Ethiop. J. Agric. Sci. 32(2) 57-69 (2022)

The Emergence of Products of New Breeding Techniques and Challenges to Ethiopia's Biosafety Regulatory Regime

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Abstract

In Ethiopia, before starting research and development on any genetically modified organisms (GMOs), approval and written permission must be obtained from the Ethiopian Environmental Protection Authority (EPA). The Authority derives this power from the Biosafety (Amendment) Proclamation No. 896/2015 ratified by the House of Peoples Representatives. EPA forms its opinion based on data provided by the applicant, inspection of laboratories and field trial sites. An addition to this decision-making repertoire is the advice from the National Biosafety Advisory Committee (NBAC) established by the Council of Ministers of FDRE under Council of Ministers Regulation No. 411/2017 on issues related to biosafety. In 2018, the Authority deregulated two Bt-cotton varieties making the country to officially embrace products of GM or biotech crops for the first time. So far, the Authority has issued permits for confined laboratory tests on bt-cotton and transgenic enset and confined field trials (CFTs) on two stacked maize hybrids (TELA^{TM}), CFT permit for 3 R-gene Late Blight Resistance (LBR) stack cisgenic potato, and CFT permit for triple gene BT-GT hybrid cotton varieties. New breeding techniques and their products are entering the global market promising high productivity to sustainable future food security. This work looked into these developments and the concomitant safety concerns and regulatory dilemmas in selected countries. It then gauged the current state of Ethiopia's biosafety framework vis-à-vis the new breeding techniques. The evidence presented here shows that Ethiopia needs to prepare guidelines for dealing with products of new breeding techniques.

Keywords: Breeding technique, biosafety regulation, and genetically modified crops

Introduction

Ensuring the sustainability of agriculture under various challenges has led to the development of alternative, oftentimes complementary techniques for crop improvement (Anders *et al.*, 2021). Over 10,000 years, improved crop varieties have been produced using various tools involving artificial selection, selective breeding, and genetic engineering collectively called genetic modification (Zhang *et al.*, 2016). Genetically engineered (GE) crops are developed through plant transformation to achieve the targeted introduction of a desirable characteristic that may not be obtainable through traditional plant breeding processes (Bell *et al.*, 2018a). Such crops undergo rigorous safety assessment first by the developer and

then by regulatory agencies prior to their commercial release following guidelines reflecting national priorities and international standards (Steiner *et al.*, 2013).

Stacked trait products are among the products of genetic engineering. According to the Organization for Economic Co-operation and Development (OECD), stacked transformation events are defined as "new products with more than one transformation event" (OECD, 2004). Stacking aims at combining different agronomic traits by introducing different novel genes in the plant genome either by conventional cross breeding (conventional stacks) or through genetic engineering; (molecular stacks) (Halpin, 2005). Globally, the most widely used genes for producing stacks are herbicide and insect tolerance genes. As stacked trait products offer multiple solutions for the farmer in one plant, they have been rapidly adopted in the United States (Jose *et al.*, 2020) with similar trends worldwide. However, the legal status of these products differs globally with the type of regulatory data required for authorization largely different. Regulatory data are generated in the laboratory, glasshouse, and confined field trials (Akinbo *et al.*, 2021).

Owing to their increasing global coverage compared to single Genetically Modified (GM) events, three general regulatory approaches to safety assessment of stacked trait products are outlined by (Bell *et al.*, 2018a). The first approach considers stacked trait products as products of conventional breeding. The fact that each individual event has already undergone extensive independent risk assessment, in countries including the USA, Canada, and New Zealand the regulation of conventional stacks does not require additional regulatory data. Under the second approach, stacked GM events are considered as GMOs and should go through the same regulatory pathway as single event GMOs. This approach is followed in the EU, Republic of Korea, Taiwan, and Mexico. Japan follows the third approach which considers the characteristics of the traits being stacked in a product to determine whether additional data is required for safety assessment. In most African countries, there is a lack of clarity on which approach is being followed (Akinbo *et al.*, 2021). Regardless of that, South Africa approved the first stacked traits of Bt and HT in 2007, a year after it amended its GMO Act.

Following the development of stacked trait products, regulators were interested to know if such products are substantially equivalent to their conventional counterparts or if additional safety assessment data is required to determine their legal status. Several studies were conducted to demonstrate the compositional equivalence of stacked trait products with their conventional counterparts (Bell *et al.*, 2018b; Steiner *et al.*, 2013).

In recent years, novel breeding tools have emerged as the most promising options for developing new crop varieties of agricultural crops. These tools employ precise and efficient genome editing techniques that work by introducing single- or double-strand breaks at specific loci of a target genome through which DNA can be replaced, inserted or deleted by a range of site-directed nucleases (SDN) (Eckerstorfer *et al.*, 2021) or sequence-specific nucleases (SSNs) (Voytas, 2013) including clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) (Jinek *et al.*, 2012), transcriptional activator-like effector nuclease (TALEN) (Zhang *et al.*, 2013) and zinc finger nuclease (ZFN) (Zhang *et al.*, 2010), base editing, prime editing (Abdullah *et al.*, 2020), and CRISPR-Cpf1 (Alok *et al.*, 2020). Genome editing literature demonstrate that cereal crops such as sorghum (Jiang *et al.*, 2013), rice (Zhou *et al.*, 2014), wheat (Upadhyay *et al.*, 2013), and maize (Liang *et al.*, 2014) have been improved for various agronomic traits using the CRISPR system. According to (Numan *et al.*, 2021), the potential of CRISPR-Cas-mediated gene-editing in trait improvement in tef is being studied. Sooner or later products of novel breeding tools could knock at our lands. In the context of these developments, therefore, examining the scope of the current regulatory regime of Ethiopia and highlighting areas of improvement is essential.

There is consensus among the global scientific community about the principles and importance for the safety assessment of GMOs (Kok *et al.*, 2014). However, differences are evident in the way, for instance stacked trait products and products of new breeding techniques are regulated. For instance, the European Union and the USA regulate products of novel breeding tools differently. As leaders in this area of science, their position has a rippling effect on other regulatory regimes in the world (Lassoued *et al.*, 2018, 2020). A detailed review on the global legislative landscape on GM crops in general is described in (Turnbull *et al.*, 2021). In the ensuing sections, the regulation of stacked trait products and products of new breeding techniques in selected countries is given together with an expert opinion on how Ethiopia's regulatory regime can smoothly make changes based on scientific consensus. This review is important as it provides key evidence for our regulators to consider preparing guidelines for conducting research and commercialization of products of new breeding techniques in Ethiopia.

Regulation of stacked gm events and gene edited products

The European Union (EU)

Broadly speaking, the EU follows the precautionary principle (Nations, 1992) and regulates GMOs, including imports (Can GMOs Deliver for Africa? | Center For Global Development). Within the EU, transgenic lines containing stacked GM events are assessed as new GMOs, although not all aspects of the safety

assessment for single GM events are deemed to be as relevant for stacked GM events (Guidance for risk assessment of food and feed from genetically modified plants, 2011). The European Food Safety Authority (EFSA) guidance document has been revised with respect to stacked GM events. In fact, after conducting detailed assessment of 20 different stacked GM events, EFSA has concluded that the crossing of single GM events using conventional methods did not result in interactions that warrant additional safety assessment of the stacked trait products (Kok *et al.*, 2014). As a result, the EU bases its approval of stacked GM events on a case-by-case risk assessment basis.

The use of gene editing technology in plant breeding is increasing globally. Gene edited plants have already been produced and are expected to flood the market in the years to come (Table 1). However, the main question lingering in the minds of regulators is if gene edited crops are considered as GMOs according to the existing biosafety regulatory regimes. The legal status of conventional GMOs is clear and often in line with the definition given in the Cartagena Protocol. However, most national, and international legislations do not explicitly refer to products of genome editing due to its novelty and diversity of products (Menz *et al.*, 2020). Regardless of that, for the EU, plants developed by gene editing are considered genetically modified organisms (GMOs) (Eckerstorfer *et al.*, 2021) and hence are subject to regulation with the existing regulatory framework until changes are made and guidance documents are published.

The United States of America

In the United States, genetically engineered major field crops; maize, soybeans and cotton were commercially introduced in 1996, with adoption rates increasing rapidly in the years that followed (ISAAA -2017). Currently, most fields planted by these crops are dominated by stacks trait products containing herbicide tolerance (HT) and insect tolerance (Bt). In contrast to the EU, stacked trait products are not considered GMOs under the US and Canada regulatory regime and no separate regulatory approval is necessary for commercializing hybrid stacks generated by crossing approved GM lines. According to data presented at (https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx) adoption of genetically engineered maize and cotton in the years between 2000 and 2022 shows an increasing trend with stacked varieties taking the lion’s share.

Table 1. List of selected genome-edited major food crops, genome editing technique used, and targeted traits.

Crop	Genome editing technique	Trait/gene	Reference
Tef	SDN1 CRISPR/Cas	Lodging tolerance	(Beyene *et al.*, 2022)
Rice	SDN1 CRISPR/Cas	Bacterial resistance	(Kim *et al.*, 2022; Liao *et al.*, 2022)
	SDN1 CRISPR/Cas	Drought and salt tolerance	(Santosh Kumar *et al.*, 2020)
	SDN1 CRISPR/Cas	Viral resistance	(Macovei *et al.*, 2018)
Wheat	SDN1 CRISPR/Cas	Fungal resistance	(Brauer *et al.*, 2020)
	SDN1 CRISPR/Cas	Increased yield potential	(Zhang *et al.*, 2021)
	SDN1 CRISPR/Cas	Increased spikelet number and delayed heading date	(Chen *et al.*, 2022)
Sorghum	SDN1 CRISPR/Cas	Leaf inclination angle	(Brant *et al.*, 2021)
Maize	SDN1 CRISPR/Cas	Drought tolerance	(Shi *et al.*, 2017)
	SDN1 CRISPR/Cas	Increased total kernel number or kernel weight	(Kelliher *et al.*, 2019)
	SDN1 CRISPR/Cas	Drought tolerance	(Njuguna *et al.*, 2018)
Barley	SDN1 CRISPR/Cas	Nitrogen use efficiency	(Karunarathne *et al.*, 2022)
	SDN1 CRISPR/Cas	Spike architecture	(de Souza Moraes *et al.*, 2022)

Due to its product-oriented regulatory policy, the United States regulatory regime has not been changed with the emergence of gene edited plants. However, through scientific discussions among the various regulatory bodies such as USDA-APHIS, EPA and FDA, the regulatory framework has been revised in 2019 to give a clear pathway for applicants. According to this revision, gene edited products are exempted from regulation when the changes in the plant's genome are deletions of any size, targeted substitutions of a single base pair, and solely introductions from sequences derived from the plant's natural gene pool or edits from sequences which are known to correspond with the plant's natural gene pool. So far, the first genome-edited canola cultivar *Cibus* and a soybean cultivar have been grown and marketed by US farmers without any formal approval and more products are expected to emerge in the years to come.

People's Republic of China, India, and Switzerland

China, Ethiopia's main development partner, has not yet released any changes to its regulatory regime regarding products of novel breeding. However, scientific discussion on the regulation of gene-edited plants started in 2015. In China, genome-edited plants, mainly rice and maize are currently in confined field trials (Chen *et al.*, 2018; YANG *et al.*, 2019). Recent studies show that the Chinese

scientific community attaches considerable importance to the field of gene editing in plants (Zhou *et al.*, 2021). China is also leading in terms of the number of articles on genome editing followed by the USA (Siwo, 2018). After conducting a series of discussions, India drafted guidelines for regulating gene edited plants while Switzerland is among the countries conducting scientific debates and planning to modify their current GMO regulation amid the emergence of gene edited plants (Menz *et al.*, 2020). Taken together, the scientific discussion conducted by several countries show the importance of initiating such discussions to timely inform our regulatory regime thereby avoiding any delays to the approval process should the country decide on investing in the new breeding techniques and import products thereof.

A unique attribute of the Chinese regulatory system is that getting an imported GMO approved in China is possible if and only if the GMO has been approved in its country of origin for the same purpose (Jin *et al.*, 2019). In contrast, the authorization process for imported GMOs in Ethiopia starts with an applicant submitting a written application to the EPA but approval in its country of origin is not an explicit requirement. However, the Authority asks applicants to provide risk assessment data endorsed by the competent authority in the country of origin.

African countries

The regulatory landscape in Africa seems to be still developing and not that proactive to emerging products of biotechnology as is in the US. However, some countries such as South Africa have instated laws relating to GMOs covering the environment and consumer protection that include product labeling. Regarding stacked trait products, no changes were made to the existing regulations in most African countries including Ethiopia. However, regarding products of new breeding techniques, recent literature shows that Africans have started the scientific discussion and debate with Kenya and Nigeria leading the continent by developing guidelines for handling gene edited products (Entine *et al.*, 2021). In the following sections, the current state of the Biosafety Amendment Proclamation of Ethiopia vis-à-vis the emergence of new breeding techniques and their products is presented.

The Federal Democratic Republic of Ethiopia

According to the Biosafety Amendment Proclamation No.896/2015 sub-article 1, a ‘modified organism means, any biological entity which has been artificially synthesized, or in which the genetic material or the expression of its traits has been changed by the introduction of any foreign gene whether taken from another organism, from a fossil organism or artificially synthesized’. Like the EU’s definition of a GMO (Directive 2001/18), this definition considers both the process and the product. However, the definition is different from the definition of ‘modified organism’ as defined in the Cartagena Biosafety Protocol. Most countries use the term ‘living modified organisms’ (LMOs) as defined in the CBP

while Ethiopia's Biosafety Framework uses the term 'modified organism'. However, in both laws the term 'modern biotechnology' is defined the same way. It is known that Ethiopia follows the precautionary principle (Nations, 1992) and regulates GMOs before environmental release. Several countries have clarified their position on stacked trait products, and some have eliminated additional data requirements for regulating such products based on several years of scientific data and experience. Without the presence of science based written clarification from the regulators, stacked trait products are considered GMOs in Ethiopia. This year, two stacked maize hybrids (TELA^{TM}) were evaluated under CFT and officially registered in Ethiopia. In developing these maize varieties, the Water Efficient Maize for Africa (WEMA) project used a combination of conventional and marker-assisted breeding and transgenic technologies (https://www.aatf-africa.org/tela-maize-project/). The application seeks the deliberate release of genetically modified stack maize with traits for water use efficiency and resistance to stem borer pests in Ethiopia. The stacking was done by conventional cross breeding whereby maize with events MON8740 (developed through Agrobacterium-mediated transformation of maize embryonic cells) was crossed with maize with events MON810 (developed by introducing plasmid DNA into plant tissue through particle acceleration method using a gene gun). The Authority considered risk assessment data of the individual events and not the stack events for granting the permit for CFT under Ethiopian conditions.

Experience from several regulations show that safety assessment data on the single GM events is sufficient to allow applicants conduct CFTs of conventional stacked GM events (Kok *et al.*, 2014). As more applicants are seeking authorization of introduction of stacked trait products into Ethiopia, it is important that EPA clearly clarify its stance regarding stacked trait products in its directives to make the decision-making process scientific. As eloquently suggested by (Kok *et al.*, 2014), the directives could explicitly state that for stacked trait products produced by conventional breeding, applicants are not required to provide complete risk assessment data on stacked trait products if the single events have already been elaborately assessed. An additional note could be included stating that specific data on any stacked GM event will only be required if there is a scientific rationale for it for instance, if there is negative interaction among the individual events/inserted genes.

Ethiopia's regulatory regime does explicitly refer to products of gene editing due to its novelty and diversity of products like most national and international legislations in the world. However, in the past three years, eight countries have introduced guidelines for regulating gene edited products (Menz *et al.*, 2020). For instance, Kenya and Nigeria have published guidelines for regulating gene editing (Table 2). Ethiopia has not yet determined whether genome editing will be evaluated differently or treated the same as GMOs under its Biosafety Law. However, amid the fast-evolving nature of breeding technologies, it is time for

Ethiopia to start the scientific discussion necessary for crafting guidelines on stacked trait products and products of new breeding techniques such as gene editing be it for research and/or environmental release.

Table 2. Status of selected countries on regulation of genome edited organisms. Argentina is the first country that enacted regulatory criteria to assess if organisms resulting from new breeding techniques (NBTs) are to be regarded as genetically modified organisms (GMOs) or not (Whelan and Lema, 2015).

Country	Guidelines/regulations	Reference
Kenya	Guidelines for determining the regulatory process of genome edited organisms and products in Kenya (2021)	https://www.biosafetykenya.go.ke/
Nigeria	National Biosafety Guidelines on Gene Editing (2022)	https://nbma.gov.ng/our-guidelines/
Argentina	Resolution 173/2015	https://www.argentina.gob.ar/agricultura/bioeconomia/biotecnologia/conabia
India	Guidelines for Safety Assessment of Genome Edited Plants (2022)	(https://dbtindia.gov.in)
Brazil	Resolution 16/2018	(Kuiken and Kuzma, 2021)
Paraguay	Resolution No. 565 (2019)	(Kuiken and Kuzma, 2021)
The EU	Under consultation	https://www.europarl.europa.eu/
USA	No separate guideline**	https://www.usda.gov/

Until 2022, a total of 21 crop species have been edited using CRISPR and TALENs genome editing tools. Of these, rice has been edited for 45 different traits followed by tomato for which 16 genes/traits were edited. An updated list of crops gene edited with either SDN1 CRISPR/Cas or TALENs can be found here (https://www.eu-sage.eu/index.php/). Ethiopian teff (Eragrostis tef) is among the recent food crops gene edited for lodging tolerance using SDN1 CRISPR/Cas (Beyene *et al.*, 2022).

Conclusion

This work covered the regulatory dilemmas around stacked trait products and products of new breeding techniques such as genome editing. Stacked trait products have specifically been regulated or deregulated in different ways in different countries. Some countries introduced guidelines for the type of data required for stacked trait products while others consider such products as non-GM requiring no further assessment. Despite the existence of several stacked trait products on the global market for decades and an active confined field trial at home, our current regulatory regime has yet to clearly provide supplementary direction and/or guidance for applicants wishing to work on or introduce stacked trait products. This is important in that it makes the decision-making process scientific.

The existing biosafety frameworks in several countries may lack the scope for dealing with products of new breeding techniques such as gene editing. Historically, amendments to biosafety frameworks are not new. For instance, the

EU biosafety framework introduced in 1990 underwent major amendments in 2001, 2003, 2013, and 2015 to respond to developments related to transgenic crops (Eckerstorfer *et al.*, 2021). In 2017, the Government of the Russian Federation issued Resolution amended Russia's regulatory framework for the registration of GMOs and products derived thereof. South Africa amended its GMO Act 15/1997 in 2006. Similarly, the Ethiopian Biosafety Framework introduced in 2009 has been amended in 2015 making it easier to conduct basic research and conduct confined field trials on GMOs. The fast emergence of gene edited plants is challenging regulators in several countries and has resulted in local, regional, and international debates to determine whether such products are regulated or not.

Neither the Ethiopian Biosafety Amendment Proclamation nor the accompanying directives (guiding documents) mention stacked GM events. However, applications containing such products have already been submitted to the EPA. Regardless of the status of those applications, therefore, it is important that EPA clearly include in the existing directives, the definition, the different forms of stacked GM events, and which additional data applicants may need to provide for evaluation and environmental release of such products. Based on the national priorities and international standards, EPA could choose to adopt one of the three general regulatory approaches to safety assessment of stacked trait products outlined by (Bell *et al.*, 2018b).

In 2018, recognizing the increasing impact of emerging new breeding technologies such as genome editing on the global economy, the OECD held a conference on genome editing and its applications in agriculture, implications for health, environment and regulation (Friedrichs *et al.*, 2019). The main objective of the conference was to provide a clearer understanding of the regulatory considerations raised by products of genome editing suggesting the importance of timely scientific dialogue to facilitate innovations involving products of novel breeding. That same year, the WTO Committee on Sanitary and Phytosanitary Measures (CSPM) involving delegations from several countries in North and South America affirmed that gene edited crops are substantially similar to conventional cultivars (Menz *et al.*, 2020) and trade barriers for such products should be avoided.

These developments show that the global impact of gene edited crops is imminent and that regulators in various jurisdictions explore opportunities for science-based dialogue. The fast development and adoption of products of new breeding techniques in Ethiopia needs to be supported by a science based and evolving regulatory regime. Therefore, it is suggested that EPA initiate the scientific discussion/debate on the regulatory dilemmas surrounding products of novel breeding in the context of national priorities and international standards and use

the outcomes of these dialogues to introduce guidelines clarifying its position on products of new breeding techniques. This would make the decision-making process scientific and would pave the way for the research community to quickly capture the benefits of products of novel breeding. Following the global trend in scientific progress in biotechnology, it is important that the regulatory regime develop a culture of anticipation regarding regulatory concerns that could be raised over products of new breeding techniques and start engaging the scientific community early on to make the necessary adjustments to the regulatory regime and to give timely direction to applicants. Improving agricultural productivity is a major policy goal for Ethiopia and timely provision of a clear regulatory pathway for applicants accelerates the approval process and is crucial to quickly capture the benefits of products of new breeding techniques.

Acknowledgement

The author would like to thank the Program for Biosafety Systems (PBS) in Ethiopia for allowing this work to be presented at their genome editing workshop held on the 1st of September 2021 at Hyatt Regency, Addis Ababa, Ethiopia.

References

Abdullah Jiang Z, Hong X, Zhang S, Yao R, and Xiao Y. 2020 *CRISPR base editing and prime editing: DSB and template-free editing systems for bacteria and plants*. *Synth. Syst. Biotechnol.*, **5**, 277–292.

Akinbo O, Obukosia S, Ouedraogo J, Sinebo W, Savadogo M, Timpo S, et al. 2021. *Commercial Release of Genetically Modified Crops in Africa: Interface Between Biosafety Regulatory Systems and Varietal Release Systems*. *Front. Plant Sci.*, **12**, 314.

Alok A, Sandhya D, Jogam P, Rodrigues V, Bhati KK, Sharma H, and Kumar J. 2020. *The Rise of the CRISPR/Cpf1 System for Efficient Genome Editing in Plants*. *Front. Plant Sci.*, **11**, 264.

Anders S, Cowling W, Pareek A, Gupta KJ, Singla-Pareek SL, and Foyer CH. 2021. *Gaining Acceptance of Novel Plant Breeding Technologies*. *Trends Plant Sci.*, **26**, 575–587.

Bell E, Nakai S, and Burzio LA. 2018a. *Stacked Genetically Engineered Trait Products Produced by Conventional Breeding Reflect the Compositional Profiles of Their Component Single Trait Products*. *J. Agric. Food Chem.*, **66**, 7794–7804.

Bell E, Nakai S, and Burzio LA. 2018b. *Stacked Genetically Engineered Trait Products Produced by Conventional Breeding Reflect the Compositional Profiles of Their Component Single Trait Products*. *J. Agric. Food Chem.*, **66**, 7794–7804.

Beyene G, Chauhan RD, Villmer J, Husic N, Wang N, Gebre E, et al. 2022. *CRISPR/Cas9-Mediated Tetra-allelic Mutation of the 'Green Revolution' SEMIDWARF-1 (SD-1) Gene Confers Lodging Resistance in Tef (Eragrostis tef)* . *Plant Biotechnol. J.*

Brant EJ, Baloglu MC, Parikh A, and Altpeter F. 2021. *CRISPR/Cas9 mediated targeted mutagenesis of LIGULELESS-1 in sorghum provides a rapidly scorable phenotype by altering leaf inclination angle*. *Biotechnol. J.*, **16**.

Brauer EK, Balcerzak M, Rocheleau H, Leung W, Schernthaner J, Subramaniam R, and Ouellet T. 2020. *Genome Editing of a Deoxynivalenol-Induced Transcription Factor Confers Resistance to* Fusarium graminearum *in Wheat*. *Mol. Plant-Microbe Interact.*, **33**, 553–560.

Can GMOs Deliver for Africa? | Center For Global Development.

Chen R, Xu Q, Liu Yan, Zhang J, Ren D, Wang G, and Liu Yunjun. 2018. *Generation of Transgene-Free Maize Male Sterile Lines Using the CRISPR/Cas9 System*. *Front. Plant Sci.*, **9**, 1180.

Chen Z, Ke W, He F, Chai L, Cheng X, Xu H, et al. 2022. *A single nucleotide deletion in the third exon of FT-D1 increases the spikelet number and delays heading date in wheat (Triticum aestivum L.)*. *Plant Biotechnol. J.*, **20**, 920–933.

Eckerstorfer MF, Grabowski M, Lener M, Engelhard M, Simon S, Dolezel M, et al. 2021. *Biosafety of Genome Editing Applications in Plant Breeding: Considerations for a Focused Case-Specific Risk Assessment in the EU*. *BioTech*, **10**, 10.

Entine J, Felipe MSS, Groenewald JH, Kershen DL, Lema M, McHughen A, et al. 2021. *Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world*. *Transgenic Res.*, **30**, 551–584.

Friedrichs S, Takasu Y, Kearns P, Dagallier B, Oshima R, Schofield J, and Moreddu C. 2019. *Meeting report of the OECD conference on "Genome Editing: Applications in Agriculture—Implications for Health, Environment and Regulation."* In: *Transgenic Research*, pp. 419–463. Springer International Publishing.

Global Status of Commercialized Biotech/GM Crops: 2017 - ISAAA Brief 53-2017 | ISAAA.org.

Guidance for risk assessment of food and feed from genetically modified plants. 2011. *EFSA J.*, **9**, 2150.

Halpin C. 2005. *Gene stacking in transgenic plants - the challenge for 21st century plant biotechnology*. *Plant Biotechnol. J.*, **3**, 141–155.

Jiang W, Zhou H, Bi H, Fromm M, Yang B, and Weeks DP. 2013. *Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice*. *Nucleic Acids Res.*, **41**, e188–e188.

Jin Y, Drabik D, Heerink N, and Wesseler J. 2019. *Getting an Imported GM Crop Approved in China*. *Trends Biotechnol.*, **37**, 566–569.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, and Charpentier E. 2012. *A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity*. *Science (80-.).*, **337**, 816–821.

Jose M, Vertuan H, Soares D, Sordi D, Bellini LF, Kotsubo R, and Berger GU. 2020. *Comparing agronomic and phenotypic plant characteristics between single and stacked events in soybean, maize, and cotton*. *PLoS One*, **15**, e0231733.

Karunarathne SD, Han Y, Zhang XQ, and Li C. 2022. *CRISPR/Cas9 gene editing and natural variation analysis demonstrate the potential for HvARE1 in improvement of nitrogen use efficiency in barley*. *J. Integr. Plant Biol.*, **64**, 756–770.

Kelliher T, Starr D, Su X, Tang G, Chen Z, Carter J, et al. 2019. *One-step genome editing of elite crop germplasm during haploid induction*. *Nat. Biotechnol.*, **37**, 287–292.

Kim C, Park J, Choi G, Kim S, Vo KTX, Jeon J, et al. 2022. *A rice gene encoding glycosyl hydrolase plays contrasting roles in immunity depending on the type of pathogens*. *Mol. Plant Pathol.*, **23**, 400.

Kok EJ, Pedersen J, Onori R, Sowa S, Schauzu M, De Schrijver A, and Teeri TH. 2014. *Plants with stacked genetically modified events: To assess or not to assessα*. *Trends Biotechnol.*, **32**, 70–73.

Kuiken T. and Kuzma J. 2021. *Genome Editing in Latin America: Regional Regulatory Overview*. Washington, D.C.

Lassoued R, Macall DM, Smyth SJ, Phillips PWB, and Hesseln, H. 2020. *How should we regulate products of new breeding techniques? Opinion of surveyed experts in plant biotechnology*. *Biotechnol. Reports*, **26**, e00460.

Lassoued R, Smyth, SJ, Phillips PWB, and Hesseln H. 2018. *Regulatory Uncertainty Around New Breeding Techniques*. *Front. Plant Sci.*, **9**, 1291.

Liang Z, Zhang K, Chen K, and Gao C. 2014. *Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system*. *J. Genet. Genomics*, **41**, 63–68.

Liao Yongxiang, Ali A, Xue Z, Zhou X, Ye W, Guo D, et al. 2022. *Disruption of LLM9428/OsCATC Represses Starch Metabolism and Confers Enhanced Blast Resistance in Rice*. *Int. J. Mol. Sci.*, **23**.

Macovei A, Sevilla NR, Cantos C, Jonson GB, Slamet-Loedin I, Čermák T, et al. 2018. *Novel alleles of rice* eIF4G *generated by CRISPR/Cas9-targeted mutagenesis confer resistance to* Rice tungro spherical virus. *Plant Biotechnol. J.*, **16**, 1918–1927.

Menz J, Modrzejewski D, Hartung F, Wilhelm R, and Sprink T. 2020. *Genome Edited Crops Touch the Market: A View on the Global Development and Regulatory Environment*. *Front. Plant Sci.*, **11**, 1525.

Nations U. 1992. *A/CONF.151/26/Vol.I: Rio Declaration on Environment and Development*.

Njuguna E, Coussens G, Aesaert S, Neyt P, Anami S, and Van Lijsebettens M. 2018. *Modulation of energy homeostasis in maize and Arabidopsis to develop lines tolerant to drought, genotoxic and oxidative stresses*. *Afrika Focus*, **30**, 66–76.

Numan M, Latif Khan A, Asaf S, Salehin M, Beyene G, Tadele Z, et al. 2021. *From Traditional Breeding to Genome Editing for Boosting Productivity of the Ancient Grain Teff [Eragrostis tef (Zucc.) Trotter]*.

Santosh Kumar VV, Verma RK, Yadav SK, Yadav P, Watts A, Rao MV, and Chinnusamy V. 2020. *CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010*. *Physiol. Mol. Biol. Plants*, **26**, 1099–1110.

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, et al. 2017. *ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions*. *Plant Biotechnol. J.*, **15**, 207–216.

Siwo GH. 2018. *The Global State of Genome Editing*. *bioRxiv*, 341198.

de Souza Moraes T, van Es SW, Hernández-Pinzón I, Kirschner GK, van der Wal F, da Silveira SR, et al. 2022. *The TCP transcription factor HvTB2 heterodimerizes with VRS5 and controls spike architecture in barley*. *Plant Reprod.*, **1**, 3.

Steiner H-Y, Halpin C, Jez JM, Kough J, Parrott W, Underhill L, et al. 2013. *Editor's Choice: Evaluating the Potential for Adverse Interactions within Genetically Engineered Breeding Stacks*. *Plant Physiol.*, **161**, 1587–1594.

Turnbull C, Lillemo M, and Hvoslef-Eide TAK. 2021. *Global Regulation of Genetically*

Modified Crops Amid the Gene Edited Crop Boom – A Review. *Front. Plant Sci.*, **12**, 258.

Upadhyay SK, Kumar J, Alok A, and Tuli R. 2013. *RNA-Guided genome editing for target gene mutations in wheat*. *G3 Genes, Genomes, Genet.*, **3**, 2233–2238.

Voytas DF. 2013. *Plant Genome Engineering with Sequence-Specific Nucleases*. *Annu. Rev. Plant Biol.*, **64**, 327–350.

Whelan AI and Lema MA. 2015. *Regulatory framework for gene editing and other new breeding techniques (NBTs) in Argentina*. *GM Crops Food*, **6**, 253–265.

YANG C hong, ZHANG Y, and HUANG C feng. 2019. *Reduction in cadmium accumulation in japonica rice grains by CRISPR/Cas9-mediated editing of OsNRAMP5*. *J. Integr. Agric.*, **18**, 688–697.

Zhang C, Wohlhueter R, and Zhang H. 2016. *Genetically modified foods: A critical review of their promise and problems*. *Food Sci. Hum. Wellness*, **5**, 116–123.

Zhang F, Maeder ML, Unger-Wallaced E, Hoshaw JP, Reyon D, Christian M, et al. 2010. *High frequency targeted mutagenesis in Arabidopsis thaliana using zinc finger nucleases*. *Proc. Natl. Acad. Sci. U. S. A.*, **107**, 12028–12033.

Zhang J, Zhang H, Li S, Li J, Yan L, and Xia L. 2021. *Increasing yield potential through manipulating of an ARE1 ortholog related to nitrogen use efficiency in wheat by CRISPR/Cas9*. *J. Integr. Plant Biol.*, **63**, 1649–1663.

Zhang Yong, Shan Q, Wang Y, Chen K, Liang Z, Li J, et al. 2013. *Rapid and efficient gene modification in rice and brachypodium using TALENs*. *Mol. Plant*, **6**, 1365–1368.

Zhou H, Liu B, Weeks DP, Spalding MH, and Yang B. 2014. *Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice*. *Nucleic Acids Res.*, **42**, 10903–10914.

Zhou W, Yuan Y, Zhang Y, and Chen D. 2021. *A Decade of CRISPR Gene Editing in China and Beyond: A Scientometric Landscape*. *Cris. J.*, **4**, 313–320.