HUDSON RIVER

Biotechnology

Technologies for Better Crops

Status of CRISPR & GMO legislation

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Summary

- CRISPR-edited crops are readily accepted in a number of countries in North and South America, where they are often regulated as non-GMO plants.
- Various Latin American countries strive for further harmonization of these regulations, and may -in time- adopt similar permissive regulations as are currently used in the USA, Brazil, Argentina, etc.
- Global steps are being taken to reduce the strict regulation on CRISPR-edited crops: In recent months, the UK, Switzerland and China have all taken legislative steps towards regulating CRISPR-edited crops as non-GMO.
- Asia, North and South America see most countries where GMOs are now grown, with acceptance in Africa being slow, at best, and acceptance in Europe simply poor.
- Import of GMO (mainly for feed) is near worldwide and is on the increase.

Introduction

CRISPR editing is among the most rapidly developing techniques for modern plant breeding. Its quick adoption within scientific research does not, however, keep pace with the adoption of this technique in legislation. In addition, countries widely differ in their approach to regulating plants derived from modern breeding techniques and consequently in the acceptance of CRISPR edited crops. In spite of these uncertainties, breeding companies do face the challenge to develop an answer to the question whether to pursue the development of CRISPR-edited plants. The following document was written to aid strategic decision making on of CRISPR edited plants.

CRISPR and GMO regulations

Acceptance of genetically modified plants is subjected to country-specific regulations, and many of these regulations are subject to changes, especially in the light of the various new breeding techniques that are being developed. The feasibility of CRISPR based gene editing has certainly given the debate new dynamics.

Sometimes, acceptance of new techniques can be surprisingly quick. The feasibility of CRISPR/Cas9 mediated gene editing has now been reported in a multitude of species, but its first successes date back to 2013 with successful experiments in wheat, rice, Arabidopsis and tobacco (Shan et al. 2013)(Li et al. 2013)(Jaganathan et al. 2018). Only three years later, in 2016, the US Department of Agriculture (USDA) indicated that it would not regulate the market entry of CRISPR edited button mushrooms and a Maize line (Waltz 2018), followed by soy, camelina and green bristlegrass lines in 2017. But rulings can have very different outcomes, as was the case in the EU in which CRISPR edited crops became subjected to very stringent GMO regulations (regulations for Genetically Modified Organisms), that often come with high costs to get new varieties accepted.

CRISPR can be done in various ways and ranges in effect from "simple" mutagenesis to the complete replacement of genes, depending on the precise techniques used. This greatly impacts the anticipated acceptance of CRISPR-based editing by different legislative bodies. Therefore, we will first briefly summarize the different CRISPR-editing techniques (and common classifications). Thereafter an overview is presented on how CRISPR edited crops are handled under current regulations in various countries. For ongoing trends, it is interesting to also take notice of the wider acceptance of GMOs, which is discussed thereafter.

SDN Classification of CRISPR edited plants

CRISPR editing can be performed in different ways, which affects both the effects the editing has for the genome and the way legislative regulations may treat CRISPR-editing in plants. The different approaches that can be taken using CRISPR are commonly subdivided in three classes (SDN-1, SDN-2 and SDN-3) that represent classes of increasing editing complexity (Lusser et al. 2012). Edits classified as SDN-1 represent the simplest form of editing. Such approaches typically induce the deletion of one or a few basepairs that occur after the targeted induction of a double strand break in a target region. The break leads to the random deletion of one- or a few basepairs, and the loss of gene function, or altered promoter function. Because of their localized effect and the random processes that cause the deletion of one or more basepairs, SDN-1 approaches do not differ much from more traditional mutagenic approaches like chemical or radiation induced mutagenesis. Major differences comprise that CRISPR mediated mutagenesis involves the use of a protein and guide-RNA, and that is directed onto specific genomic region rather than randomly placed.

SDN-2 edits also concern relatively small sequence alterations, but different from the random mutations that result from SDN-1 edits, the basepair changes are directed in techniques classified as SDN-2. With SDN-2 edits one does not only induce a double-strand break, but also provides a specific repair template (in the form of DNA or RNA). When this template is used for repair of the break, targeted changes of one to several base pairs can be induced onto the DNA. SDN-2 approaches can replace specific basepairs on the DNA, causing targeted changes in gene expression, or alter gene-function by changing catalytic sites of target proteins.

When the repair template is long and causes the replacement of longer sequences, like parts of genes, whole genes or even insertion of completely new sequences, CRISPR editing is classified as SDN-3. Such approaches cause effects that are closer to "classical" genetic modification approaches in which tDNA inserts are made onto chromosomes. Table 1 (adapted from Kuiken and Kuzma, 2021) summarizes these different categories.

SDN-1	Involves the unguided repair of a targeted double-strand break (DSB) by a mechanism called non-homologous end joining. The imperfect repair of DSBs can cause a mutation that in turn can lead to gene silencing, gene knock-out or changes in gene expression.
SDN- 2	Involves a template-guided repair of a targeted DSB using a sequence donor (typically a short single-stranded DNA or RNA). The donor carries one or several small mutations flanked by sequences that match both ends of the DSB, and is thus recognized as a repair template, allowing the introduction of the mutation(s) at the target site.
SDN-3	Involves a template-guided repair of a targeted DSB using a sequence donor, typically double-stranded DNA containing an entire gene or an even longer genetic element(s). Both ends of the donor are homologous to the DSB ends (and the donor sequence is usually more than 800 bp each), which therefore recognize the donor as a repair template, allowing the introduction of the gene or genetic element(s) at the target site.

CRISPR acceptance worldwide

Providing a summary of current regulations is not straightforward since countries differ widely in their regulations. Certainly, procedures for acceptance are not synchronized among countries. The novelty of CRISPR edited crops makes that legislative and regulatory bodies are often still in the process of determining their stance on CRISPR edited crops.

In what countries are CRISPR edited plants regulated as non-GMO?

Perhaps the highest level of certainty, with respect to the direct acceptance of a newly developed variety based on CRISPR-editing, is to judge countries by their most recent actions. Several countries allow CRISPR-edited crops to be grown and did explicitly exempt such crops from the (more expensive) regulations imposed on GM crops (Schmidt, Belisle, and Frommer 2020). CRISPR based crop varieties are nowadays expected to be regulated as non-GMO crops in the following regions: North America (Canada & USA) South America (Chile, Argentina, Brasil, Colombia and Paraguay) as well as Australia, Israel and Japan (Menz et al. 2020). In those countries SDN-1 plants will not be considered under GMO regulations. In recent months, the UK, Switzerland and China have all taken legislative steps in this direction as well. Canada is a global exception, as it follows a case-by-case review approach but is generally permissive to CRISPR-based editing (Menz et al. 2020). Figure 1 shows countries for which there is a high likeliness that an SDN-1 edited crop would be admitted for growth without stringent GMO regulations.

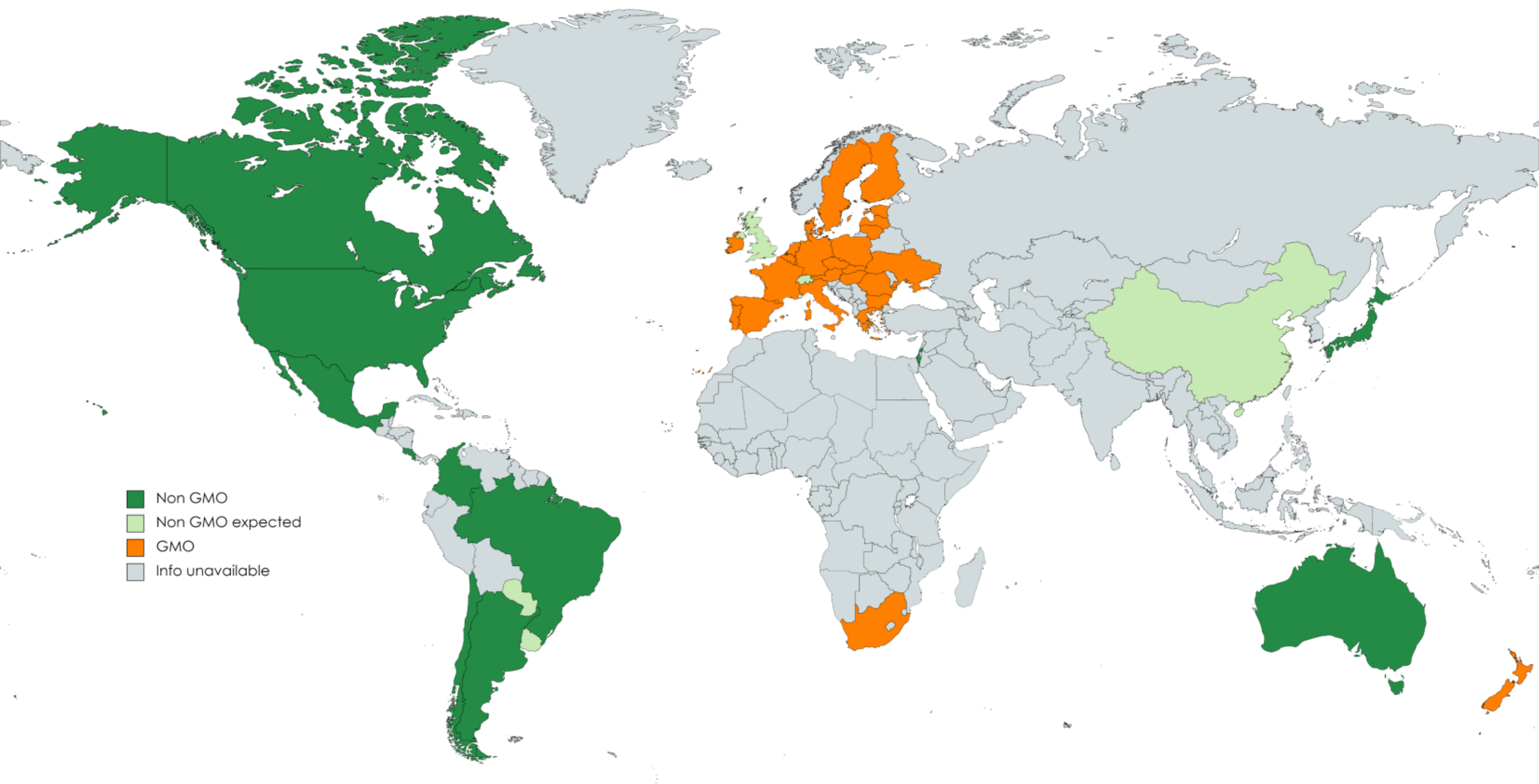

Figure 1: Map showing whether SDN-1 organisms are treated as GMO or not. Canada is an exception, where it is not the GMO status, but rather the "novelty" of a new traits is considered. Crops in these countries will generally follow similar admission procedures as non-GMO crops.

The main picture that arises is that countries in which field crops like maize and soy are important are generally permissive of SDN-1 and 2. Accurate, detailed information on regulations on especially South American countries are given in (Kuiken and Kuzma 2021) and (Gatica-Arias 2020). Countries and regions in which SDN-1 and 2 are explicitly classified as GM crops comprise the EU, New Zeeland and South Africa.

It is of note that, even though regulations for growth of SDN1 crops may be permissive, countries may have different regulations in place that restrict SDN1 plants for human consumption, because rules for growth and rules for consumption of SDN1 are regulated by different legislative authorities (e.g. like in Japan).

For strategic decision making it would be most helpful to be able to predict whether SDN-1 plants will be regulated as either GMO or non-GMO in the (near-) future. Several countries are currently in the act of adapting their GMO regulations. South Africa, but also other countries like Norway, India and the UK are actively reviewing their current regulations, also with respect to CRISPR-based editing (Schmidt, Belisle, and Frommer 2020). It is difficult, if not impossible to predict the outcomes of such legislative procedures. Sometimes, it has been possible to identify trends, like after a 2018 statement issued by a number of (mainly) American countries (Argentina, Australia, Brazil, Canada, Guatemala, Honduras, Paraguay and the USA) that expressed a strong incentive to bring trans-national uniformity to the acceptance of modern "precision biotechnology products"(Argentina et al. 2018), and current legislation is clearly in line with current affairs. Ecuador, though cautiously, does seek harmonization of regulation with surrounding countries (Turnbull, Lillemo, and Hvoslef-Eide 2021).

A wider perspective

Focusing on current CRISPR-regulation is only part of a wider picture. As mentioned, countries that economically depend on large field crops were shown to be generally permissive of SDN-1 and SDN-2, but this acceptance should perhaps not be seen separate from a wider setting of general acceptance of GM crops. Economic interests for several crops and countries are huge. Top growing countries in terms of crop area (2019) are United States, Brazil, Argentina, Canada and India(ISAAA 2019). For soy, maize, cotton and canola respectively 78%, 30, 76 and 29% of area planted in 2018 was GM (ISAAA 2019), and acceptance of such produce for (animal-) food and for clothes is widespread (Figure 2).

What countries produce & import GMO crops?

The ISAAA tracks developments over time in the acceptance of biotech crops. 29 countries worldwide planted GM crops in 2019 and another 42 different countries imported GM crops (Figure 2) (ISAAA 2019). Using more recent sources (Turnbull, Lillemo, and Hvoslef-Eide 2021) it is possible to provide the overview shown in Figure 2 that shows countries that either plant GM crops or import GM crops for feed or food. Countries allowing GM growth are mainly found in Asia and North and South America. When also including GM importing countries for either food or feed, it suggests that GM crops, in some form, are accepted worldwide, with most exceptions in African countries. GMO adoption in Africa has been remarkably slow (Turnbull, Lillemo, and Hvoslef-Eide 2021). Nevertheless, there are various countries that now allow field trials for GM crops (Burkina Faso, Ethiopia, Ghana, Kenya, Malawi, Mozambique, Nigeria, South Africa, Swaziland, Tanzania, and Uganda) (ISAAA 2019).

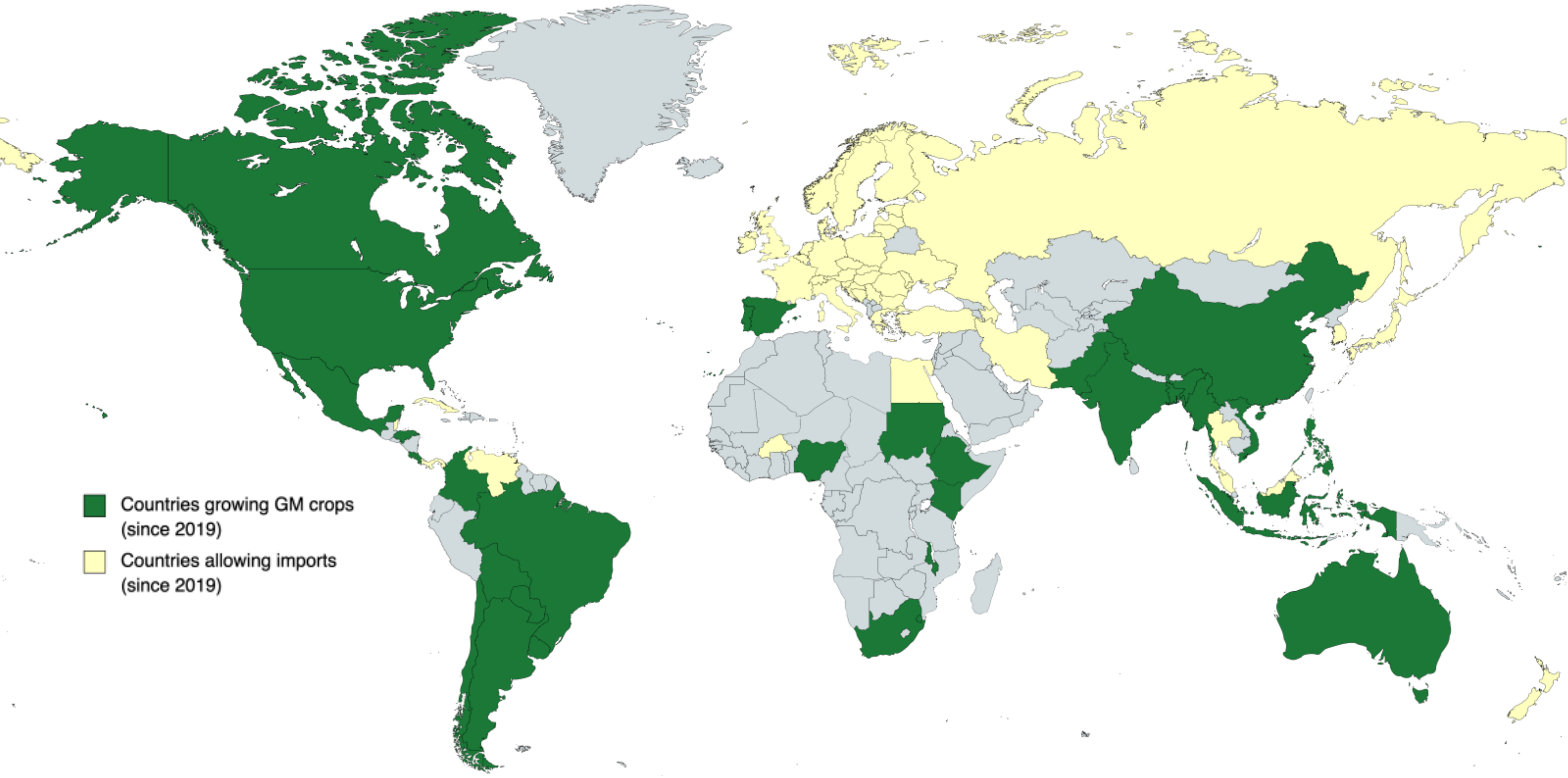

Figure 2: Countries currently producing GMO crops for either seed, feed or food (green) and countries actively importing GMO feed and/or food (yellow).

Conclusion

CRISPR-edited crops are readily accepted in a number of countries in North and South America. Various Latin American countries strive for further harmonization of these regulations, and may -in time- adopt similar permissive regulations as are currently used in the USA, Brazil, Argentina, etc. One may wonder whether harmonization of regulations will prompt those large producers to adopt less permissive regulations, especially when one considers the ease at which other countries import that produce. We think this is unlikely, but this remains speculation. Asia, North and South America see most countries where GMOs are now grown, with acceptance in Africa being slow, at best, and acceptance in Europe simply poor. Import of GMO (mainly for feed) is near worldwide and is on the increase.

Useful websites

The **global gene editing regulation tracker** can be accessed on the website of the Genetic Literacy Project: https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/

Recent developments in research and regulation on CRISPR edited crops can be accessed at the **Genome Editing Resource** on the website of ISAAA:
https://www.isaaa.org/resources/genomeediting/default.asp

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