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Letters

An increasing number of countries regulate genome editing in crops

Genome editing provides the means for targeted DNA changes in precise locations in a plant genome. Large parts of the world have already introduced regulations or guidelines for crops that were subjected to genome editing. Over the past 2 years, many more countries have introduced guidelines that enable the use of such edited lines in agriculture in a similar way as conventionally bred lines, provided they do not contain a transgene. We here summarize the recent developments across the planet.

Genome editing (sometimes called gene editing) is a widely used technology that generates DNA sequence variants at defined positions in a genome; for example, within the coding region for a protein to affect the function of a particular enzyme, transporter, or receptor, or in the promoter region to impact cell type specificity or timing of promoter activity. Depending on the target gene, such variants may improve or produce new traits; for example, disease resistance, stress tolerance, or increased yield potential. The best-known system in the genome editing toolbox is CRISPR/Cas9, for which Emmanuelle Charpentier and Jennifer Doudna, the inventors of the genetic scissors, received the Nobel Prize (Ledford & Callaway, 2020). Other excellent systems are transcription activator-like effector nucleases (TALENs) or Zinc-finger nucleases (ZFNs), which can also be used for editing (Shukla *et al.*, 2009; Li *et al.*, 2012). All three editing tools target a specific sequence in the genome and induce chromosomal breaks at or in the vicinity of the target site that are repaired imperfectly by the cell, thereby generating sequence variants at the target site (Wada *et al.*, 2020). Regulatory systems distinguish three types of genome editing by site-directed nucleases (SDNs). SDN-1 introduces small changes at the target site. SDN-2 uses template-guided repair by homologous recombination to introduce a specific DNA sequence replacement in the genome. SDN-3 inserts larger genetic elements (e.g. full genes) in a similar manner as SDN-2 (Friedrichs *et al.*, 2019). Advanced techniques, such as base editors, exchange nucleotides at the target site and, therefore, replace, for example, a cytosine for a thymine, or an adenine to a guanine (Anzalone *et al.*, 2020). Although these tools transiently introduce a nuclease to produce the variants, respective transgenes can be effectively removed by simple backcrossing to the parent. Additionally, delivery of the genome editing tools without stable transgenesis (e.g. HI-Edit, nanoparticle delivery, ribonucleoproteins) might eliminate the need to remove or test for transgenes (Kelliher *et al.*, 2019; Gong *et al.*, 2021; Puchta *et al.*, 2022).

The resulting offspring does not contain remnants of the scissors but retains the edited sequence. The resulting plant lines are thus not fundamentally different from lines that carry natural variants, and they do not contain any transgenes. It is, of course, important to use due diligence to ensure that the transgenes have effectively been removed.

Over the past decade, a broad public discourse has been held on whether nontransgenic edited plants should be treated in the same way as conventional lines, or whether the process used to generate them should be taken into account, and therefore whether edited crops should be treated as genetically modified organisms (GMOs). Different countries have taken different paths along these two possible regulations. Therefore, before the edited lines can be introduced into breeding programs, and especially be used as a product, the country that intends to use the lines has to develop a legal regulatory framework.

Two years ago we summarized the status of the regulatory landscape for genome editing (Schmidt *et al.*, 2020). At that time, the European Court of Justice (ECJ) classified all genome edited lines as GMO, whereas the USA and several other countries classified transgene-free, genome-edited lines as equivalent to conventionally bred lines. It remained open which path countries in continents with a high agricultural productivity, like Asia and Africa, would take. Especially in the last few months, several countries established new guidelines for the use of genome-edited plants, which are summarized in Fig. 1. For example, new guidelines were developed in rapid succession in Russia, in additional countries in Central and South America, in two countries in Africa, and in China and India. Notably, in Europe, the UK outlined a plan to exempt genome-edited crops from GMO regulations, and Switzerland agreed on a regulatory framework enabling the use of edited crops. In parallel, the EU is reassessing its position.

The Americas

The USA, Canada, and four South American countries had implemented regulations that classified genome-edited crops (SDN-1, -2) as equivalent to conventional breeds (Schmidt *et al.*, 2020). In 2019, the governments of Guatemala, Honduras, Ecuador, and Paraguay decided to follow the decision of many South American countries to implement a case-by-case review of genome-edited crops (Whelan & Lema, 2019).

Guatemala, Honduras

Already in 2019, Guatemala and Honduras signed a resolution that exempted new genetic combinations without stable integration of exogenous DNA from their existing GMO regulations. This resolution lays the groundwork for treating edited plants similar to conventional breeds (Gatica-Arias, 2020; Kuiken & Kuzma, 2021).

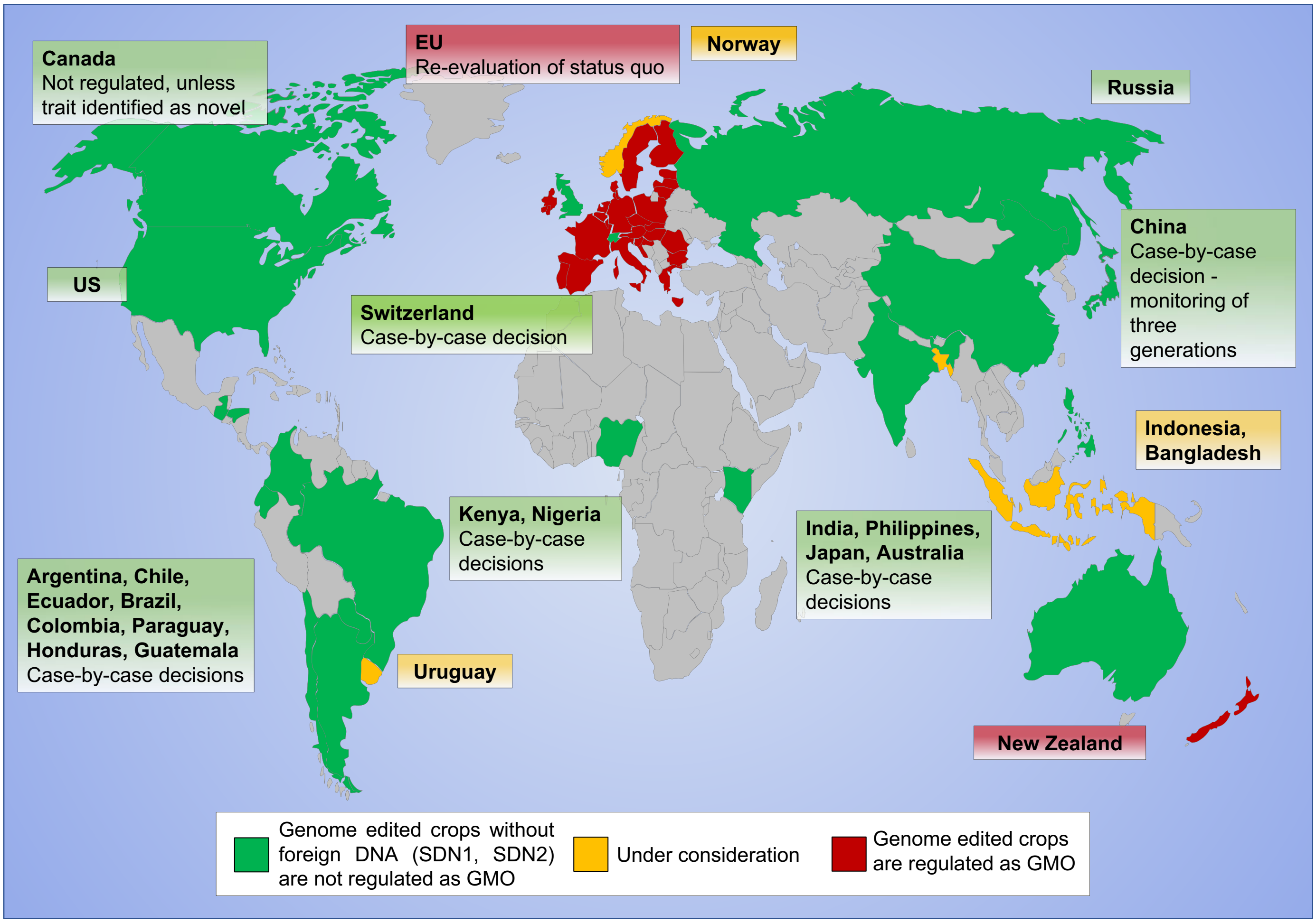

Fig. 1 Current state of genome editing legislation (June 2022). GMO, genetically modified organism; SDN, site-directed nuclease. The illustration is simplified and does not necessarily represent all countries. [Correction added after online publication 16 July 2022: the figure, and associated legend, have been updated.]

Ecuador

Ecuador introduced guidelines for genome editing in 2019 and was among the first countries to specify that only organisms containing foreign DNA should be classified as GMO (Entine *et al.*, 2021). This legislation paved the way for the explicit exclusion of crops generated by SDN-1 and crops where genes are only transferred between closely related organisms (*cis*-genesis).

Paraguay

In 2019, the Paraguayan Government issued regulations that allow exemption of genome-edited products from GMO regulations after consultation with the National Biosafety Commission. Applications must include detailed documentation on potential off-target effects, and proper validation of the absence of foreign DNA (Gatica-Arias, 2020).

Asia

In 2019, Japan was the only Asian country that did not distinguish between traditional breeding methods and genome editing in terms of safety (Normile, 2019). After the successful implementation of the Russian regulations, several more Asian countries followed in 2022.

China

In January 2022, the Ministry of Agriculture published preliminary guidelines for a safety evaluation of genome-edited plants that do not contain exogenous DNA (Mallapaty, 2022). In the application process, the Chinese Government focuses on a safety evaluation regarding the risk of genome-edited plants on the environment and divides the risk into four categories. The draft rules stipulate that once gene-edited plants have completed pilot trials, a production certificate can be obtained, eliminating the need for further time-consuming field trials. Key information requested includes a detailed description of the trait, risks and benefits, how it was generated, evidence for the absence of vector sequences, and validation of the stability of the trait over three generations (USDA Report Number CH2022-0015).

India

On 30 March 2022, the Indian Government signed the Office Memorandum ‘Exemption of the Genome Edited plants falling under the categories of SDN1 and SDN2 from the provisions of the Rules, 1989' (indianexpress.com). The memorandum states that work with genome-edited plants must be carried out under strict safety precautions until it can be ensured that exogenous introduced DNA is no longer present. The guidelines cover genome-edited plants produced by SDN-1 and SDN-2. If validated to be free of transgenes, they are exempted from the current GMO regulations and can be released as a new variety and used for further development and evaluation. In May 2022, the Department of Biotechnology, Government of India, released 'Guidelines for the Safety Assessment of Genome Edited Plants, 2022', which provides detailed guidance on the regulatory requirements. Notably, Bangladesh, Nepal, Sri Lanka, and Cambodia have 'seeds without borders' agreements in place that will likely lead to harmonization of genome editing guidelines (Gauchan & Joshi, 2019).

Philippines

In May 2022, the Philippine Department of Agriculture issued the Joint Department Circular no. 1, which provides the regulatory framework for the use of genome-edited plants (http://biotech.da.gov.ph). The circular excludes plants from the existing GMO regulations that do not contain a novel combination of genetic material obtained through biotechnology. After a crop is determined non-GMO, a certificate will be issued to the developer (USDA Report no. CH2022-0026). The government states that the regulations aim at accelerating the development of plant varieties and reducing costs for farmers and possible impact on the environment.

Russia

In 2019, a decree of the President of Russia (Resolution of 22 April 2019 no. 479) established funding for genome editing and classified transgene-free edited crops as equivalent to those generated by conventional breeding (Dobrovidova, 2019).

Africa

The African Union Agenda 2063 aims at utilizing genome editing to improve agricultural productivity and crop resistance. So far, two countries, namely Nigeria and Kenya, have implemented regulations for a case-by-case review of genome-edited crops.

Nigeria

As the first country in Africa, Nigeria authorized guidelines on genome editing in December 2020 through its National Biosafety Management Agency. Decisions will be made on a case-by-case basis: if edited lines do not contain a new combination of genetic material, they can be classified as conventional varieties or products.

Kenya

In February 2022, Kenya's National Biosafety Authority published guidelines that provide the framework for exemptions of genome-edited organisms and products from the Biosafety Act, paving the path to a case-by-case approval that would treat them as conventional varieties or breeds.

The African Union Agenda 2063 provides a pan-African vision, and other African countries may decide to implement comparable guidelines as developed in Nigeria and Kenya.

Europe

European Union

An ECJ decision in 2018 classified genome-edited plants as GMOs. More recently, the EU began to reevaluate its position and has performed a study examining the implementation of EU legislation on genome editing (europarl.europa.eu). The Commission collected feedback in 2021 and plans a public consultation for the second quarter of 2022, with adoption of possibly new rules by autumn 2023 (ec.europa.eu). The exact outcome remains open.

Norway

The Norwegian Government established a public committee on genome technology in 2020 with the task of publishing a comprehensive overview of gene technology by June 2022 (Kjeldaas *et al.*, 2021). A public consultation on proposed revisions to Norway's Gene Technology Act has led to a recommendation to ease regulations on edited crops.

Switzerland

In March 2022, the Swiss parliament decided to allow exemptions for genome editing in plant breeding, under the condition that there is a clear benefit for farmers, consumers, and the environment over conventional breeding (www.parlament.ch). Under the new rules, transgene-free edited lines would not fall under the genetic engineering law and would not be treated or declared as GMO. However, detailed regulations have still to be developed and are expected to be in place by mid-2024.

United Kingdom

It is expected that the UK will publish their guidelines in June 2022. Initially, genome-edited crops will be exempt from the GMO field trial regulations, followed by exemption from the definition as GMO. Notably, the statutory instrument that would enable field trials defines 'qualifying higher plants' as genome-edited plants that could have been produced by traditional breeding techniques or could have arisen through natural processes (www.gov.uk). It is expected that genome-edited plants can then be effectively placed in field trials and obtain commercial approval without case-to-case review (Stokstad, 2021).

Detailing and harmonization of the guidelines of different countries

In many cases, details of the procedure for classification of crops as nonregulated are being worked out. Overall, we expect that key aspects will be comparable, however it will be important to harmonize the detailed regulations across the planet. A key necessity is the elimination of the scissors and the guides used for editing, which become obsolete after editing process is completed. Several rounds of backcrossing can be used to remove any transgene remnants, but appropriate due diligence will be highly important to exclude carryover of transgene fragments, even if they are not harmful. Details on the required evidence will likely be different for different countries, yet we recommend that scientists and companies should use state-of-the-art technologies to prevent unintended release of a transgenic plant. Since transformation can lead to insertion of vector backbone, appropriate measures need to be taken to identify such rare insertions, which were, for example, detected in hornless cows (Young *et al*., 2020). Some countries also require trait validation over three generations.

In summary, as compared with our previous overview, many countries in the Americas and Asia have implemented legislation that regulates genome-edited crops, and now also two African countries have followed suit (Schmidt *et al*., 2020).

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