality (SENASA), the National Direction of Agricultural Food Markets (DNMA), and the National Institute of Seeds (INASE). CONABIA evaluates the impact of GM plants on the agricultural ecosystem. SENASA, with the help of a technical advisory committee, determines the safety of food or feed derived from GM plants for, respectively, human or animal consumption. DNMA assesses their commercial impacts, and INASE is responsible for registering and controlling commercially marketed seeds (11, 121).

2.4. Regulatory Framework, Brazil

Under a 2005 biosafety law, the Brazilian National Biosafety Council (CNBS) is responsible for GM-plant regulation. The ERA and food and feed safety assessments are conducted by the National Technical Commission of Biosafety (CTNBio). CNBS generally views CTNBio assessments as conclusive and considers only administrative appeals that are of national interest, including social or economic issues (60, 107).

2.5. Regulatory Framework, India

As an extension of the Environment Protection Act of 1986, India implemented the 1989 Biosafety Rules, which cover the entire area of genetic engineering research as well as the large-scale application of GMOs and products derived thereof (79). The two main agencies responsible for implementation of the rules are the Ministry of Environment, Forests and Climate Change and the Department of Biotechnology. Six competent authorities are defined by the rules, and the Genetic Engineering Appraisal Committee is the apex body, which grants approval for the manufacture, sale, import, and export of all GMOs and products thereof, including foodstuff (79).

2.6. Regulatory Framework, the European Union

The regulatory framework within the EU was triggered by the technology that was applied to introduce a certain characteristic or trait. EU legislation differentiates GM plants intended to be introduced into the environment (deliberate release) from those intended to be used as food or feed.

2.6.1. Approval for deliberate release. Approval for the deliberate release of a GM plant is regulated by Directive 2001/18/EC,[1] which has been amended by Directive (EU) 2018/350.[2] The approval process involves all member states, the EC, and, if necessary, the European Food Safety Authority (EFSA) (**Figure 2**). The application is submitted to the competent authority of the member state where the GM plant is to be placed on the market for the first time. The member state's competent authority issues a risk assessment report indicating whether or not the GM plant under consideration may be placed on the market. In the case of a favorable decision, the assessment report is passed over to the other member states via the EC. Both member states and the EC scrutinize the assessment report and may pose objections. In cases where no objections are raised or when objections can be overcome, the GM plant is approved by the competent authority that performed the initial risk assessment. If no agreement can be reached or if the assessment report supports a rejection of the application, the EFSA has to provide a scientific opinion taking into account the scientific objections raised by the competent authorities of member states. Based on the EFSA's opinion, the EC presents a draft decision to the regulatory committee. In the case that no qualified majority is reached in favor of it, the decision is passed over to the Council of

[1] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, 2001 O.J. (L 106) 1–39.

[2] Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms, O.J. (L 67) 30–45.

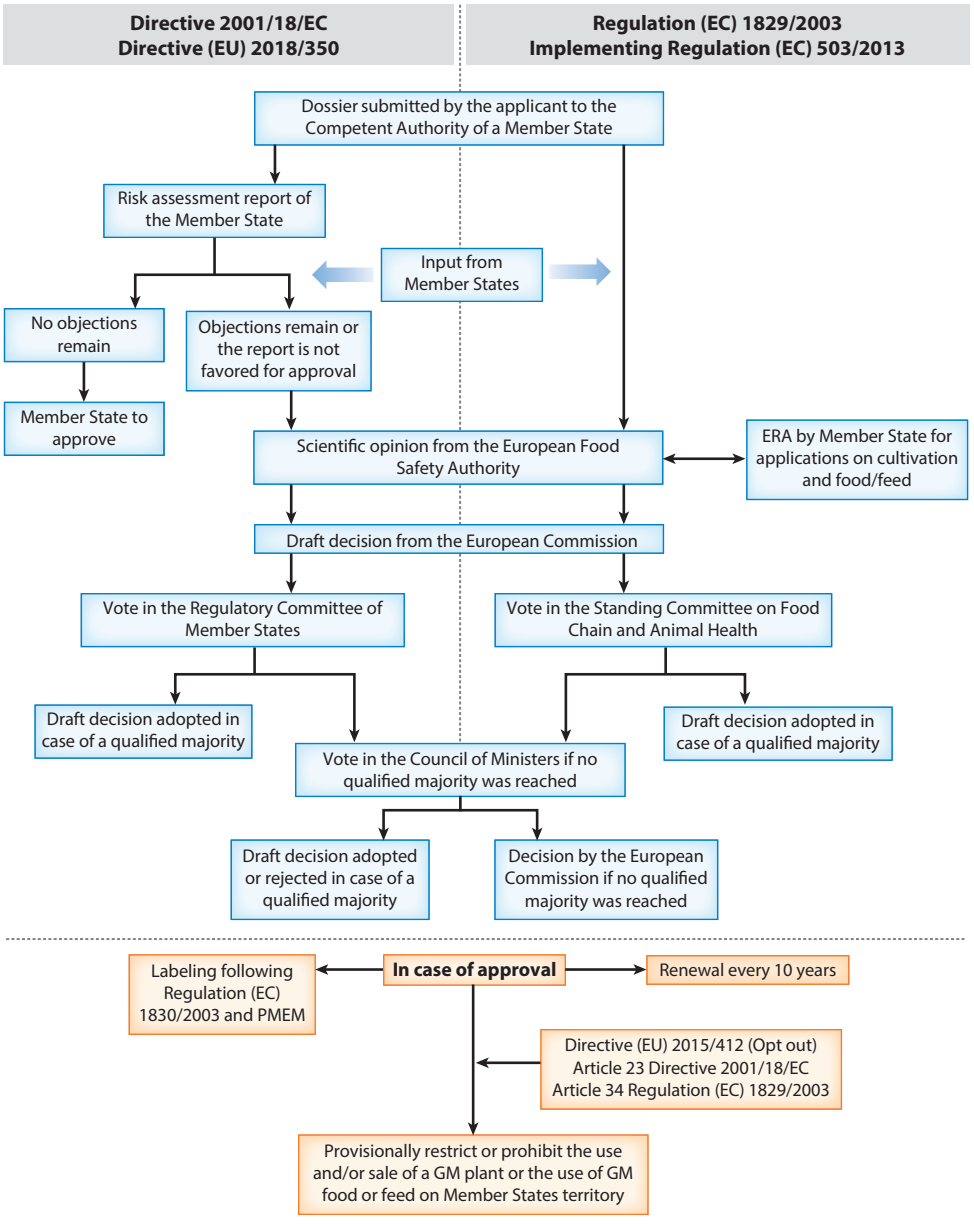

Figure 2

Approval and postapproval processes for the deliberate release and marketing of GM crops within the EU. Depending on the intended scope of an application (deliberate release versus food and feed use), different directives/regulations apply within the EU regulatory framework. Approval for the deliberate release of a GM plant (*left*) is regulated by Directive 2001/18/EC amended by Directive (EU) 2018/350. The approval of GM food and feed (*right*) is regulated by Regulation (EC) 1829/2003 and Implementing Regulation (EU) 503/2013. The regulatory framework not only covers the approval process but also applies for already approved GM plants (postapproval process, *below*). Abbreviations: EC, European Commission; ERA, environmental risk assessment; EU, European Union; GM, genetically modified; PMEM, post market environmental monitoring. Figure adapted from Reference 25.

Ministers. If the Council of Ministers does not adopt or reject the draft decision by qualified majority, the EC has to decide.

2.6.2. Approval for food and feed purposes. The approval of GM food and feed is regulated by Regulation (EC) 1829/2003[3] and Implementing Regulation (EU) 503/2013.[4] The application is submitted to a competent authority of a member state, which forwards it to EFSA, and EFSA conducts the risk assessment while considering the scientific opinion of the member states. If cultivation is covered by an application, EFSA asks a competent authority to perform the ERA, following Directive 2001/18/EC and Directive (EU) 2018/350. Based on EFSA's scientific opinion, a draft decision is made by the EC and presented to the Standing Committee on Food Chain and Animal Health. If no favorable decision is reached by a qualified majority, then the draft decision is passed over to the Council of Ministers. If the Council of Ministers does not adopt or reject the draft decision by qualified majority, the EC has to decide.

2.6.3. Postapproval considerations. Upon approval of a GM plant, EU legislation stipulates specific labeling and traceability requirements. These are applied to all food and feed products consisting of, containing, or produced from GM plants (including oils). A labeling threshold of 0.9% was established for authorized products, provided that these traces are adventitious or technically unavoidable [Regulation (EC) No 1830/2003].[5] Furthermore, postmarket monitoring is requested in order to "trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as or in products after they have been placed on the market" (Directive 2001/18/EC). In addition, the EU legislation foresees the possibility of the evocation of safeguard clauses and emergency measures if new scientific information becomes available to challenge a former risk conclusion and restrict or prohibit the marketing of a respective GM plant in member state territories [Directive 2001/18/EC and Regulation (EC) 1829/2003]. The cultivation of MON 810 maize was banned by several member states; however, the available scientific information used to justify the ban did not, based on EFSA's judgment, invalidate previous risk assessment conclusions or risk management recommendations (27). Even though the European Court of Justice concluded, "where it is not evident that genetically modified products are likely to constitute a serious risk to human health, animal health or the environment, neither the Commission nor the Member States have the option of adopting emergency measures such as the prohibition on the cultivation of maize MON 810" (18, pp. 1–2), the national bans remained. In March 2015, the opt-out Directive[6] came into force, amending Directive 2001/18/EC and enabling member states to prohibit or restrict the cultivation of GM plants in their territories based on societal concerns, such as socioeconomic impacts, or in consideration of public policy. Based on that Directive, 19 member states (partially)

[3]Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed, 2003 O.J. (L 268) 1–23.

[4]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006 Text with EEA relevance, O.J. (L 157) 1–48.

[5]Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC, O.J. (L 268) 24–28.

[6]Directive (EU) 2015/412 of the European Parliament and of the Council of 11 March 2015 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of genetically modified organisms (GMOs) in their territory, O.J. (L 68) 1–8.

excluded their territories from the cultivation of specific GM varieties (48). To provide a more predictable situation for both farmers and the market, a group of European biotech specialists and legal experts proposed an opt-in mechanism, which would enable countries to apply GM crop traits if desired (45). In its recent ruling, the EU Court of Justice (20) did not follow the opinion of the Advocate General (19) that organisms obtained by mutagenesis are generally exempted from the obligations in the GMO Directive[1] but instead decided that these organisms are GMOs indeed subject to the obligations. However, the Court exempted organisms obtained by mutagenesis techniques conventionally used in a number of applications and having long safety records. The ruling demonstrates that the European GMO regulatory framework is inadequate and needs to be updated. In its statement on a scientific perspective on the regulatory status of products derived from gene editing and the implications for the GMO Directive, the Group of Chief Scientific Advisors requested "to improve EU GMO legislation to be clear, evidence-based, implementable, proportionate and flexible enough to cope with future advances in science and technology in this area" (49, p. 6). To achieve this, the Group of Chief Scientific Advisors recommends "revising the existing GMO Directive to reflect current knowledge and scientific evidence, in particular on gene editing and established techniques of genetic modification. This should be done with reference to other legislation relevant to food safety and environmental protection" (49, p. 6).

Protection goal: the objectives of environmental policies, typically defined in law or regulations; environmental components that should be protected

Ecosystem services concept: a framework distinguishing ecosystem service categories: provisioning (e.g., food/feed), regulating (e.g., pollination), cultural (e.g., recreation), and supporting (e.g., nutrient cycling)

3. RISK AND SAFETY ASSESSMENT OF GENETICALLY MODIFIED PLANTS

3.1. Environmental Risk Assessment

In the ERA that precedes the commercial release of any new GM plant, the risk to components of the environment that are protected by relevant laws or policies is assessed (26, 98). A common protection goal to be addressed in most jurisdictions is biodiversity, a term that is too vague and generic to be scientifically assessed. The ecosystem services concept has been found to be very useful to translate the policy protection goal of biodiversity into more specific, operational protection goals (26, 58). According to the Millennium Ecosystem Assessment (90), four categories of ecosystem services can be differentiated: provisioning, regulating, supporting, and cultural services. One valued regulating service is the biological control of pest insects provided by their natural enemies (i.e., predators and parasitoids). As described previously, in the problem formulation phase of the ERA, conceptual models are constructed that describe pathways whereby the GM plant could harm the specific protection goals, i.e., arthropod abundance or ecological functions provided by arthropods (**Figure 1**). This allows the development of risk hypotheses that are then tested in the analysis phase of the ERA. A detailed description of this process and how to derive testable risk hypotheses is provided elsewhere (e.g., 58, 62, 88).

For the cultivation of GM plants carrying an insecticidal trait [e.g., which produce an insecticidal protein, such as a Cry protein from *Bacillus thuringiensis* (*Bt*)], the risk to biological control organisms can be grouped in three categories: (*a*) The plant transformation process may have introduced potentially harmful, unintended changes; (*b*) the insecticidal protein may directly affect nontarget species (toxicity); and (*c*) indirect effects on biological control may occur because of changes in crop management or to crop-based arthropod food webs.

The risk of unintended changes caused by the transformation process (the first category of risk) is typically addressed by a weight-of-evidence approach that considers information from the molecular characterization of the particular GM event and from a comparison of the composition and agronomic and phenotypic characteristics of the GM plant with its conventional

counterpart(s) (56, 58). The approach aims to identify unintended changes that are potentially harmful and which would need to be assessed in more detail in the ERA. There is increasing evidence that the process of genetic engineering generally has fewer effects on crop composition compared with traditional breeding methods (65). The current approach appears to be sufficiently conservative, particularly because offtypes are typically eliminated during the many years of breeding and selection during the development of a GM variety (71, 83, 102).

Nontarget organism (NTO): an arthropod species that is not intended to be affected by the potential stressor under consideration

A more realistic risk is that the insecticidal protein produced by the GM plant could be toxic to nontarget species that are exposed under field conditions (risk in the second category). Thus, a typical risk hypothesis addressed during ERA is that "the insecticidal protein does not harm [nontarget arthropods] at the concentration expressed in the field" (92, p. 205). This hypothesis is subsequently tested within different tiers that progress from laboratory studies representing highly controlled, worst-case exposure conditions (tier 1) to bioassays with more realistic exposure to the toxin (tier 2) and field studies (tier 3) (57, 92). Moving to a higher tier is considered relevant only if adverse effects are detected at the lower tier or if unacceptable scientific uncertainty remains. Because not all nontarget organisms (NTOs) that are potentially at risk can be tested from a practical viewpoint, a representative subset of species is selected for assessment. Three main criteria are used to select those test species (97):

1. Potential sensitivity: Species with the highest likelihood of being sensitive to the arthropod-active compound, based on the known spectrum of activity of the active ingredient, its mode of action, and the phylogenetic relatedness of the test and target species.
2. Relevance: Species should be representative of valued taxa or functional groups that are most likely to be exposed to the arthropod-active compound in the field. Knowledge about the natural enemies present in a particular crop, their relevance to biological control, and their biology and ecology is used to select representative test species (72, 95).
3. Availability and reliability: Suitable life stages of the test species must be obtainable in sufficient quantity and quality, and validated test protocols should be available that allow consistent detection of adverse effects under ecologically relevant parameters.

We now have more than two decades of experience assessing the nontarget risk of GM plants that produce insecticidal proteins derived from *Bt* (Cry and VIP proteins). Key in this assessment are the laboratory toxicity studies that feed nontarget species concentrations of the insecticidal protein, which exceed the level of exposure under field conditions. Such studies, however, need to be carefully designed to avoid erroneous results, i.e., false negatives (which would lead to the release of a GM plant that is potentially harmful) and false positives (which would trigger additional testing and confuse the ERA) (24, 93, 94).

There is evidence that the insecticidal proteins produced by GM crops grown today have no unintended effects on NTOs outside the order (in case of Lepidoptera-active proteins) or family (in case of Coleoptera-active proteins) of the target insect(s) (96). The tiered risk assessment approach appears to be sufficiently conservative (29) and also works for alternative and novel modes of action, such as RNAi-based resistance (91). Due to the fact that *Bt*-transgenic GM crops provide a much-targeted pest control and help reduce the amount of insecticides used, these plants often lead to higher biodiversity in crop fields (60, 80, 96).

3.2. Toxicology

Foods and feeds derived from GM crops need to be shown to be as safe as those derived from their conventional counterparts prior to commercialization. Within the EU, Regulation (EC)

1829/2003 and, since 2013, the Implementing Regulation (EU) 503/2013 lay down specific requirements to be considered during risk assessment. Implementation of these regulations is facilitated by guidance documents provided by the EFSA (e.g., 33, 36, 38–41) and include a focus on the potential toxicity of an introduced trait or unintended changes that go beyond the intended effect of the genetic modification. In order to identify such "potential adverse effects on the whole genetically modified food/feed or address remaining uncertainties," the Implementing Regulation (EU) 503/2013 (p. 31) requests the mandatory conduct of 90-day rodent feeding trials with whole food or feed and contradicts the currently prevailing paradigm that such studies might only be considered on a case-by-case basis, i.e., if the molecular, compositional, phenotypic or agronomic analyses were indicative of an adverse effect (34, 35). Based on the Organisation for Economic Co-operation and Development (OECD) Test Guideline 408 Repeated Dose 90-day Oral Toxicity Study in Rodents, 90-day feeding trials are frequently performed to determine the toxicological potential of single substances. The underlying principle is that the highest dose level should be chosen to induce toxicity but not death or severe suffering. Furthermore, a dose response should be established and the no-observed-adverse-effect level (NOAEL) should be identified (82). Based on the NOAEL or similar measures, an acceptable daily intake level can be determined to which humans may be exposed daily over their lifetimes without an appreciable health risk (42). When applying this principle to the testing of whole food or feed, several limitations should be considered. (*a*) The highest dose that can be administered is determined by the need to provide nutritionally balanced diets and the satiety of the test animals. Thus, the dose may be too low to identify potential hazards associated with the test food or feed (33). (*b*) With regard to unintended effects, it is unlikely that substances that occur in small amounts and/or that have a low toxic potential will cause any observable toxicity because they would be below the NOAEL and would be unlikely to impact human or animal health at normal intake levels (34). (*c*) In the absence of a targeted test hypothesis, it is impossible to determine a priori an effect size of potential toxicological relevance and consequently to empower the study in such a way that an effect can be detected (33). The Implementing Regulation (EU) 503/2013 contains a review clause stating that "[t]he Commission shall review the requirement to perform 90-day feeding studies in rodents with whole genetically modified food/feed […] on the basis of new scientific information" (p. 7). Furthermore, "The Commission shall in particular monitor the outcome of the research project called GRACE (GMO Risk Assessment and Communication of Evidence)" (p. 7). The mandatory nature of a 90-day animal feeding trial with whole GM food/feed assumes that its performance generally provides an added value for the risk assessment of GM crops per se and reduces the level of uncertainty. However, this is contradicted by the conclusions of the GRACE consortium, which state, among other things, that if preceding analyses have not identified a trigger, 90-day feeding studies do not innately provide added value to a risk assessment nor do they increase the confidence in the data provided because significant differences in measured end points may have been generated randomly. Furthermore, their mandatory performance cannot be justified in light of the legally required replacement, reduction, and refinement approach (61). Nevertheless, after performing the review as stipulated in the Implementing Regulation (EU) 503/2013, the EC came to the conclusion that the mandatory nature of such studies should be maintained as "…there remain difficulties to define, with the necessary precision, the level of uncertainties […] which would trigger the requirement for the 90-day studies on a case by case basis", and "…the majority of Member States supported the Commission's conclusion to maintain the 90-day feeding study requirement […] as a necessary additional safety layer" (47, A.03). This clearly demonstrates the failure of EU member states and the EC to implement evidence-based regulations, further ignoring the legal requirement to replace, reduce, and refine the use of laboratory animals as requested

by Directive 2010/63/EU.[7] Furthermore, a recently published and poorly designed research study fueled the debate about further prolonging the exposure period of GM whole food and feed in order not to miss any potential adverse effect (104). The study was heavily criticized by the scientific community and various agencies involved in food safety assessment (for more details, see 70). The scientific justification and added value of long-term feeding trials were analyzed by another EU-funded project called GM Plant Two Year Safety Testing (G-TwYST). The G-TwYST consortium concluded, among other things, that (*a*) without a targeted hypothesis, the performance of 90-day and long-term trials does not provide additional information supporting risk assessment, and (*b*) in contrast to the study performed by Séralini et al. (104), no carcinogenic or other adverse effects could be observed after an exposure period of two years with NK603 maize (63). The results from both G-TwYST and GRACE show that "[n]either the 90-day nor the long-term animal studies revealed any health risks of the GM maize tested" and that "the added scientific value of animal feeding studies without a targeted hypothesis is very limited and does not significantly reduce remaining uncertainties." Therefore, the researchers determined, "we do not see the need to continue with the mandatory requirement to conduct untargeted animal feeding studies for each novel GM plant" (64, p. 1).

RNA-directed DNA methylation (RdDM): the epigenetic and sequence-specific methylation of plant DNA caused by small interfering RNAs, Argonaute proteins, de novo DNA methyltransferase, and additional special factors

3.3. Risk and Safety Assessment of RNA Interference–Based Genetically Modified Plants

RNAi encompasses a variety of natural sequence homology-dependent gene silencing processes in eukaryotic organisms. Basically, two different RNAi mechanisms, both initiated by double-strand RNA (dsRNA), which is then cleaved by RNase III–type Dicer-like (DCL) enzymes into small RNAs, can be discriminated (6, 106): posttranscriptional gene silencing (PTGS) and transcriptional gene silencing (TGS).

In plants, small RNAs are usually categorized into two major classes, small interfering RNAs (siRNAs) and microRNAs, depending on their biogenesis (3, 8). **Figure 3** gives an overview of the small RNA classes involved in different gene silencing pathways in plants.

While PTGS involves cytoplasmic mRNA cleavage and translational repression (9), the place of TGS action is the nucleus. Here, 24-nucleotide (nt) siRNAs bound to Argonaute proteins enter a complex pathway resulting in epigenetic modifications at sequence-specific target loci, including RNA-directed DNA methylation (RdDM) and histone modifications (76). TGS is thought to be involved in transposon silencing, genome structure, and stress adaptation.

Posttranscriptional RNAi is an efficient tool for studying plant gene function and has been used for crop improvement for a long time. For RNAi-mediated gene silencing, dsRNA has to be produced as a trigger. This can be achieved via genetic modification by the introduction of sense, antisense, or hairpin (hp) constructs homologous to the respective target gene or by infection with a recombinant plant virus carrying part of the target gene in an approach termed virus-induced gene silencing. An early example of an RNAi-based GM plant is the FLAVR SAVR tomato with reduced polygalacturonase expression and delayed fruit softening (89). More recently, RNAi has been applied to obtain GM plants with improved nutritional value and enhanced product quality. Some of these plants have been deregulated and commercialized in several countries. They include soybean with high oleic acid and low linoleic acid, nonbrowning Arctic™ apple, and potato with reduced acrylamide formation and black spot resistance (105).

[7] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Text with EEA relevance), O.J. (L 276) 33–79.

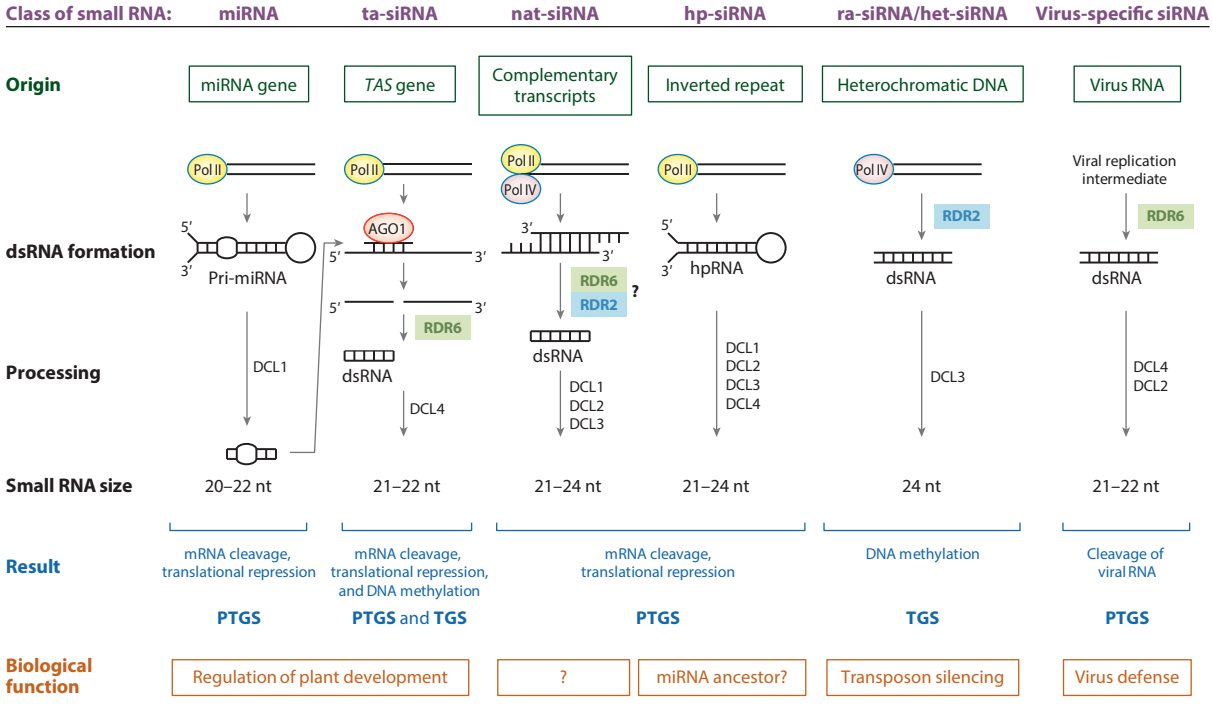

Figure 3

Overview of gene silencing pathways in plants and biogenesis of different small interfering RNA (siRNA) classes. MicroRNAs (miRNAs) are derived from endogenous gene transcripts with imperfect double-stranded stem loop structure [primary (pri)-miRNA] after successive processing by Dicer-like 1 (DCL1) into shorter precursor (pre)-miRNA and into mature miRNA. Plant miRNAs target mRNAs with near-perfect sequence homology, resulting in either mRNA cleavage or translational inhibition (9). As many miRNAs are involved in the regulation of transcription factors, they have important roles in plant development, partly through the action of secondary *trans*-acting siRNAs (ta-siRNAs) which originate from *TAS* genes (reviewed in 73). Ta-siRNAs are an example of secondary siRNAs because their biogenesis is initiated by a miRNA targeting a primary transcript (ARGONAUTE 1, AGO1) followed by RDR6-mediated double-strand RNA (dsRNA) synthesis. Natural siRNAs (nat-siRNAs) are derived from the hybridization of separately transcribed complementary RNAs from opposite DNA strands. Formation of many but not all nat-siRNAs depends on RDR2 or RDR6 (3). Hairpin (hp)-siRNA derived from long hairpin precursors are believed to potentially evolve into miRNAs. They are similar to exogenous hp constructs introduced by genetic modification (3). The largest class of siRNAs naturally occurring in plants are repeat-associated (ra)-siRNAs or heterochromatic (het)siRNAs, which require RNA polymerase (Pol) IV and RDR2 for accumulation and which act at the transcriptional level. siRNAs produced from dsRNA synthesized by RDR6 using viral replication intermediates as a template can trigger an antiviral response to an infecting homologous virus. Posttranscriptional gene silencing (PTGS) in plants can be amplified using small RNA as a primer for converting single-strand RNA into new dsRNA, which is subsequently processed by DCLs into secondary siRNAs. Several factors, such as aberrant or overexpressed target transcripts, promote secondary siRNA formation (3).

RNAi has also been used for the generation of GM plants with resistance to viruses (54) and, more recently, for achieving resistance to bacterial crown gall disease (1). Further applications of RNAi are the control of fungal infections and plant pests through the expression of dsRNA that targets essential pathogen and pest genes using host-induced gene silencing (HIGS) approaches (118). In GM maize MON 87411, an inverted repeat sequence of a 240–base pair fragment of the western corn rootworm (*Diabrotica virgifera virgifera*) *Snf7* gene introduced as a plant-incorporated protectant (PIP) causes downregulation of the targeted *DvSnf7* gene. When western corn rootworm feeds on the plant, gene silencing eventually leads to insect mortality. This GM maize has been approved for commercial cultivation in the United States and Canada (126).

Off-target effect: a DNA repair event that occurs outside the intended target sequence and leads to a detectable mutation

In HIGS, dsRNA and siRNAs generated by the plant RNAi machinery are transferred from the plant to pathogens or pests (118). Insects, however, seem to take up only longer dsRNA fragments efficiently. At the same time, dsRNA is degraded in many insects by specific nucleases, thereby limiting successful RNAi applications for insect control especially in Lepidoptera (reviewed in 123). Therefore, there have been activities to generate transplastomic plants in order to produce large amounts of long dsRNA in the chloroplasts where it is protected from Dicer activities (123).

RNAi-based GM plants are internationally regulated according to respective gene technology regulations. However, there is still ongoing discussion about the relevant risk assessment issues specific to RNAi-based GM plants (13). One of the specific risks associated with RNAi is that siRNAs may pair with partially complementary mRNAs from off-target genes in the GM plant or in organisms interacting with it. Although bioinformatics is helpful for predicting potential off-target genes (124), there is a general consensus that its value for risk assessment is limited due to gaps in crop genome sequences, sequence variations between plant varieties, and additional factors involved in target site binding and dsRNA processing. It should also be kept in mind that plant genomes are dynamic and that RNAi is a natural process, implying that off-target base-pairing can also occur for endogenous small RNAs in the course of evolution and conventional breeding. Possible unintended adverse effects on agronomic performance, plant composition, or nutritional value resulting from off-target silencing in an RNAi crop can be detected during comparative analysis, which is a common part of regulatory approaches for GM plants (13, 53).

Off-target gene silencing may also theoretically occur in other organisms exposed to the RNAi plant, e.g., during food and feed consumption by humans and farm animals. However, there is a long history of the safe consumption of small RNAs naturally produced in plants, including those matching human genes (105). Several reasons account for this apparent nonfunctionality of ingested small RNAs from plants, including uptake barriers, degradation, and differences between plant and mammalian RNAi-pathway components (53).

The risk of off-target gene silencing in interacting organisms is more pronounced in the case of plants with RNAi-based PIPs where NTOs related to the target pest may contain genes with sufficient sequence homology. However, as has been stressed previously, siRNA targeting of mRNAs and the resulting gene silencing cannot be predicted by sequence alone, although generally it can be stated that the risk of off-target silencing increases with the length of a dsRNA due to increased chances for multiple 21-nt matches (123, 126). One possible first approach in NTO testing is to select organisms based on their phylogenetic relatedness to the target organism in order to characterize the spectrum of lethal activity of a specific dsRNA (4). An important point to consider in the evaluation of risks to NTOs is exposure, which is one factor in the ecological risk equation (115). This means that relevant NTOs should be selected not only according to taxonomic relatedness but also depending on relevant exposure scenarios. Environmental exposure to dsRNA is determined by expression levels in the plant and dsRNA stability in different environmental settings. As shown by Dubelman et al. (30), dsRNA is rapidly degraded in soil, although its half-life depends on soil characteristics. In transplastomic plants, dsRNA amounts in leaf tissue are much higher than in nuclear-transformed plants, although roots, tubers, and pollen are essentially free of transgenic dsRNA, implying that pollinators and pollen-eating insects are not exposed (123). Taking into account relevant routes and levels of exposure, an ERA protocol has been developed for *DvSnf7* dsRNA, including for laboratory studies with a set of NTOs from different functional groups, such as pollinators or biocontrol species (5).

One general consideration during the evaluation of RNAi-based GM plants is the possible switch from PTGS to TGS. Posttranscriptional RNAi can be accompanied by the methylation of homologous DNA, including the RNAi trigger. For transgenes, it has been found that methylation

Table 1 Presumed regulatory classification of plants modified by RNA-directed DNA-methylation techniques

Production of promoter-targeting dsRNA	Result	Classification according to definitions in European GMO legislation[a]	
		Intermediate organism	Transgene-free progeny
Plant transformation with dsRNA construct	Stable transgene integration and inheritance of dsRNA construct	GMO	
Plant transformation with dsRNA construct, followed by transgene segregation through outcrossing	Stable transgene integration in the primary transformant (intermediate organism); selected transgene-free progeny with epigenetic modifications	GMO	No GMO (if plant is free of recombinant DNA)
Transient introduction of dsRNA construct by an agroinfiltration method that facilitates siRNA transport to distal organs	Transient dsRNA production in infiltrated cells; transport of processed siRNAs to new leaves; maintenance of epigenetic modifications in in vitro regenerants and their progeny	No GMO (DNA not integrated in plant genome)	No GMO
Transient introduction of dsRNA construct by infection with a VIGS vector	Transient dsRNA production in infected tissues; after transgenerational viral clearance virus-free progeny with epigenetic modifications	GMO (infecting virus is a GMO)	No GMO
Direct introduction of free dsRNA	SiRNA-mediated epigenetic modifications that are transmitted to offspring plants	No GMO	

[a]Classification is mainly based on the generally accepted view that epigenetic modifications also occur naturally and do not lead to genetic changes.
Abbreviations: dsRNA, double-strand RNA; GMO, genetically modified organism; siRNA, small interfering RNA; VIGS, virus-induced gene silencing.

sometimes spreads along the transcribed sequence into the adjacent promoter. Such a switch could lead to shutoff of dsRNA production and reduced RNAi efficiency in the course of vegetative or generative plant propagation (13). For transgenes, epigenetic switches from PTGS to TGS correlated with de novo methylations of promoter and 5′-transcribed regions have been observed during prolonged vegetative plant propagation (28, 52).

As stressed previously, TGS is a form of siRNA-mediated gene silencing where gene expression is specifically shut down by RdDM and other epigenetic modifications. Because this silencing mechanism does not require continued presence of the dsRNA trigger, there have been attempts to use it as a new GM-free breeding technique. To obtain transgene-free TGS plants, dsRNA targeting a specific promoter can be transiently expressed after agroinfiltration or inoculation with viral vectors, or it can be produced in GM plants. In the latter case, the dsRNA-producing transgene construct is then segregated by outcrossing, while promoter methylations are maintained. **Table 1** shows presumed regulatory classifications of different types of RdDM plants. So far there are no commercial applications of this technique, partly because several obstacles limit stable and heritable TGS of endogenous genes (68).

4. THE PRODUCTION, DETECTION, AND IDENTIFICATION OF GENOME-EDITED PLANTS

4.1. Techniques for Genome Editing

In the scientific literature, the term genome editing was coined in 2005 by Urnov et al. (114), and, since then, it has been used for a number of different alterations and mutations in the genomes of animals, fungi, and plants. The technical tools used for genome editing in plants can be specific

oligonucleotides that foster the mismatch repair pathway, site-directed nucleases (SDNs), or a combination of both (7, 37, 99). In this section, we present and discuss the different techniques used for genome editing, different outcomes of the application of these techniques, and problems associated with the detection and identification of genome-editing events.

Site-directed nuclease (SDN): nuclease guided to a specific DNA sequence for cleavage

Oligo-directed mutagenesis (ODM): an intentional mispairing of an oligonucleotide and genomic target sequence to induce a specific mutation by subsequent repair

Double-strand break (DSB): simultaneous breakage of both DNA strands induced, e.g., by nucleases, by torsional stress during replication or transcription, or by cellular repair processes

The use of oligo-directed mutagenesis (ODM) for mutating a plant genome was first described in the late 1990s (7). The technique has different names, such as gene-repair oligonucleotides and rapid trait development system, and serves usually for the introduction of point mutations. In recent years, a combination of ODM and the application of SDNs has led to an improvement in the specific editing of genes (99).

Also since the 1990s, it has been known that DNA double-strand breaks (DSBs) enhance recombination at the broken locus, which may or may not involve sequence homology (2, 84). Using artificial cutting sites introduced a priori in plant genomes for meganucleases such as *ISce-I*, several groups showed that the introduction of a DSB enhances especially homologous recombination (HR) by at least an order of magnitude (85). Subsequently, researchers using artificial zinc-finger nucleases (ZFNs) could achieve endogenous gene targeting but with low efficiency (23). However, over the past few years, the development and application of meganucleases, ZFNs, transcription activator-like effector nucleases (TALENs) designed in a more sophisticated manner, and, most recently, CRISPR/Cas9 systems increased the editing efficiency and resulted in various site-directed gene-editing events in a growing number of plants.

The designations of SDN-1, SDN-2, and SDN-3 represent different ways to influence the repair of a DSB through SDNs (37) (**Figure 4**). In SDN-1, the DSB is introduced at a specific

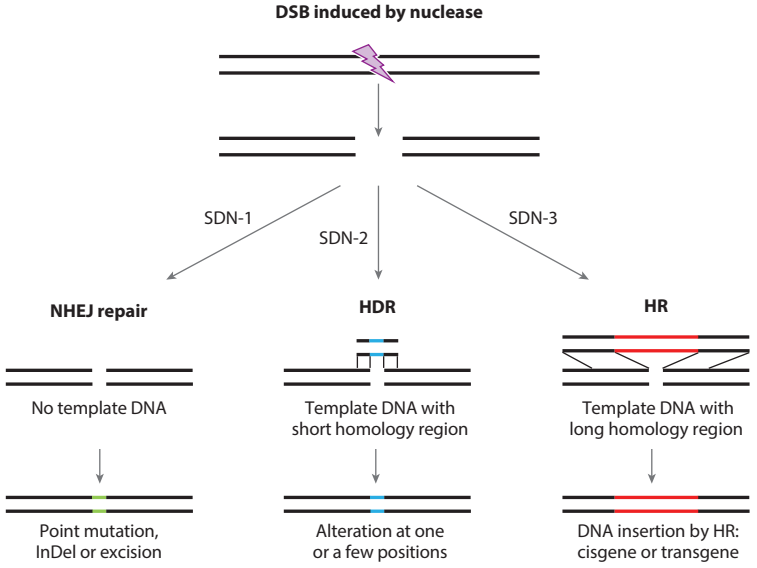

Figure 4

Different forms of double-strand break (DSB) repair after application of a site-directed nuclease (SDN). Three ways of repairing the introduction of a DSB in DNA are depicted. SDN-1: Without any homologous DNA around, the DSB is almost exclusively repaired by nonhomologous end joining (NHEJ), leading to point mutations or small insertions or deletions (InDels) that occur just by chance. SDN-2: Small alterations are introduced via homology-directed repair (HDR) using a short-template DNA. SDN-3: This mechanism exploits the homologous recombination (HR)-pathway for introduction of a new DNA sequence flanked by long arms of DNA that have to be homologous to the genomic sequence around the DSB.

genomic site and the repair is performed by host cell repair mechanisms without any further intervention; that is, no template DNA is added. In SDN-2, a template DNA has to be added that is homologous to the break area. This template DNA contains bases that differ from those in the original genome sequence, leading to a specifically altered genomic sequence that is copied into the break during the repair process. The difference between the original and desired sequences may be as small as one or a few nucleotide(s). However, SDN-2 is not useful for the introduction of larger sequences (e.g., transgenes or regulatory sequences) because the mechanism used for SDN-2-driven repair is not HR but rather a form of nonhomologous end joining termed microhomology-mediated end joining. This is a mechanism that does not work efficiently on extensively resected DSBs but uses microhomologies ranging from 6 to 20 nt to copy a template into the DSB (122, reviewed in 117). In SDN-3, long stretches of template DNA have to be added with homology of around 500 nt or greater to the areas upstream and downstream of the introduced DSB (86). Between the long, homologous sequences that are essential for HR, virtually every sequence can be located using a transgene, cisgene, synthetic gene, or regulatory sequence (**Figure 4**).

4.2. Outcome and Detection of the Different Site-Directed Nuclease Techniques

Considering the different approaches to and outcomes of using SDN techniques, we want to raise the point that the term genome editing, which is often used for all applications ranging from ODM to SDN-1 through SDN-3, should be differentiated more in scientific and public discussions (31). As mentioned before, usually SDN-1 is applied for the induction of point mutations to interrupt a gene or for the deletion of sequences. Application of SDN-2 involves template DNA to introduce a specific alteration of a single or a few base pair(s). In both cases, the resulting plants are indistinguishable from natural mutations and could be produced in principle by conventional crossing procedures or undirected mutagenesis. The application of SDN-3 is still rather complicated and inefficient. SDN-3 needs a long, perfectly homologous region of template DNA and a successful HR event. Therefore, the main application of SDN-3 is to introduce a new sequence at a specific location in the genome of the host resulting in transgenic or cisgenic plants.

The detection of alterations provoked by these different SDN and ODM approaches is often possible by simple PCR followed by directed sequencing or by whole-genome sequencing. However, in the case of ODM, SDN-1, and SDN-2, the detectable genomic alteration cannot be attributed to a specific method of production: It may have arisen by natural mechanisms, such as spontaneous mutation, by somatic or meiotic replication failure, or by conventional crossbreeding. Only in the case of SDN-3 does the newly inserted sequence provide the possibility of identifying plants likely produced by genome editing (**Table 2**).

As previously mentioned, it is impossible to identify the technique by which a small genomic alteration was produced, and this impossibility might be linked to the future regulatory status of GM plants in the EU. A plant or any other organism containing only genetic material that has been altered in a way that occurs naturally is, according to article 2 of Directive 2001/18/EC, not a GMO. Following this basic definition in article 2, an altered organism that is indistinguishable from a naturally bred one should consequently not be a matter of European GMO regulation (21). In the case of SDN-3, there are at least two different possible outcomes. Firstly, insertion of a DNA sequence from a noncrossable counterpart would result in a transgenic organism and could therefore be a matter of regulation. Secondly, the perfect replacement of an allele in a breeding line with the allele of a crossable wild type from the same plant species would result in a plant that could theoretically result from conventional crossing followed by a very high number of

Zinc-finger nuclease (ZFN): a designed zinc-finger protein recognizing and binding to a specific DNA sequence fused to a nuclease, usually *Fok*I from *Flavobacterium okeanokoites*

Transcription activator-like effector nuclease (TALEN): bacterial transcription factor binding specific DNA bases; artificially fused to a nuclease protein (e.g., *Fok*I)

CRISPR/Cas9 system: adaption of the prokaryotic immune system to introduce directed and intended DNA double-strand breaks in genomes via guide RNA

Cisgenic plant: a plant whose modified gene and controlling elements are derived from the same species or from a crossable plant

Table 2 Detection and identification of genomic alterations made with different GE techniques

GE technique	Common genome alteration	Detection	Detection method	Identification of GE method possible[a]
ODM	Few specific base changes	Yes	PCR; sequencing	No
SDN-1	+1 base insertion; small or large deletions	Yes	PCR; sequencing	No
SDN-2	Few specific base changes	Yes	PCR; sequencing	No
SDN-3	Insertion and/or replacement of large sequences	Yes	PCR; sequencing; southern blot	Yes

[a]In most cases, the clear attribution of the genomic alteration to a specific GE technique is not possible.
Abbreviations: GE, genome editing; ODM, oligo-directed mutagenesis; SDN, site-directed nuclease.

backcrossing steps. In this case, GMO regulation of the plant produced by SDN-3 would be logically inconsistent because it contains only genetic material that can arise by natural crossing procedures.

5. SYNTHETIC BIOLOGY AND GENE DRIVES

SynBio can be seen as a rapidly developing, diverse collection of modern technologies aiming to transmit the application of standardized engineering techniques to biology (75, 112). SynBio is generally separated in five major fields: (*a*) use of artificial genes and genomes, (*b*) metabolic engineering, (*c*) design of genetic signal circuits, (*d*) creation of protocells and minimal genomes, and (*e*) xenobiology. Some definitions also include genome editing and gene drives (**Figure 5**). SynBio applications have been used in prokaryotes, yeast, and cell cultures and are now being introduced to algae, plants, and other higher organisms to create artificial genetic elements or new metabolic pathways (22, 74). Although applications of SynBio are being used in a number of organisms, there is still no commonly accepted definition for this collection of technologies (66, 125).

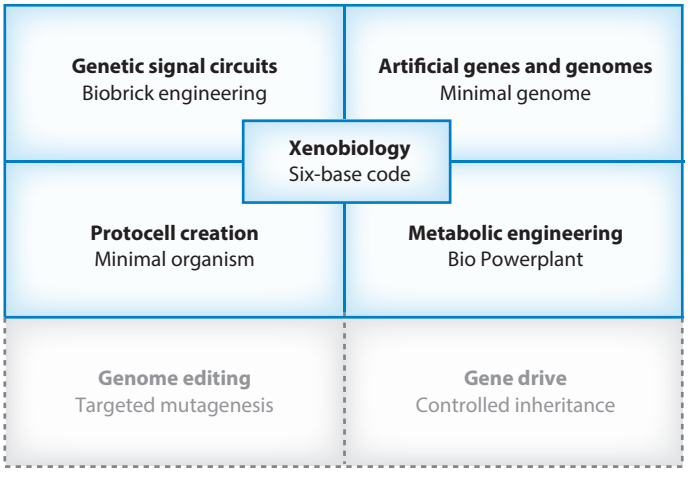

Figure 5

The major fields of synthetic biology. Topics covered by most definitions are shown in blue, and topics covered only by selected definitions are shown in gray. Example applications are written below the topics. Figure inspired by Reference 111.

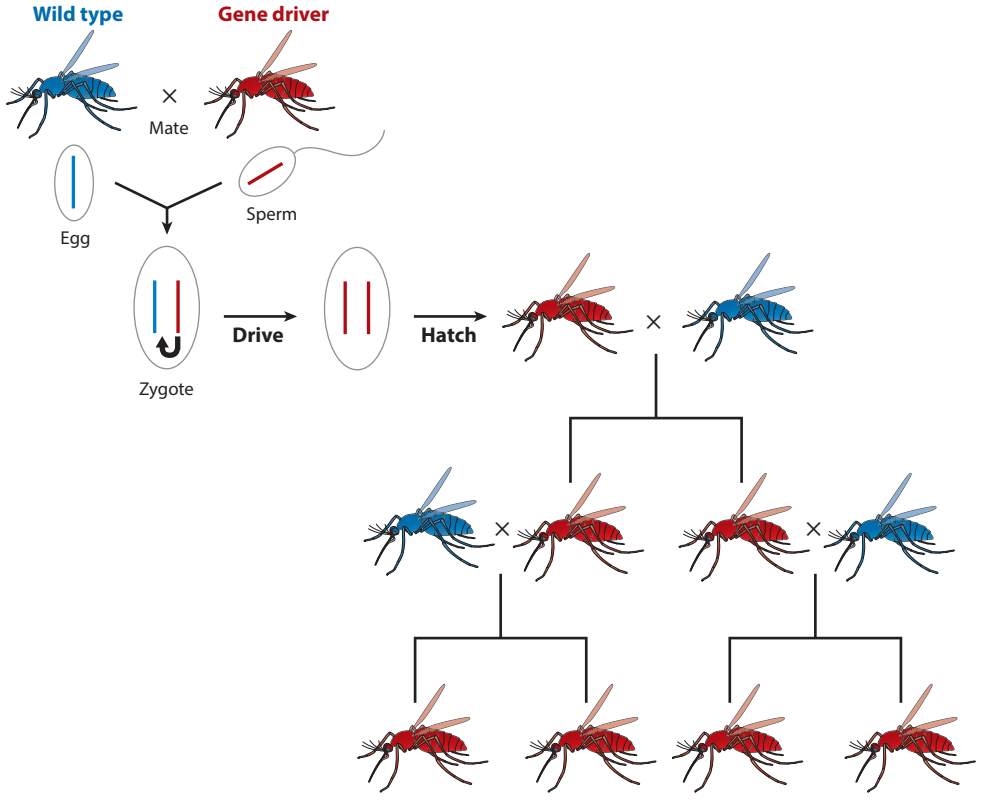

Figure 6

Principles of an engineered gene drive system with non-Mendelian inheritance. During mating, the genetic compositions of the wild type and gene driver organisms will be mixed. In a cellular state, the developing zygote will carry the driving allele in a heterozygous manner. The gene drive will cut the wild-type allele and introduce itself into it. After hatching, the organism will carry the driver in a homozygous manner, and, when it breeds with a wild type, the cycle will repeat.

In contrast, the definition for gene drive is commonly accepted as "systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced" (17, p. 1). For functional gene drives, four basic criteria have to be met. There needs to be: (*a*) sexual reproduction, (*b*) a relatively short generation time, (*c*) stability of the driving genetic elements, and (*d*) a population structure that is appropriate to the desired outcome (17). Plants do not meet these criteria in most cases, and consequently the US National Academy of Sciences sees only minor possibilities for direct gene drives in plants; however, applications for plant pests, such as insects, are possible. In a recent publication, Neve (81) discusses this question even further and highlights potential applications and existing limitations for gene drives in agriculture. It should be highlighted that in the many debates on regulation and assessment of gene drives only engineered gene drive systems using a defined driver such as CRISPR/Cas9 (**Figure 6**) are considered. Naturally occurring systems already in use, such as *Wolbachia*, are out of scope as they are not products of modern biotechnology (12, 46, 55). Because engineered gene drives contain foreign genetic material, such systems are always GMOs and have to be regulated as such. In a recently published paper, Turner et al. (113) anticipate that existing risk assessment frameworks are also applicable to the products of gene drive technologies that persist in the

environment and spread across national borders and that governance structures surrounding the involvement of civil society in regulatory processes must be administered in a more transparent and defined manner.

Debates about the definition and scope of SynBio have been ongoing for years. In an opinion on SynBio, the EU collected more than 35 definitions and presented its own operational definition as "the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms" (100, p. 27). This broad definition is comprehensive and could be interpreted as covering the whole fields of biotechnology, genetic engineering, and genome editing. The EC agreed on such a broad operational definition because criteria such as complexity, speed, and number of independent modifications are not suitable to unambiguously differentiate gene technology and SynBio from one another. Other authors and organizations have noted the absence of a commonly agreed upon definition and have referred instead to the developing collection of techniques or have raised concerns about an overly broad definition that would categorize most biotechnological approaches as SynBio (50, 66). SynBio should be seen more as a toolbox, and an organism created by the use of SynBio should be different from any organism that could occur in nature. The epistemic novelty of SynBio lies in the systematic use of engineering approaches to intentionally design artificial organisms (87).

This lack of a commonly agreed upon definition is challenging for risk and safety assessment in the field under current guidance because the scope is imprecise and different interpretations of guidance documents are possible.

The EC's broad definition allows Europe to take advantage of current methods for risk assessment and of safety guidelines for gene technology work mainly defined within the European Directives 2001/18/EC and 2009/41/EC[8] as well as EC Decision 2000/608/EC.[9] The Scientific Committee on Emerging and Newly Identified Health Risks considers this framework suitable for the anticipated short-term and intermediate products of SynBio (including gene drives). But experts have also raised concerns that the current guidance is not adequate to regulate and assess SynBio products; e.g., some protocells are not yet viewed as living organisms because they are not capable of growth and replication and xeno-nucleic acids may or may not be considered heritable material (44, 101, 110). To avoid future risk-governance deficits for SynBio, experts in the field recommend advancing risk assessment methods alongside developments in SynBio and adapting regulations in a flexible manner so that they remain timely (101).

In the United States, SynBio products and engineered gene drives are regulated by applying the existing policy and regulatory frameworks for biotechnology. SynBio is regulated by the EPA's Toxic Substances Control Act Section 5, the APHIS Plant Protection Act Sections 412 and 414, the FDA's Food, Drug and Cosmetic Act Chapter 5, and the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (the latter which amended the NIH Guidelines for Research Involving Recombinant Nucleic Acid Molecules to include SynBio). This framework seems to be suitable to regulate the short-term and intermediate products of SynBio. It is expected that with the new coordinated framework on biotechnology, which is still being developed, SynBio products can also be regulated and assessed properly, and no deficits in assessment should occur.

[8]Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (Recast) (Text with EEA relevance), O.J. (L 125) 75–97.

[9]Commission Decision of 27 September 2000 concerning the guidance notes for risk assessment outlined in Annex III of Directive 90/219/EEC on the contained use of genetically modified micro-organisms (notified under document number C(2000) 2736) (Text with EEA relevance), O.J. (L 258) 43–48.

In Canada, SynBio products are treated like any other novel product of biotechnology and are overseen by Health Canada.

In Asia, some countries apply existing guidance to the regulation of SynBio products. For example, Singapore governs SynBio using its biosafety guidelines for GMOs and the current Biological Agents and Toxins Act (112). Other Asian countries, such as China, do not have a dedicated regulation in place (101).

In South America, some countries apply their existing frameworks to SynBio products and regulate them in most cases like GMOs. In Argentina, for example, SynBio is regulated by existing Resolution 173 of the Secretariat of Agriculture, Livestock and Fisheries (119).

For SynBio organisms, various genetic safeguards are being developed that could be considered in future assessments. One such safeguard is engineered auxotrophy, which applies in cases where organisms are not capable of producing all essential compounds for their survival on their own. Other examples are kill switches or gene flow barriers (reviewed in 78). Unfortunately, such engineered safeguards are error prone because mutations can inactivate them. For this reason, single safeguards are considered insufficiently reliable for SynBio (101).

6. OUTLOOK

The progress in modern biotechniques has been widely recognized as a revolution in our ability to edit plant genomes and has consequently challenged our views and interpretation of current regulatory systems. Policy developments for agricultural innovations should be transparent, proportional to risk, and fully informed by the advancing scientific evidence and experience worldwide. To be able to explore the enormous potential of modern biotechniques for sustainable agriculture and benefit of the bioeconomy, it is crucial to clarify the status of certain new plant breeding techniques, including genome editing, and to resolve current legislative uncertainties.

Scientific breakthroughs to advance food and agricultural research by the year 2030 should include the ability to carry out routine gene editing of agriculturally important organisms and allow for precise and rapid improvement of traits that are important for productivity and quality. The US National Academies of Sciences, Engineering, and Medicine recommend establishing an initiative to exploit the use of genomics and precision breeding and to encourage the acceptance and adoption of these breakthrough technologies. This will require the incorporation of "insights gained from social science and related education and communication efforts with producers and the public" (51, p. 5). This initiative and other similar efforts elsewhere would be worthwhile advancements to help modernize our food and agricultural system.

SUMMARY POINTS

1. A variety of techniques are available to select and introduce desirable traits in plants ranging from conventional breeding techniques and genetic engineering to a growing number of modern biotechniques, including genome editing.
2. Products of genetic engineering are a reality in our daily lives, whether as industrial and medicinal applications or for animal and human consumption.
3. The increased precision now possible in plant breeding using genome-editing techniques represents a big change from conventional breeding approaches, which in large part relied on random, uncontrolled chemical- or radiation-induced mutagenesis, and from genetic engineering that relies on unpredictable insertions of isolated genes into the plant genome. If conducive regulatory and social conditions are in place, genome

editing could substantially increase the positive impacts of plant breeding on human welfare and sustainability.

4. The development and use of modern biotechniques are regulated by different countries and communities of states according to their national laws and governance structures. The legal frameworks require submission of comprehensive scientific evidence about the biology of the organism and its safety with regard to human and animal health and the environment into which it will be released.
5. The decision of whether to classify plants with new traits as genetically modified organisms (GMOs) or living modified organisms (LMOs) has dramatic consequences for research and development in different jurisdictions, especially in the European Union. The legal interpretations of biotechnology regulations by several countries tend to exclude most or all genome-edited plants from GMO regulation. Classification of a plant as a GMO or LMO is not a safety-related issue per se.
6. The assessment of safety can only realistically be made on a case-by-case basis and depends on features of the end product; genetically and phenotypically similar plants deriving from the use of different techniques are not expected to present significantly different risks. Consequently, the aim should be to regulate the specific agricultural trait or product rather than the technology by which that trait or product is produced.
7. Plants modified by modern biotechniques should be excluded from specific regulations if their genetic changes are similar to or indistinguishable from those of conventionally bred plants and if no novel, product-based risk can be identified.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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