

Food and Agriculture Organization of the United Nations

Gene editing and agrifood systems

Gene editing and agrifood systems

Food and Agriculture Organization of the United Nations Rome, 2022

Required citation: FAO. 2022. *Gene editing and agrifood systems*. Rome. https://doi.org/10.4060/cc3579en

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO in preference to others of a similar nature that are not mentioned.

The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of FAO.

ISBN 978-92-5-137417-7 © FAO. 2022



Some rights reserved. This work is made available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; https://creativecommons.org/licenses/by-nc-sa/3.0/igo/legalcode).

Under the terms of this licence, this work may be copied, redistributed and adapted for non-commercial purposes, provided that the work is appropriately cited. In any use of this work, there should be no suggestion that FAO endorses any specific organization, products or services. The use of the FAO logo is not permitted. If the work is adapted, then it must be licensed under the same or equivalent Creative Commons licence. If a translation of this work is created, it must include the following disclaimer along with the required citation: "This translation was not created by the Food and Agriculture Organization of the United Nations (FAO). FAO is not responsible for the content or accuracy of this translation. The original [Language] edition shall be the authoritative edition."

Disputes arising under the licence that cannot be settled amicably will be resolved by mediation and arbitration as described in Article 8 of the licence except as otherwise provided herein. The applicable mediation rules will be the mediation rules of the World Intellectual Property Organization http://www.wipo.int/amc/en/mediation/rules and any arbitration will be conducted in accordance with the Arbitration Rules of the United Nations Commission on International Trade Law (UNCITRAL).

Third-party materials. Users wishing to reuse material from this work that is attributed to a third party, such as tables, figures or images, are responsible for determining whether permission is needed for that reuse and for obtaining permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

Sales, rights and licensing. FAO information products are available on the FAO website (www.fao.org/publications) and can be purchased through publications-sales@fao.org. Requests for commercial use should be submitted via: www.fao.org/contact-us/ licence-request. Queries regarding rights and licensing should be submitted to: copyright@fao.org.

For	eword	V
Ack	nowledgements	vii
Abb	previations and acronyms	viii
Exe	cutive summary	X
1	Introduction	1
2	Advances in plant and animal breeding	3
	Summary	3
	A brief history of plant and animal breeding	3
	Principles of gene editing	5
	Some illustrative applications of gene editing	5
	Disease resistance in plants	6
	Agronomically important traits	
	Ouality traits	
	Quantitative trait regulation	
	Crop domestication	
	Aquacunure Microorganisms	12 12
3	Gene editing – potential hazards, benefits and impacts on the environ and society Summary Gene editing and human hunger Gene editing and human health	13
	Allergenicity Toxicity	
	Composition analysis	
	Gene editing and the environment	16
	Issues associated with gene-edited plants	
	Cons editing and enimal welfere	10
	Gene editing and annual wenare and distribution of benefits	19 10
	Economic impact at farm bouched level	
	Economic impact at input market level	
	Differentiated markets	
	Societal factors and public acceptance	
	Gene editing and fundamental ethical considerations	25
4	Governance and regulation	28
	Summary	28
	Sanitary and phytosanitary regulations	
	A timeline of national approaches	29

iii

Multilateral instruments	33
Cartagena Protocol	
The International Plant Protection Convention	
The Organisation for Economic Co-operation and Development	35
Other key issues	35
Identification	
Off-target changes and unintended insertions	35
Property, access and benefit sharing	36
Access and benefit sharing	
Intellectual property	
Gene-edited products considerations	
Patents and gene-editing methods	
Economic interventions	
Innovation funding	
Trade interventions	

Summary	39
Public sector	39
Private sector	40
Intellectual property considerations	42
Public-private and transformative partnerships	42
Identifying and managing conflicts of interest in public-private partnerships	43

6 The way forward	45
-------------------	----

eferences

Appendices

Appendix A	64
Appendix B.1a Case study – Powdery mildew resistance	69
Appendix B.1b Case study – PRRSV-resistant pigs	70
Appendix B.1c Case study – CRISPR-Cas9 and fish	71

The promise of CRISPR for food security

When my colleagues and I first described CRISPR genome editing in 2012, my thoughts about its potential impact focused on human health. The genetic mutation that causes sickle cell disease, for example, had long been known, but we had no way to address it until the discovery of CRISPR technology. Over time, it has become increasingly clear to me that the agricultural and environmental applications of CRISPR hold the potential for the most widespread impact. Genetic diseases, as unfortunately common as they are, do not touch everyone the way agriculture does. Everyone must eat.

In the decade since CRISPR genome editing emerged, scientists have developed a toolkit to tackle the most pressing issues facing humanity and the planet. With the capacity to precisely edit the genomes of crop plants, we can alter nutritional content to combat malnutrition, remove toxins from staple foods like cassava, increase yields to fight hunger, and improve pest resistance, reducing the need for agrochemical inputs. Edited products could also introduce adaptations to address drought and flood resistance, increase biodiversity, and help to capture more carbon, restoring farm soils and improving the fertility of marginal lands. The benefit of CRISPR extends beyond the development of products. As a research tool it can be used to conduct genetic screens, unlocking new biological pathways and expanding our knowledge of the genome and the functional impact of mutations, all of which provide us with new options for future applications.

It is important that we understand our place in history, both the challenges we face today, and ones we have overcome in the past. In late 2022, the number of humans on the planet surpassed 8 billion. The Earth is being stretched for resources as we strive to provide for the needs of humanity, while simultaneously reducing our impact on the global climate. Technologies have helped us through challenging times before. Starting in the 1960s, the Green Revolution reduced global hunger and poverty, but it also brought new challenges and unexpected consequences, including overuse of agrochemicals and monocropping. How can we learn from history to anticipate problems that may arise and take pre-emptive action to ensure positive outcomes for all? Growing anthropogenic pressures on the planet necessitate that we consider all technological options that could sustain human life and preserve the environment. This report provides the international community with guideposts for areas we must address as the first genome edited agricultural products make their way to farmers' fields and our tables.

One area of particular concern for me has been ensuring the equitable distribution of benefits. In the past, high regulatory burdens on agricultural technologies had the effect of consolidating the most sophisticated tools in the hands of a few large companies. This impacted the types of products developed, prices, and global access, and individual farmers have not shared equally in the benefits. Beyond its ease of use, its precision, the reduced cost and shortened timeline for development, CRISPR stands out as a powerful democratizing tool which can be used by scientists globally. I envision a distributed model of crop development in which local scientists can address issues of suitability of crops to agroecological contexts, distribution of benefits, access to genetic resources and sovereignty. In addition, local scientists understand and share the values of their neighbours and have the trust of their communities. This approach has the added benefit of supporting a diversity of crops, particularly neglected varieties, with potentially outsized impacts on malnutrition and hunger. Driven by this goal, the institute that I founded, the Innovative Genomics Institute, in partnership with the African Union and African Plant Breeding Academy, launched an African CRISPR course to equip local researchers with the necessary skills to develop their own genome edited varieties. By partnering with CGIAR and

NARS scientists we can further advance breeding of crops critical to feeding much of the world's population like cassava, sorghum, millet and banana.

Around the world, governments have taken a practical approach towards regulation: in cases where changes made via genome editing are synonymous with changes that could have been made using conventional breeding, products undergo similar safety assessments. For edits that lead to insertions of DNA, governments can rely on over three decades' worth of experience in transgenics. This clear regulatory approach has spurred investment by academic and public-sector developers who now have a path to product approvals.

Any review of genome editing technology should be accompanied by a discussion on ethics. Valid concerns have been raised over the desire for an unaltered natural environment, maintenance of ancient germplasm, as well as animal rights, and cultures around the world will have questions about how the technology might comply or conflict with religious beliefs. Platforms for societal engagement are much needed to facilitate discussions on ethics, and to educate the public on the technology. Individuals should have the ability to choose products that meet their needs and adhere to their belief systems. A consumer of the future may choose an edited variety because they prioritize sustainability, animal welfare, have allergies, or prefer the taste. A farmer of the future may choose to grow an edited crop because it preserves a favoured variety while making it more resilient to a changing climate, or because it increases yield, or captures carbon from the atmosphere. A successful path to the future is one that includes informed choice.

It is my hope that this report will serve as a guiding document for those seeking to responsibly and equitably deploy genome editing technologies. All of human health depends on agriculture. It has been just over 10 years since CRISPR genome editing emerged as a tool; now it is time to wield it wisely.

vi

ennife Doudra

Jennifer A. Doudna

Professor, University of California Berkeley and Founder of the Innovative Genomics Institute, United States of America This Issue Paper was developed by a team of co-authors with oversight from the FAO Chief Scientist Office. The text was subjected to multiple phases of review followed by revision, involving the Chief Scientist Office, FAO Task Force on emerging biotechnologies and external peer reviewers.

Writing team:

Caixia Gao,¹ Enoch Kikulwe,² Jennifer Kuzma,³ Martin Lema,⁴ Preetmoninder Lidder,⁵ Jonathan Robinson,⁵ Justus Wessler⁶ and Kevin Zhao¹

The commitment and dedication of the members of the FAO Task Force on emerging biotechnologies is deeply appreciated – their valuable inputs and feedback contributed to the development, review and finalization of this issue paper.

The following **external experts** are gratefully acknowledged for contributing their time and expertise during the peer review of this document:

Margaret Karembu (ISAAA-AfriCenter, Kenya), Elspeth MacRae (Bioeconomy Futures, New Zealand), Anne Ingeborg Myhr (NORCE, Norway), Peter Thygesen (Office of the Gene Technology Regulator, Australia), Eliane Ubalijoro (Future Earth, Canada) and Ren Wang (Chinese Academy of Agricultural Sciences, China).

Please note that reviews and suggestions provided by the external experts do not constitute an endorsement, and the named reviewers should not be understood to agree with everything in the paper.

Special thanks go to Ludovica Mei (FAO) for production coordination, Max Kardung (Wageningen University & Research) for liaising and coordinating with the co-authors and Adriana Brunetti (FAO) for the design and final layout of the publication.

- ² Alliance of Bioversity International and CIAT
- ³North Carolina State University
- ⁴National University of Quilmes
- ⁵ Food and Agriculture Organization of the United Nations (FAO)

⁶Wageningen University & Research

¹ Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences

Abbreviations and acronyms

ABE	adenine base editors
ASF	African swine fever
AIRCA	Association of International Research and Development Centers for Agriculture
APHIS	Animal and Plant Health Inspection Service (United States of America)
Bt	Bacillus thuringiensis
CAC	Codex Alimentarius Commission
CBD	Convention on Biological Diversity
CBE	cytosine base editors
CIAT	International Center for Tropical Agriculture
CJEU	Court of Justice of the European Union
CPB	Cartagena Protocol on Biosafety
CRISPR	clustered regularly interspaced short palindromic repeats
CSF	classical swine fever
CTNBio	National Technical Biosafety Commission (Brazil)
DNA	deoxyribonucleic acid
DSB	double-strand break
ECJ	European Court of Justice
EPA	Environmental Protection Authority (New Zealand)
EPA	Environmental Protection Agency (United States of America)
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia-New Zealand
GABA	gamma-aminobutyric acid
GFP	green fluorescent protein
GHG	greenhouse gas
GM	genetically modified
GMO	genetically modified organism
HDR	homology directed repair
HICs	high-income countries
HR	homologous recombination
IAEA	International Atomic Energy Agency
IPBES	International Panel for Biodiversity and Environmental Services
ICA	Agricultural Institute (Colombia)
ICBA	International Center for Biosaline Agriculture
icipe	International Centre for Insect Physiology and Ecology
IP	Intellectual Property
IPCC	Intergovernmental Panel on Climate Change
IPPC	International Plant Protection Convention
IPR	Intellectual Property Rights
ISAAA	International Service for the Acquisition of Agri-biotech Applications
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture

viii

LMICs	low- and middle-income countries
LMO	living modified organism
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Japan)
MARA	Ministry of Agriculture and Rural Affairs (China)
MHLW	Ministry of Health, Labour and Welfare (Japan)
MMEJ	microhomology-mediated end joining
MoE	Ministry of Environment (Japan)
NAFTA	North American Free Trade Agreement
NARS	national agricultural research systems
NASEM	United States National Academies of Science, Engineering and Medicine
NBMA	National Biosafety Management Agency (Nigeria)
NBA	National Biosafety Authority (Kenya)
NBT	new breeding technique
NCPB	National Committee on Biosafety (Philippines)
NGO	non-governmental organization
NHEJ	non-homologous end joining
NORCE	Norwegian Resource Centre
ODM	oligonucleotide-directed mutagenesis
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PBI	Plant Breeding Innovations (Philippines)
PLT	Patent Law Treaty
PRRS	porcine reproductive and respiratory syndrome
PRRSV	porcine reproductive and respiratory syndrome virus
R&D	research and development
RNA	ribonucleic acid
SAG	Agriculture and Livestock Service (Chile)
SDG	Sustainable Development Goal
SENSA	Honduran National Service of Agrifood Health and Safety
SDN	site-directed nuclease
TAL	transcription activator-like
TALE	transcription activator-like effector
TALENS	transcription activator-like effector nucleases
TRIPS	Agreement on Trade-Related Aspects of Intellectual Property Rights
UPOV	International Union for the Protection of New Varieties of Plants
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
WGS	whole genome sequencing
WHO	World Health Organisation
WIPO	World Intellectual Property Organization
WTO	World Trade Organization
ZFN	zinc finger nuclease

ix

Many of the agrifood systems that feed the world are severely impacted by global warming, extreme weather events, degradation of land and water resources, conflict, pandemics and demographic shifts. The effects have been particularly felt by the most vulnerable communities and individuals, many of whom depend on agriculture for their livelihoods. Disruptions to global agrifood systems have resulted in widespread hunger, malnutrition and inequality. Elimination of hunger and improvement in nutrition will require major transformations of agrifood systems in many parts of the world. Innovative applications of science and technology will play significant roles in the necessary transformations. Gene-editing technology, including CRISPR (clustered regularly interspaced short palindromic repeats), represents one of the most recent advances in genetics and its application to plant and animal breeding, and is set to contribute to improvements in many aspects of agricultural production. It has the potential to help satisfy the increasing global demand for food and agricultural products.

This science- and evidence-based Issue Paper on gene editing and agrifood systems presents a balanced discussion of the most pertinent aspects of gene editing, including the consequences for human hunger, human health, food safety, effects on the environment, animal welfare, socioeconomic impact and distribution of benefits. Intrinsic ethical concerns and issues of governance and regulation are addressed, and the roles of the public and private sectors, alone and in partnership, are summarized. Various scenarios are also presented for how gene editing might be used in the future to help transform agrifood systems.

Plant and animal breeding began through the mechanisms of natural selection and was directed and hastened through the activities of ancient agriculturalists, who, with no knowledge of the mechanisms of heredity, guided the processes of domestication through their selections of superior crops and animals. Scientific breeding, relying on knowledge of genetics and statistics, is only little over a century old and represented an improvement in speed and precision. Gene editing is the latest advance in this continuum, further increasing precision in crop and livestock breeding. CRISPR-Cas, for example, enables parts of a genome to be targeted precisely and cut. Insertions and deletions of genetic material at the cut site allow a germline to be developed that will result in a plant or animal expressing desired traits. Applications of gene editing are discussed in terms of their merits and demerits for various traits introduced into crops, livestock and fish that enhance production.

Gene editing has the potential to improve food security, nutrition and environmental sustainability but issues of safety must be considered. Identification of potential problems associated with new products is important to ensure their secure and sustainable use and satisfy consumers. The environment, biodiversity and human health could be negatively influenced by release of gene-edited products and therefore regulation must be enacted. Considerable information has been gained from previous experiences with transgenic plants and animals that is relevant to gene editing and its products. Gene editing can be inherently more precise than other methods used to date, which could reduce the likelihood of any harmful effects on human health and the environment.

The economic impact of gene editing will be determined by availability of products, particularly seeds, to small-scale producers, especially in low- and middle-income countries. It is possible that gene editing could reduce farm management costs, but impact at the household level will depend on numerous additional factors, many of which will be situation specific. Social and ethical concerns, including public trust in scientists and developers will be important, as will considerations of risk and benefit distributions, and questions about naturalness and differing cultural values. Intrinsic

X

ethical concerns and animal welfare will also have to be considered when developing and deploying gene-edited products.

Governance aspects of gene-edited products include sanitary and phytosanitary regulations. To date, there has been a variety of approaches taken by national bodies, differing markedly in stringency. While many treat gene-edited products similarly to products derived from genetic modification, others do not. This has consequences for international trade and commerce. Governments must focus on taking a well thought out regulatory approach that attempts international harmonization to the extent possible. Following establishment of national regulatory protocols, other governance issues can be tackled, including trade impacts, intellectual property attributions and facilitation of access and distribution. At the multilateral level, the range of governance aspects can be addressed through various specialized mechanisms and bodies.

Gene editing research takes place in the private and public sectors, but their objectives and incentives can differ. The private sector generally prioritizes marketable products and profits, whereas the public sector is often less constrained and allows greater academic freedom. Issues of ownership of technologies and products, including those from gene editing, are seldom straightforward. Intellectual property considerations are as relevant to gene-editing procedures and products as they are to other technologies. However, many of the aspirations of public and private organizations are compatible, and collaboration between the two sectors is possible and can be beneficial. The principal issue is to ensure that those for whom geneediting technologies and products offer a solution to current constraints can afford and access them.

Gene editing is not a stand-alone technology and is not the only solution for the problems currently faced by agrifood systems. It should be integrated into plant and animal breeding systems and used alongside other improved practices and technologies. The products of gene editing should be available to those that need them most, and the crops and livestock that are important to small-scale producers living in vulnerable environments must receive the attention they merit. As the Nobel Laureate Jennifer Doudna said, "One risk that is often overlooked is the real possibility that some of the advances we make in genome editing will benefit a small fraction of society. With new technologies this is often the case at first, so we have to consciously work from the start to make new cures and agricultural tools that are accessible and affordable."

Previous modifications to agrifood systems, including the Green Revolution, have not necessarily been easy, and not without trade-offs, but innovative applications of science and technology have regularly resulted in substantial improvements in productivity. Gene editing may represent a further step towards the transformation of agrifood systems so that they can withstand better the current pressures and those they will face to an even greater extent in the future. It is important that interventions involving application of gene editing result in developments that are appropriate and sustainable and that are effective within the limits set by the environment.

FAO is ready to play a leading role in this important area of scientific and technological advance by providing a neutral forum for constructive dialogue and exchange of knowledge and by promoting discussion on the applications of gene editing to agriculture and food production.

xi

1 Introduction

Humans have had a binding relationship with the natural world that has endured for over one hundred millennia. As omnivores, with a plant- and meat-based diet, this special relationship has been, and continues to be, one of mutual dependence for food and feed. However, the relationship extends beyond diet to include all aspects of the environment in which plants and animals exist. Without plants, crop plants in particular, humankind cannot survive. Because of the special relationship, new technologies that impinge on agriculture stir vigorous debate. Gene editing is among the most recent technologies to do such.

Currently, agrifood systems, which ultimately feed the global population, are facing unprecedented pressures. While agrifood systems have always been vulnerable to biotic and abiotic stresses, especially, but not exclusively, changeable weather patterns, the combined effects of climate change, degradation of land and water resources, conflict, pandemics and demographic shifts, have exacted a severe toll on many of the most vulnerable producers and consumers. The result has been widespread hunger, malnutrition and inequality, and systems of production, often traditional, and frequently in marginal areas, no longer function efficiently and often fail completely. The effects have been felt in both terrestrial and aquatic environments.

Over recent decades, global food production steadily increased, life expectancy improved, hunger was in decline, infant and child mortality rates fell, and global poverty levels contracted. However, past progress is now threatened. The world is not on track to meet the Sustainable Development Goals (SDGs). To meet the goals set by SDG 2, zero hunger and improved nutrition, will require major transformations of agrifood systems in many parts of the world. Millions of people are now being pushed into acute poverty and food insecurity. As many as 828 million people were affected by hunger in 2021 - 46 million people more than a year earlier and 150 million more than in 2019 (FAO, IFAD, UNICEF, WFP and WHO, 2022). In 2021, acute food insecurity reached a new high, 193 million people experienced crisis levels or worse, an increase of 40 million over the previous high recorded in 2020 (WFP, 2020).

The number of people without access to a healthy diet rose significantly from 2019, reaching almost 3.1 billion in 2020, reflecting the impacts of rising consumer food prices caused by various forces, particularly the COVID-19 pandemic. An estimated 45 million children under the age of five suffered from wasting, increasing risk of child mortality appreciably. In addition, 149 million children under the age of five suffered from stunted growth and development due to lack of essential nutrients in their diets, while 39 million were overweight.

To satisfy the global demand for food and agricultural products by 2050, FAO has projected that agricultural output will need to increase by between 40 and 53 percent, compared with a 2012 baseline value (FAO, 2018). Other estimates indicate that to feed 9.7 billion people in 2050, crop production needs to increase by 56 percent compared with a 2010 baseline value, while demand for animal-based foods is anticipated to increase by nearly 70 percent (World Resources Institute, 2019).

Food production currently accounts for about half of all habitable land (UNEP, 2019), while approximately a third of agricultural land is degraded (FAO and ITPS, 2015). Soil erosion exceeds soil formation rate despite soil productivity having increased in many parts of the world (IPCC, 2019). While water-use efficiency has improved, agriculture still accounts for 70 percent of freshwater withdrawals worldwide and is the primary source of nutrient runoff; 3.2 billion people live in agricultural areas with severe water shortages or scarcity (FAO, 2020). Agrifood systems rely on soil and water and there is competition for these resources with sectors outside agriculture.

The world is not on course to meet the requirements set by the Paris Agreement. Current agrifood systems are responsible for 34 percent of total anthropogenic greenhouse gas (GHG) emissions (Crippa *et al.*, 2021). These directly, and negatively, affect climate and amplify risks associated with biodiversity loss and other issues. Extreme weather events are expected to increase in frequency and severity and increasing temperatures will change distribution and acuteness of pests and diseases. Changing rainfall patterns will result in droughts and flooding, both being detrimental to agricultural production. The rural poor, especially those in low- and middleincome countries (LMICs), are disproportionately affected by climate change because their livelihoods largely depend on agriculture. Biodiversity is also severely threatened, with nearly a million species at risk of extinction (IPBES, 2019). Many components at genetic, species and ecosystem levels that provide vital services to agrifood systems are in decline.

The evolution of humankind and agrifood systems has been one of adaptation and change. Problems have been solved in numerous ways, including moving to more productive environments, changing management practices, developing new techniques, etc. Early agriculturalists built on the results of evolutionary forces by selecting seeds of the best crops, and superior animals, to perpetuate their production. They were unaware that they were exploiting naturally occurring mutations that conferred favourable traits on their crops and livestock. Long before the mechanisms of heredity were established in the nineteenth century, farmers practised plant and animal breeding to increase production. The development of genetics as a scientific discipline formalized the breeding processes and increased their precision. The principles were applied to numerous key crops and domestic animal species to great effect. Further application of results from research in biology and statistics continued to feed into breeding processes, all the while increasing precision. Understanding of genetics at the molecular level, including whole genome sequencing, provided breeders with more refined tools, which increased the efficiency of generating adapted plant and animal germplasm. This step represented even greater precision and efficiency.

Gene-editing/genome-editing technologies, including CRISPR (clustered regularly interspaced short palindromic repeats), represent one of the latest advances in genetics and its application to plant and animal breeding. Gene editing allows modification of a genome more precisely than other forms of breeding. Gene editing can reduce the breeding time needed to produce a new variety or breed and reduce research and development costs. Gene editing also represents an opportunity to address a range of difficult problems, including those associated with developing durable resistances to diseases, pests and abiotic stresses. It also offers new options for developing adapted traits in neglected and underutilized crop species. Gene editing can play a role in enhancing nutritional composition of crops and improving efficiency of feed conversion and reduction in methane and nitrogen emissions in livestock. In addition, gene editing is used to develop microorganisms for precision fermentation to produce food ingredients, additives and biopolymers.

This Issue Paper on gene editing is science- and evidence-based and is forward-looking. It is not intended to be an exhaustive review or a metaanalysis. Gene editing regarding microorganisms is not discussed. The paper takes a broad and interdisciplinary approach, which is necessary because application of gene editing, as with any technology, involves balancing benefits, costs and uncertainties. These are discussed in terms of consequences for human hunger, human health, food safety, effects on the environment, animal welfare, socioeconomic impact and distribution of benefits. Intrinsic ethical concerns are also addressed. The gene-editing technology is outlined, and a brief history of plant and animal breeding is presented. Issues of governance and regulation are addressed and the roles of the public and private sectors, alone and in partnership, summarized. Finally, various scenarios are suggested for how this new technology might be used in the future to improve agrifood systems.

The methodological approach followed for the Issue Paper drew on robust scientific knowledge summarized by the authors. The major issues to be addressed were identified by the authors and an FAO Task Force on emerging biotechnologies. To secure a high-quality product, external experts reviewed the paper. This represented a double check to ensure that important issues were not overlooked.

This Issue Paper attempts to discuss the most important topics associated with gene editing, considering FAO's vision of a world free from hunger and malnutrition, where food and agriculture contribute to improving the living standards of all, especially the poorest, in an economically, socially and environmentally sustainable manner. In keeping with FAO's role as providing a neutral forum for constructive dialogue and exchange of knowledge, this Issue Paper addresses the merits and demerits of gene editing. It does not take a side in the discussion and neither advocates nor provides recommendations.

2 Advances in plant and animal breeding

SUMMARY

Plants and animals evolved under conditions of natural selection acting on spontaneous mutations. Mutations, the building blocks of evolution, determine which individuals survive and which do not, favourable mutations being retained and perpetuated. Mutations and chromosomal and more recently artificial, selection acts. The advent of settled agriculture represented a time of increased directional selection, the ancient agriculturalists choosing seeds of the best plants to sow in subsequent seasons. Crops were domesticated through continuous rounds of selection among wild species. Likewise, the best individuals among livestock animals were retained for breeding purposes. Increases in crop and livestock production were achieved without any understanding of the underlying mechanisms of heredity. Understanding of how traits are under genetic control came about in the mid-nineteenth century and this information was applied to breeding better crops and livestock. Important traits were demonstrated to be under the control of single genes in some cases and multiple genes in others. With the development of genetical and statistical understanding, plant and animal breeding became more precise and more efficient. Elucidation of the molecular mechanisms of gene action added further precision and provided breeders with additional tools. Gene-editing technologies represent the latest step in this continuum, potentially increasing precision in breeding crops and livestock. The technologies, including CRISPR-Cas, enable specific areas of a genome to be targeted precisely and cut. Insertions and deletions of genetic material at the cut site alter protein production, ultimately allowing a germline to be developed that will result in a plant or animal expressing sought-after traits. Applications of gene editing are discussed for traits introduced into plants and animals.

A brief history of plant and animal breeding

Plant and animal breeding build on the results of natural selection, the forces that fuel evolution. The building blocks of evolution are spontaneous mutations, changes in an individual's DNA (deoxyribonucleic acid). Mutations can be molecular changes or the losses or duplications of molecular material. Hybridization among individuals with different mutations creates the diversity on which natural selection, and more recently artificial selection, acts.

Wild cereal grains were being harvested and ground in the Jordan Valley over 20 000 years ago, and similar relatively sophisticated management of food plants took place in other parts of the world. Early agriculturalists had no understanding of genetics and yet unwittingly they selected among plants and animals, leading to domestication of some amenable species by 12 000 years ago. Such unintentional, and later deliberate, selection represent the cornerstone of plant and animal breeding. Desirable plant traits that were consciously selected included upright habit and non-shattering heads in cereals, and docility in animals, but many other traits valuable to farmers were maintained, and undesirable ones removed. Tremendous progress was made over thousands of years in the absence of any understanding of the mechanism of heredity. However, it was established, albeit unknowingly, that breeding progress could only be made if there was variation in a population and screening and selection for traits could be practised within and among those populations.

Understanding the genetic mechanism of heredity came about through the work of Gregor Mendel in the mid-nineteenth century that was rediscovered in 1900 (Stenseth, Andersson and Hoekstra, 2022). Plant breeding from thereon assumed scientific status and became more precise. Scientific breeding replaced empirical breeding, which for several hundred years had relied in trialand-error approaches. Selection among progeny of deliberately hybridized parents or those with induced mutations replaced mass screening of progeny from largely random populations from open pollinations or spontaneous mutations. As more became known about genetic control of

3

traits, it was realized that many important traits were controlled not by simply inherited single genes but by gene complexes, polygenes. This is referred to as quantitative genetics. Research in genetics and statistics furnished breeders with tools to help them manipulate both types of inheritance. Biometrical genetics represented a mathematical approach to breeding and allowed increased precision in operations.

The aim of plant and animal breeders is to use variation to produce modified, improved, varieties. This relies on exploiting variation, which can either exist naturally or be induced. Existing variation can include breeding lines, cultivars, landraces and wild relatives. Induced variation comprises mutations and wide crosses. Mutations can be induced using chemicals or radiation, while wide crosses can be sexual or somatic, and hybrids can also be developed within or between species and genera.

The application of science to plant breeding led to major advances in cereal production in the 1960s. Wheat and rice varieties had genes for dwarfism bred into them that altered their harvest indices and meant that more resources were directed to the grain, and they did not lodge when supplied, often under irrigation, with relatively large applications of fertilizer. The new high-yielding varieties, through shuttle breeding, also had daylength insensitivity bred into them. The agrifood systems of many countries were changed because of the Green Revolution technologies, particularly those of Mexico and India (Pingali, 2012). In those countries yields rose (Gollin, Hansen and Wingender, 2021), hunger was averted (von Der Goltz et al., 2020), incomes rose, pressure on land diminished (Stevenson et al., 2013) and infant mortality declined (Bharadwaj et al., 2020). The advances made were not, however, solely attributable to plant breeding efforts; new agronomic practices were required, including increased irrigation and agrochemical use. Nonetheless, the breeding component has been calculated to account for about 20 percent of yield growth between 1960 and 1980 and approaching 50 percent of the yield increase between 1980 and 2000 (Evenson and Gollin, 2003; Qaim, 2016).

The Green Revolution was not entirely positive, it did have some negative socioeconomic and environmental consequences, partially offsetting the productivity gains. These included increased indebtedness of small-scale producers, some of whom lost their farmland, resulting in considerable rural-urban migration (Ponting, 2007). Large-scale adoption and intensified production of single crops (monocropping), using fertilizer- and waterintensive methods, had far-reaching agricultural and environmental impacts, including lowered groundwater tables, soil and water pollution and increased greenhouse gas emissions (Foley *et al.*, 2011).

With developments in the understanding of genetic processes at the molecular level, plant and animal breeders were supplied with a range of new tools to aid their work. This heralded the era of genomics; entire genomes being sequenced. Now breeders were able to select for variation at the molecular level. Selection was no longer confined to selecting among phenotypes, what the material looked or behaved like, but at the genotype level, which gene sequences were present or absent. One of the tools this work supplied was transgenesis, whereby plant, animal, prokaryote and synthetic genes could be manipulated to express desired traits in adapted material. Gene cloning and recombinant DNA technology, genetic engineering, became widely used in plant and animal breeding, and particularly in associated research. This suite of new technologies represented a further advance but created extensive and impassioned debate, which continues, on the extrinsic merits and demerits of the technology, the risk of creating an oligopoly and control of the global agrifood system, regulatory and Intellectual Property Rights (IPR) ramifications and on its biosafety and ethics.

Gene-editing technologies represent the latest step towards increasing precision in breeding crops and livestock and are a natural sequel to genomic and transgenic technologies. Technologies, including CRISPR-Cas, enable specific areas of a genome to be targeted precisely and cut. Insertions and deletions of genetic material at the cut site alter protein production, ultimately allowing a germline to be developed that will result in a plant or animal expressing sought-after traits. Foreign genes need not necessarily be inserted into a host genome, there are protocols for removing foreign material under some circumstances. As with all new technologies, there is discussion of the benefits, risks and consequences from a range of viewpoints, which will be addressed in this Issue Paper.

Figure 1 summarizes developments in plant breeding technologies, from conventional crossing

4



Figure 1 Developments in plant breeding technologies

to gene editing, indicating the time saving represented by gene editing over other approaches.

Principles of gene editing

Gene-editing approaches rely on distinct protein-DNA interactions to target specific areas of the genome. Various protocols can be used. The discovery and engineering of CRISPR has simplified the process of rapidly and efficiently targeting protein domains in areas of interest in a genome (Doudna and Sternberg, 2017). The gene-editing process comprises two components, targeting a DNA site of interest in the nucleus of a living cell and editing it. Endogenous cellular DNA replication and repair secures the editing event.

Site-directed nuclease (SDN) gene editing involves the use of different DNA-cutting enzymes (nucleases) that cut DNA at fixed locations using various DNA binding systems. After the cut is made, cellular DNA repair mechanisms recognize the cut and repair the damage, using one of two pathways that are naturally present in cells. SDN-1 relies on the endogenous capacity to repair breaks in DNA. Insertions and deletions around the cut site change protein synthesis mechanisms so that a targeted protein can be knocked out, its expression terminated. SDN-2 uses a foreign donor nucleic acid template to perform a precise edit at the cut site, which is incorporated into the host genome. The process is inefficient however and the result is often the same as for the SDN-1 protocol. SDN-3 also relies on foreign donor DNA that is inserted into the cut site. However, unlike SDN-2, which elicits small, precise changes, SDN-3 can insert large fragments of DNA, including entire genes. SDN-3 is also, depending on circumstance, inefficient and there are newer, more precise, gene-editing technologies available, such as base editing. Fundamentally SDN-1, SDN-2 and SDN-3 respectively effect DNA disruption, DNA correction and DNA insertion.

Site-directed small mutations can also be achieved by oligonucleotide-directed mutagenesis (ODM). ODM uses a short stretch of nucleic acid, which has a homologous sequence to the target site, but which contains a point or small mutation. This type of gene editing is, therefore, comparable with SDN-2 (Sprink *et al.*, 2016).

A detailed description of some of the gene-editing technologies is provided in Appendix A and sitedirected nuclease are summarized in Box 1.

Some illustrative applications of gene editing

Gene-editing technologies can be used in practical plant and animal breeding to make some of the component processes more efficient and more precise. They are also of considerable use in research to generate information in support of plant and animal breeding. Although many gene-edited organisms have been created or are in the pipeline,

Box 1 Site-directed nucleases

SDN-1: Techniques using site-directed nucleases with the objective of generating localized random base pair changes, deletions or short random insertions (indels), as a result of error in the cell gene repair mechanism based on non-homologous end joining (NHEJ). No exogenous DNA repair template is used in these applications.

SDN-2: Techniques using site-directed nucleases with the objective of generating a localized predefined point mutation or deletion/addition, because of co-introducing a repair DNA molecule that is homologous to the targeted area and is expected to act as a repair template. Repairing is achieved by

very few have been commercialized to date. Gene-edited products that have already entered the market include a gamma-aminobutyric acid enriched tomato, two CRISPR edited fish in Japan, and soybean with improved fatty acid composition in the United States of America (Waltz, 2022).

Table 1 provides an overview of applications of gene-editing technologies in agrifood systems.

Disease resistance in plants

Host plant resistance to pathogens is controlled by various genetic mechanisms. One mechanism, involving so-termed S genes, controls resistance to powdery mildew (*MLO* resistance), a disease that infects a broad range of crops, including wheat and barley. Fifty years ago, Ethiopian barley landraces collected in the 1930s were identified as *MLO* resistant and the resistance did not conform to the classic gene-for-gene system (Jørgensen, 1992). Induced mutation was used to develop *mlo* genes for barley effective against all isolates of the pathogen. Powdery mildew isolates with elevated *MLO* aggressiveness were produced in barley that were not found in nature.

Natural mutations that decrease the levels of the *MLO* protein were identified in barley, which render those barley cultivars resistant to powdery mildew disease. Researchers demonstrated that the use of SDN technologies, which are used for gene editing, can knock out three copies of the *MLO* gene in wheat to develop resistance (Wang *et al.*, 2014). Subsequent studies established that simply knocking out the gene in wheat resulted in decreased crop yields, however. Research homologous recombination (HR). SDN-2 generates changes spanning few base pairs in genetic elements (promoters, coding sequences, etc.) that pre-exist in the host genome.

SDN-3: Techniques using site-directed nucleases with the objective of generating a localized pre-defined insertion/deletion/replacement of entire genetic elements (promoters, coding sequences, etc.), or entire genes, because of co-introducing a large DNA molecule to be inserted in the target area. DNA molecule may or may not be homologous to the targeted area, and its insertion can take place either by HR or by NHEJ.

subsequently identified a critical additional wheat genome edit that increases wheat yields and rescues any negative pleiotropic growth defects resulting from the *MLO* knockout (Li *et al.*, 2022) (see Appendix B.1a).

Rice blast disease reduces rice yields worldwide. Gene editing a critical site in the rice genome enables generation of new rice varieties with durable rice blast resistance (Wang *et al.*, 2016). Bacterial blight also reduces rice yields. A small, targeted deletion edit in the rice genome results in superior improved resistance (Oliva *et al.*, 2019).

These are just three examples of many where gene editing has the potential to improve disease resistance in crop plants to a range of pathogens and pathogen types.

Herbicide tolerance

Herbicide tolerance in crops is an important trait, particularly in intensive agrifood systems. Transgenic breeding for herbicide tolerance relied heavily on the introduction of herbicide tolerance genes from bacteria and various plant species into major crops such as maize and soybean. Geneediting approaches can also be applied to develop new crop varieties with dependable herbicide tolerance. Unlike transgenic breeding, gene editing can be used to edit host plant genomes directly, without introducing extraneous DNA. Precise editing, including base editing and prime editing, can target specific amino acid sequences in genes of interest and thereby confer tolerance. These technologies demonstrate the capacity to develop new plant varieties rapidly by targeting key areas

Species	Trait	Research organization	Sources	
Improved food and feed quality				
Camelina	Improved fatty acid composition	Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, USA	[1]	
Lettuce	Increased vitamin C content	State Key Laboratory of Plant Cell and Chromosome Engineering, Center for Genome Editing, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China	[2]	
Oilseed rape	Improved fatty acid composition	National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China	[3]	
Potato	Reduced acrylamide formation	Cellectis plant sciences Inc., New Brighton, MN, USA	[4]	
Soybean	Improved fatty acid composition	Calyxt, Roseville, MN, USA	[5]	
Tomato	High content of γ-aminobutyric acid (GABA)	Sanatech Minato-ku, Tokyo, Japan & University of Tsukuba, Ibaraki, Japan	[6]	
Wheat	Low gluten content	Instituto de Agricultura Sostenible (IAS-CSIC), Córdoba, Spain	[7]	
		Wageningen University, Wageningen, Netherlands	[8]	
Wild tomato	<i>De novo</i> domestication – High antioxidant content	Several universities from Brazil, Germany and the USA	[9]	
Brewer's yeast	Flavour improvement in fermented beverages	Centre of Microbial and Plant Genetics, Leuven, Belgium	[10]	
Improved agro	onomic properties			
Alfalfa	High yield	National Institute of Agricultural Technology, Argentina	[11]	
Banana	Fungus protection	Queensland University of Technology, Brisbane, Australia	[12]	
	Protection against bacterial wilt, fusarium wilt and banana streak virus	International Institute of Tropical Agriculture, Nigeria	[13]	
	Protection against bunchy top virus	Agricultural Research Council, Pretoria, South Africa	[14]	
Cacao	Protection against fungal disease	Pennsylvania State University, USA	[15]	
Cassava	Reduced cyanide levels Virus resistance	University of California, Berkeley, CA, USA	[16], [17]	
Cherry	Virus resistance	Department of Horticulture, Plant Biotechnology Resource and Outreach Center, Michigan State University, East Lansing, MI, USA	[18]	
Citrus	Protection against citrus canker	Chinese Academy of Sciences, China	[19]	
Cucumber	Protection against multiple viruses	Department of Plant Pathology and Weed Research, ARO, Volcani Center, Bet-Dagan, Israel	[20]	
Flax	Herbicide tolerance	Cibus, San Diego, CA, USA	[21]	
Grapevine	Drought tolerance	Stellenbosch University, Stellenbosch, South Africa	[14]	
Maize	Fungus resistance	DuPont Pioneer, Johnston, IA, USA	[22]	
Oilseed Rape	Herbicide tolerance	Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou China	[23]	
Potato and sugar beet	Disease-resistant varieties	Russian Academy of Sciences, Russian Federation	[24]	
Rice	Salt tolerance	National Institute for Plant Biotechnology, New Delhi, India	[25]	
	Fungus protection	Department of Genetics, Development & Cell Biology, Iowa State University, Ames, Iowa, USA	[26]	
	Salt tolerance	Key Laboratory of Rice Genetic Breeding of Anhui Province, Rice Research Institute, Anhui Academy of Agricultural Sciences, Hefei, 230031, China	[27]	

7

Table 1 Applications of gene editing technologies in agrifood systems

Species	Trait	Research organization	Sources
Sorghum	Increased protein content	University of Queensland, Queensland, Australia	[28]
	Striga resistance	Kenyatta University, Kenya	[41]
Soybean	Nematode resistance	Evogene, Rehovot, Israel & TMG, Cambé, Brazil	[29]
Tomato	Bacterial resistance	Department of Plant and Microbial Biology, University of California, Berkeley, USA	[30]
	Provitamin D3 enhanced	John Innes Centre, Norwich, United Kingdom	[40]
Wheat	Fungus protection	State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China	[31]
Applications in animal breeding			
Chicken	Protected against avian leukosis virus	Czech Academy of Sciences, Prague, Czech Republic	[32]
Dairy cattle	Hypoallergenic milk	National Institute of Agricultural Technology, Argentina	[33]
	Polled	Recombinetics, St. Paul, Minnesota, USA	[38]
Fish (tiger puffer and red sea bream)	Increased growth	Regional Fish Institute, Kyoto, Japan & Kyoto University, Japan & Kindai University, Japan	[34]
Goat	High-yielding cashmere goats	State Key Laboratory of Reproductive Regulation & Breeding of Grassland Livestock, Inner Mongolia University, Hohhot, 010000, China	[39]
Salmon	Sterility and disease resistance	Norwegian Institute of Marine Research, Bergen, Norway	[35]
Swine	Double muscled	Seoul National University, Seoul, Republic of Korea	[39]
	Increased tolerance to cold temperatures and leaner meat	Chinese Academy of Sciences, Beijing, China.	[36]
	Protection against swine fever	Roslin Institute, Edinburgh, United Kingdom	[37]

Sources:

[1] Ozseyhan, M. E., Kang, J., Mu, X. & Lu, C. 2018. Mutagenesis of the FAE1 genes significantly changes fatty acid composition in seeds of Camelina sativa. *Plant Physiology and Biochemistry*: PPB, 123, 1–7. https://doi.org/10.1016/j.plaphy.2017.11.021

- [2] Zhang, H., Si, X., Ji, X., Fan, R., Liu, J., Chen, K., Wang, D. & Gao, C. 2018. Genome editing of upstream open reading frames enables translational control in plants. *Nature Biotechnology*, 36(9): 894–898. https://doi.org/10.1038/nbt.4202
- [3] Huang, H., Cui, T., Zhang, L. et al. 2020. Modifications of fatty acid profile through targeted mutation at BnaFAD2 gene with CRISPR/ Cas9-mediated gene editing in Brassica napus. Theoretical and Applied Genetics, 133: 2401–2411. https://doi.org/10.1007/s00122-020-03607-y
- [4] Clasen, B.M., Stoddard, T.J., Luo, S., Demorest, Z.L., Li, J., Cedrone, F., Tibebu, R., Davison, S., Ray, E.E., Daulhac, A., Coffman, A., Yabandith, A., Retterath, A., Haun, W., Baltes, N.J., Mathis, L., Voytas, D.F. & Zhang, F. 2016. Improving cold storage and processing traits in potato through targeted gene knockout. *Plant Biotechnology Journal*, 14: 169–176. https://doi.org/10.1111/pbi.12370
- [5] Animal and Plant Health Inspection Service, USDA. 2022. Plant-Trait-Mechanism of Action (MOA) combinations that have been determined by APHIS not to require regulation under 7 CFR part 340. https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permitsnotifications-petitions/confirmations/moa/moa-table
- [6] GABA-enriched tomato is first CRISPR-edited food to enter market. 2022. https://www.nature.com/articles/d41587-021-00026-2.epdf?no_publisher_access=1&r3_referer=nature
- [7] Sánchez-León, S., Gil-Humanes, J., Ozuna, C.V., Giménez, M.J., Sousa, C., Voytas, D.F. & Barro, F. 2018. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnology Journal*, 16: 902–910. https://doi.org/10.1111/pbi.12837
- [8] Wheat can be made gluten safe for people with coeliac disease by using gene editing. 2019. https://www.wur.nl/en/newsarticle/wheatcan-be-made-gluten-safe-for-people-with-coeliac-disease-by-using-gene-editing.htm
- [9] Zsögön, A., Čermák, T., Naves, E.R., Notini, M.M., Edel, K.H., Weinl, S. & Peres, L.E.P. 2018. De novo domestication of wild tomato using genome editing. *Nature Biotechnology*, 36(12): 1211–1216. https://doi.org/10.1038/nbt.4272
- [10] Mertens, S., Gallone, B., Steensels, J., Herrera-Malaver, B., Cortebeek, J., Nolmans, R. *et al.* 2019. Reducing phenolic off-flavors through CRISPR-based gene editing of the FDC1 gene in Saccharomyces cerevisiae x Saccharomyces eubayanus hybrid lager beer yeasts. *PLoS* ONE 14(1): e0209124. https://doi.org/10.1371/journal.pone.0209124
- [11] Editar el genoma de la alfalfa para aumentar su rendimiento. 2019. http://ria.inta.gob.ar/sites/default/files/actualidadimasd/3-editar_el_genoma_de_la_alfalfa_para_aumentar_su_rendimiento.pdf

8

[12] Dale, J., James, A., Paul, J.Y. et al. 2017. Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. Nature Communications, 8: 1496. https://doi.org/10.1038/s41467-017-01670-6

- [13] African scientists urge use of gene editing to improve crops. 2019. https://allianceforscience.cornell.edu/blog/2019/09/african-scientistsurge-use-gene-editing-improve-crops/
- [14] Developments in Delegations on Biosafety Issues, ENV/CBC/MONO(2021)19. 2021. https://www.oecd.org/officialdocuments/ publicdisplaydocumentpdf/?cote=env/cbc/mono(2021)19&doclanguage=en
- [15] Fister, A.S., Landherr, L., Maximova, S.N. & Guiltinan, M.J. 2018. Transient expression of CRISPR/Cas9 machinery targeting TcNPR3 enhances defense response in theobroma cacao. *Frontiers in Plant Science*, 9: 268. https://doi.org/10.3389/fpls.2018.00268
- [16] Genome editing of the staple crop cassava to eliminate toxic cyanogen production. 2020. https://innovativegenomics.org/projects/ genome-editing-staple-crop-cassava-eliminate-toxic-cyanogen-production/
- [17] Gomez, M.A., Lin, Z.D., Moll, T., Chauhan, R.D., Hayden, L., Renninger, K., Beyene, G., Taylor, N.J., Carrington, J.C., Staskawicz, B.J. & Bart, R.S. 2019. Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. *Plant Biotechnology Journal*, 17(2): 421–434. https://doi.org/10.1111/pbi.12987
- [18] Song, G.-q., Sink, K.C., Walworth, A.E., Cook, M.A., Allison, R.F. & Lang, G.A. 2013. Engineering cherry rootstocks with resistance to Prunus necrotic ring spot virus through RNAi-mediated silencing. *Plant Biotechnology Journal*, 11: 702–708. https://doi.org/10.1111/ pbi.12060
- [19] Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L. & Zou, X. 2017. Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene Cs LOB 1 promoter in citrus. *Plant Biotechnology Journal*, 15(12): 1509–1519. https://doi.org/10.1111/pbi.12733
- [20] Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., Sherman, A., Arazi, T. & Gal-On, A. 2016. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Molecular Plant Pathology*, 17(7): 1140–1153. https:// doi.org/10.1111/mpp.12375
- [21] Sauer, N.J., Narváez-Vásquez, J., Mozoruk, J., Miller, R.B., Warburg, Z.J., Woodward, M.J., Mihiret, Y.A., Lincoln, T.A., Segami, R.E., Sanders, S.L., Walker, K.A., Beetham, P.R., Schöpke, C.R. & Gocal, G.F.W. 2016. Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. *Plant Physiology*, 170(4): 1917–1928. https://doi.org/10.1104/ pp.15.01696
- [22] Shi, J., Gao, H., Wang, H., Lafitte, H.R., Archibald, R.L., Yang, M., Hakimi, S.M., Mo, H. & Habben, J.E. 2017. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnology Journal*, 15(2): 207–216. https:// doi.org/10.1111/pbi.12603
- [23] Wu, J., Chen, C., Xian, G., Liu, D., Lin, L., Yin, S., Sun, Q., Fang, Y., Zhang, H. & Wang, Y. 2020. Engineering herbicide-resistant oilseed rape by CRISPR/Cas9-mediated cytosine base-editing. *Plant Biotechnology Journal*, 18(9): 1857–1859. https://doi.org/10.1111/pbi.13368
- [24] Dobrovidova, O. 2019. Russia joins in global gene-editing bonanza. Nature, 569(7756):319–320. https://doi.org/10.1038/d41586-019-01519-6
- [25] Farhat, S., Jain, N., Singh, N., Sreevathsa, R., Dash, P.K., Rai, R. & Rai, V. 2019. CRISPR-Cas9 directed genome engineering for enhancing salt stress tolerance in rice. *Seminars in Cell & Developmental Biology*, 96: 91–99. https://pubmed.ncbi.nlm.nih.gov/31075379/
- [26] Li, T., Liu, B., Spalding, M. et al. High-efficiency TALEN-based gene editing produces disease-resistant rice. 2012. Nature Biotechnology, 30: 390–392. https://doi.org/10.1038/nbt.2199https://doi.org/10.1038/nbt.2199
- [27] Duan, Y.B., Li, J., Qin, R. Y., Xu, R.F., Li, H., Yang, Y.C., Ma, H., Li, L., Wei, P.C. & Yang, J.B. 2016. Identification of a regulatory element responsible for salt induction of rice OsRAV2 through ex situ and in situ promoter analysis. *Plant Molecular Biology*, 90(1-2): 49–62. https://doi.org/10.1007/s11103-015-0393-z
- [28] CRISPR-edited sorghum could provide needed protein to 500 million people in sub-Saharan Africa. 2019. https://geneticliteracyproject. org/2019/11/21/crispr-edited-sorghum-could-provide-needed-protein-to-500-million-people-in-sub-saharan-africa/
- [29] Evogene and TMG Announce Collaboration to Develop Nematode Resistant Soybean through Genome Editing. 2018. https://www. evogene.com/press_release/evogene-and-tmg-announce-collaboration-to-develop-nematode-resistant-soybean-through-genome-editing/
- [30] de Toledo Thomazella, D.P., Brail, O., Dahlbeck, D. & Staskawicz, B.J. 2016. CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. *BioRxiv*, 064824. https://doi.org/10.1101/064824
- [31] Wang, Y., Cheng, X., Shan, Q. et al. 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology, 32: 947–951. https://doi.org/10.1038/nbt.2969
- [32] Koslová, A., Kučerová, D., Reinišová, M., Geryk, J., Trefil, P. & Hejnar, J. 2018. Genetic resistance to avian leukosis viruses induced by CRISPR/Cas9 editing of specific receptor genes in chicken cells. *Viruses*, 10(11): 605. https://doi.org/10.3390/v10110605
- [33] Edición génica en animales para la producción de leche hipoalergénica2. 2021. https://www.argentina.gob.ar/inta/tecnologias/ediciongenica-en-animales-para-la-produccion-de-leche-hipoalergenica
- [34] Japan embraces CRISPR-edited fish. 2022. Nature Biotechnology, 40: 10. https://doi.org/10.1038/s41587-021-01197-8
- [35] 10 Unusual Applications of CRISPR Gene Editing. 2020. https://www.labiotech.eu/best-biotech/crispr-applications-gene-editing/
- [36] China's CRISPR push in animals promises better meat, novel therapies, and pig organs for people. 2019. https://www.science.org/ content/article/china-s-crispr-push-animals-promises-better-meat-novel-therapies-and-pig-organs-people
- [37] CRISPR immunizes pigs against PRRS—deadly viral disease that costs \$600 million annually. 2019. https://geneticliteracyproject. org/2019/06/26/podcast-crispr-immunizes-pigs-against-prrs-deadly-viral-disease-that-costs-600-million-annually/
- [38] Carlson, D.F., Lancto, C.A., Zang, B., Kim, E.-S., Walton, M. et al. 2016. Production of hornless dairy cattle from genome-edited cell lines. Nature Biotechnology, 34: 479–481. https://www.nature.com/articles/nbt.3560
- [39] Li, X., Fei, H., Xiao, H., Hui, W., Bai, D., Wang, X., Liang, H., Cang, M., Liu, D. 2019. Generation of Tβ4 knock-in Cashmere goat using CRISPR/Cas9. International Journal of Biological Science, 15: 1743–1754. https://pubmed.ncbi.nlm.nih.gov/31360116/
- [40] Li, J., Scarano, A., Mora Gonzalez, N., D'Orso, F., Yue, Y., Nemeth, K., Saalbach, G., Hill, L., Oliveira Martins, C., Moran, R., Santino, A. & Martin, C. 2022. Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nature Plants*. https://doi.org/10.1038/s41477-022-01154-6
- [41] Mallu, T.S., Mutinda, S., Githiri, S.M., Odeny, D. & Runo, S. 2021. New pre-attachment Striga resistant sorghum adapted to African agroecologies. *Pest Management Science*, 6: 2894–2902. https://pubmed.ncbi.nlm.nih.gov/33576100/

of the host genome (Zhang *et al.*, 2020; Liu *et al.*, 2020; Li *et al.*, 2016; Gao, 2021).

Gene editing is also a viable technology for screening plant populations. Large RNA (ribonucleic acid) libraries have been employed to screen for desired traits based on a variety of known mutations in particular genes and associated phenotypes. Such a targeted gene-editing highthroughput screening approach enabled the discovery of new mutations conferring desirable and durable herbicide tolerance in a variety of crops (Li *et al.*, 2020). The methods can continue to be developed to screen for other useful traits.

Agronomically important traits

Nitrogen use efficiency is a crucial element in crop production, affecting plant growth and yield. The status of nitrogen fertilizer use efficiency has important economic consequences for smallscale producers because fertilizer represents a considerable investment. Rice mutants with impaired physiological functions were developed that were more nitrogen use efficient than the original varieties (Wang et al., 2018). In addition, through targeted gene editing, specific wheat genes were targeted and knocked out to produce new varieties with significantly enhanced nitrogen use efficiency (Zhang et al., 2021). However, wheat yields are determined by numerous aspects of the plant's physiology. The TaGW7 wheat gene has a rice homolog that influences grain weight and size. A genetic knockout of the gene in wheat resulted in new bread wheat varieties with larger, heavier kernels (Wang et al., 2019).

Soil salinity, which causes salt stress, is a frequent barrier to crop production. A single knockout of a gene controlling a specific protein's production in tomatoes generated new tomato varieties with enhanced tolerance to salt under experimental conditions (Tran *et al.*, 2021). Furthermore, upregulation of a maize gene (ARGOS8) enhances drought tolerance in the crop. Using targeted insertion technologies, the upregulated ARGOS8 gene was incorporated into the genome and in field trials the improved variants had enhanced yields under drought stress conditions (Shi *et al.*, 2017).

Quality traits

The presence of prolamin, a glutenin, in wheat causes the autoimmune disease celiac, which affects between 1 in 170 to 1 in 100 individuals on a global basis (Fasano and Catassi, 2012).

The glutenin gene family in wheat has numerous members. Because many proteins are implicated in the production of prolamins, it is not possible to leverage traditional breeding approaches to develop wheat varieties with reduced levels of prolamin. Application of gene-editing technologies, however, could result in the simultaneous edit of 35 genes in the wheat gluten family, producing transgene-free cultivars that could significantly reduce immune reactivity (Sánchez-León *et al.*, 2017).

Wheat is mostly used as a raw material to produce foods, including bread, that require high temperatures during preparation. Hightemperature baking of wheat converts the small endogenous molecule asparagine into acrylamide, which is known to be associated with increased cancer risks. The genes encoding the asparagine biosynthesis pathway were identified and it was hypothesized that a genetic perturbation of this pathway would reduce acrylamide levels produced during baking. Genetic knockout of the asparagine synthetase gene in wheat resulted in a near complete reduction in levels of asparagine, which greatly improves wheat's nutritional profile (Raffan *et al.*, 2021).

Vegetable oils are an important global commodity, and their nutritional profiles greatly affect human health (Ghodsi and Nosrati, 2020). Soybean oil is produced in large volumes for human consumption, but its properties include low levels of monounsaturated oleic acid. The protein encoded by the gene for fatty acid desaturase 2 (FAD2) converts oleic acid to polyunsaturated linoleic acid. Through targeted gene editing to knock out this gene, new soybean varieties are being developed with substantially increased levels of oleic acid (Al Amin et al., 2019) and low levels of linoleic acid. This builds on the work of Demorest et al. (2016), who developed a soybean line with low levels of trans-fatty acids. A higher consumption of oleic acid significantly reduces the risk of cardiovascular disease in humans (Kris-Etherton et al., 1999). Premium high-quality gene-edited soybean oil became available in the United States of America in 2019 (Tome, 2021). Targeted mutagenesis, using CRISPR-Cas9, was also used to develop a healthpromoting tomato, Sicilian Rouge, with high levels of gamma-aminobutyric acid (GABA) (Nonaka et al., 2017), which is sold in Japan for general consumption (Waltz, 2021). In 2023, nutrientenriched mustard leaf will become available in the United States of America (Smith, 2021).

Potatoes are an important food source for people around the world, but they do not store well. To extend the storage life of potatoes and enable year-round consumption, many growers use cold storage. However, sugars accumulate when potatoes are exposed to low temperatures. These, in turn, are transformed into acrylamide when processed at high temperatures. The genetic knockout of the vacuolar invertase gene (VInv) substantially decreases levels of sugars accumulated in cold-stored potatoes and thereby extends their storage life (Zhu *et al.*, 2014).

Quantitative trait regulation

Numerous agronomically important traits are controlled quantitatively, by polygenes, and cannot be edited through simple genetic knockouts. To modify such traits requires changes in the expression levels of several genes, rather than simply switching a single gene on or off. Geneediting technologies can edit cis-regulatory genetic elements to fine-tune expression levels and expression patterns of multiple genes (Rodríguez-Leal et al., 2017; Zhang et al., 2018a; Xing et al., 2020). Cis-regulatory genetic elements are regions of DNA located near targeted genes. Edited mutations alter expression levels or patterns of the targeted gene. Editing such regions for specific tomato genes produces a variety of growth forms and canopy architectures, enhancing genetic diversity (Rodríguez-Leal et al., 2017) and generating useful germplasm for future breeding efforts.

A further level of protein expression regulation is governed by the translation levels of genes. The manipulation of open reading frames (ORFs) located near a primary coding sequence has a major effect on gene expression (Zhang *et al.*, 2018a; Xing *et al.*, 2020). Through incorporation or removal of nearby ORFs, a suite of expression levels for specific genes can be generated. Using this approach, new varieties of lettuce with various levels of vitamin C and new varieties of strawberries with various levels of sugar have been developed (Zhang *et al.*, 2018a; Xing *et al.*, 2020).

Kumar *et al.* (2022) reviewed the developments to date on gene editing of a broad range of crops with respect to nutrient enrichment. This included enrichment for vitamin A, vitamin E, iron and zinc. They summarized over seventy cases of quality improvements in more than twenty crops, including several crops typically grown by smallholder farmers, such as sorghum, groundnut, pomegranate, sweet potato, cassava and banana. Although sixteen cases of use of CRISPR-Cas9 were listed for tomato, and twenty-one for rice, carrot, eggplant, camelina, apple and grape were also listed.

Crop domestication

The genetic base of some crops is narrow. Exploiting the genetic diversity of wild crop relatives can furnish new and useful genetic traits, which can be introgressed into elite varieties. However, making and managing wide crosses is difficult and resource demanding.

A supplementary approach might be to domesticate wild crop relatives directly to produce commercial cultivars. Traditional domestication processes are slow, requiring millennia, as evolution progresses through gradual accumulation of spontaneous mutations. Making such genetic changes to crop relatives using established technologies is impractical, if not impossible. However, expanding knowledge of the genetics of crop domestication has enabled exploration of the use of gene-editing technologies to speed up the domestication process.

CRISPR-Cas technology was employed to edit multiple domestication-related genes in wild tomato simultaneously (Li *et al.*, 2018). Specifically, partial domestication of the wild tomato allowed creation of new varieties suitable for current field production, comparable with currently grown elite tomato varieties. The newly domesticated wild tomatoes maintain their highly desired stress resistances.

In addition to the domestication of wild tomato, a recent study described potential *de novo* domestication of wild rice to obtain new commercial rice varieties. Wild rice, in comparison with widely grown elite rice varieties, has broad genetic diversity. Wild rice is characterized by its large biomass and strong environmental adaptability. Using multiplex gene editing, researchers improved six agronomic traits, including seed shattering, awn length, plant height, grain length, stem thickness and heading date in wild rice (Yu *et al.*, 2021).

In addition to wild crop relatives, many currently important crops have not been exhaustively domesticated. So termed orphan crops and neglected and under-utilized species have not been exploited to their full potential. Many are adapted to harsh environments and conditions, with the potential to grow on marginal land, unsuitable for standard crop cultivation. Application of gene editing has been explored to increase the utility of orphan crops such as sorghum, millet, cowpea and quinoa (Gao, 2021).

Livestock

Gene-editing technologies have been used to produce numerous new lines in pigs, cattle, sheep and goats. The gene-edited individuals can potentially be used as bioreactors (Liu *et al.*, 2013), disease models (Tan *et al.*, 2013), founder animals for genetic lines with enhanced productivity (Proudfoot *et al.*, 2015) and organ donors (Hauschild *et al.*, 2011; Li *et al.*, 2015). Gene editing was successfully applied to pig zygotes to produce live gene-edited pigs (Lillico *et al.*, 2013). Others modified the genome of Holstein– Friesian cattle, thereby engineering a heritable genome modification that facilitated resistance to tuberculosis (Wu *et al.*, 2015).

Applications to livestock production include polled cattle, porcine reproductive and respiratory syndrome (PRRS) virus-resistant pigs (see Appendix B.1b); pigs with improved resistance to African swine fever (ASF); sheep, goats, and cattle with increased lean muscle yield; thermotolerant cattle with the slick trait, and sheep with increased wool length and yield. Other examples include cows and goats with changed milk composition. A transgenic calf was produced whose milk contained no protein β -lactoglobulin, a major milk allergen. An application in pets produced mini-pigs (Cyranoski, 2015) and faster running beagles (Brinegar *et al.*, 2017).

Cows with increased resistance to tuberculosis have been researched using gene-editing technologies (Van Eenennaam *et al.*, 2021). Wu *et al.* (2015) added the mouse gene SP110 to a specific location in the bovine genome and created transgenic cattle with increased resistance to tuberculosis. Classical swine fever (CSF) is another devastating disease, leading to large economic losses in the pig industry, and CSF-virus resistant pigs is a further application of gene-editing technology. Applications in the poultry industry include resistance to avian leukosis virus (Koslová *et al.*, 2018). Honey bees resistant to the parasitic *Varroa* mite are also being researched with geneediting technologies (Mondet *et al.*, 2020).

Aquaculture

Two commercial applications of CRISPR in fish have been developed in Japan (see Appendix B.1c). Both studies leveraged the knockout of a single gene to increase overall size. The leptin receptor gene in tiger puffer was knocked out, which increased appetite and consequently weight of the fish. Red sea bream had its myostatin protein knocked out, which because of suppressed muscle growth allowed the fish to grow larger while consuming the same amount of food (Ohama *et al.*, 2020).

Microorganisms

Although not a focus of this Issue Paper, gene editing has been used to develop microorganisms to control some plant pests and diseases, improve soil status, and to help in food processing (Wesseler et al., 2022). Examples of applications of such engineered microorganisms include resistance to plant pathogens (Glandorf, 2019), bioremediation of soils and biostimulants such as products that enhance nitrogen fixation and nutrient up-take (Gosal, Kaur and Kaur, 2020), and alternatives to current plant protection agrochemicals (Scheepmaker et al., 2016). In the food industry as well as in the forest industry and related bio-based industries, gene editing is used to produce new bacteria and enzymes, for instance new enzymes can increase the fermentation efficiency in food processing (Deckers et al., 2020). New bacteria and enzymes are also important for developing plant-based alternatives to meat and cellular meat and play an important role in processing biological resources for the development of bioeconomy products such as biochemicals as alternatives to those based on fossil fuels (Clomburg, Crumbley and Gonzalez, 2017).

3 Gene editing – potential hazards, benefits and impacts on the environment and society

SUMMARY

Gene editing has the potential to transform agrifood systems and improve food security, nutrition and environmental sustainability, but for gene-edited food items, as for conventionally produced foods, there are potential hazards. Identifying potential hazards and assessing the corresponding risks prior to ensure their safe and sustainable use, as well as assuage public concerns. Potential impact categories include effects on the environment, biodiversity and human health in terms of food safety and nutrition. Suitable methods for assessing gene-edited products will be important to ensure the safety of gene-edited products while not placing an onerous regulatory burden on small developers and innovators. The economic impact of gene editing depends on the extent to which gene-edited products become available to small-scale producers, especially in LMICs, and those further down the supply chain. Gene editing also has the potential to reduce expenditures on crop protection and decrease labour demand. A potential positive impact at the farm-household level will depend on input quality, including that of seeds. There are social and ethical concerns associated with gene editing that are influenced by trust in scientists and developers, concerns about risk and benefit distributions, and questions about naturalness and differing cultural concerns and animal welfare, will have to be considered when deploying gene-edited products.

Gene editing represents an advance in technology that has the potential to transform some aspects of the world's agrifood systems for the better. However, as with all technological advances, in addition to the perceived benefits of gene editing there are potential hazards associated with specific processes of production. Some of the risks can be anticipated, but there might also be unintended consequences that will need to be managed. Although the consequences of introducing gene editing into agricultural processes are likely to be complex and interrelated, some elemental divisions can be made. One of the prime questions concerns whether gene editing has the capacity to relieve global hunger, albeit partially. It is also relevant to ask whether human health will be impacted positively through consumption of gene-edited food and whether the environment will change in terms of ecosystem stability because of introducing geneediting technologies. Gene editing, for specific traits that may be introduced in livestock, could also raise questions about animal welfare. Moreover, if benefits of gene editing are realized, and agrifood systems are transformed, how will the benefits be distributed? The discussion of these issues will include both practical and ethical considerations, and answers to key questions are unlikely to be straightforward. At the same time, these issues do not differ from those associated with established breeding methods, including mutation breeding and transgenesis.

Gene editing and human hunger

Food shortages are not the sole cause of hunger. Hunger and poverty are inextricably linked, and the causes of poverty are many, including inequitable income distribution, population pressure, compromised health, inadequate education and issues surrounding land tenure. Hunger and malnutrition are determined by both the amount of food available for consumption and its quality. Whether and where gene editing can have an impact is the point at issue.

Experience with transgenic crops has, at times, been controversial. An in-depth review of crop yield data by the United States National Academies of Science, Engineering and Medicine (NASEM) established no overall increase in the rate of yield gain attributable to genetically modified (GM) crops through 2015, although some GM crops and traits, in specific environments, were associated with yield gains (e.g. Bt crops under pest pressure) (NASEM 2016a). Others suggested that GM crops contributed to food safety and improved income of smallholder farmers (ISAAA, 2019).

Yield improvements using gene editing might be possible but are likely only to be for specific crops and traits under specific environmental conditions. However, engineering more complex traits that address yield gains, such as improved photosynthesis and stress tolerance, might be achievable with gene editing (Matres *et al.*, 2021). Gene-edited crops show promise for positive local effects on hunger if they are integrated into smallscale agrifood systems, many of which are based on neglected and underutilized species, and not necessarily on the major crops that are likely to be the prime recipients of gene-editing attention.

Gene editing undoubtedly is a more precise technology than previous induced mutagenesis and genetic engineering techniques, and some of the safety issues surrounding introduction of foreign genes into organisms are no longer valid. However, what effect gene editing can have when food distribution represents a major challenge is open to debate, especially given that food production is often less of an issue than its equitable distribution and access.

Gene editing represents a tool for scientists and breeders, making their work more efficient and effective. It might mean that response to disease and pest epidemics can be hastened over current relatively time-consuming processes. Useful work has been done on gene editing and powdery mildew resistance, but this represents just one of many serious crop diseases that merit attention. If gene editing were to contribute significantly to developing disease and pest tolerant germplasm, it would be an ongoing process because resistances are overcome through evolution and adaptation of the pests and pathogens to the engineered resistances. Tan et al. (2020) provided a detailed technical account of how gene editing might aid plant breeding. However, the environment sets natural limits on production, and climate change, with accompanying extreme weather events, makes crop and animal production particularly challenging. It is also the case that food shortages frequently occur in areas of significant poverty,

high population growth rate and political instability, where plant breeding efforts, for example, are unlikely to reach their potential.

The issue of malnutrition is not the same as that of hunger. If the problem is one of an unbalanced diet, rather that one of total calorie intake, gene editing could make major contributions. For example, progress has been made, using traditional and modern biotechnological techniques in the field of biofortification. The provision of adequate levels of essential dietary elements, including minerals, by boosting their levels in traditional host plants, or by introducing them into non-traditional hosts, can have a significant impact on malnutrition. Gene editing might be used to obviate the sometimes long breeding processes that HarvestPlus,¹ for example, has used to produce nutrient-enriched crops. Kumar et al. (2022) published an extensive list of instances where CRISPR-Cas9 has been used to improve the quality of a range of crops, including cereals, vegetables and fruits. They provided information on how gene editing has been used to boost the content of vitamins A and E, iron and zinc in various crops in an attempt to tackle problems of malnutrition.

Gene editing also has potential for developing orphan crops and neglected and underutilized species to boost food and nutritional security, agrobiodiversity and improve livelihoods. Such crops are unlikely to be attractive to the private sector because they are predominantly grown by poor smallholder farmers in the more marginal environments. Consequently, funding research on these crops can be scarce and the private sector usually would not regard them as a priority for investment. There are however possibilities for organizations such as CGIAR (formerly the Consultative Group on International Agricultural Research), AIRCA centres (Association of International Research and Development Centers for Agriculture) such as ICBA (International Center for Biosaline Agriculture) and icipe (International Centre for Insect Physiology and Ecology), and NARS (national agricultural research systems) to develop public goods that are unattractive to the private sector.

Gene-editing technologies are constantly evolving, providing even more precise tools for researchers and breeders. The causes of hunger

¹ https://www.ifpri.org/program/harvestplus

and malnutrition go well beyond anything that gene-editing applications can solve, and alone, gene editing is unlikely to make a significant impact on hunger and malnutrition. However, used in conjunction with other available tools, it is set to make a significant contribution to transformation of agrifood systems.

Gene editing and human health

Many direct effects of gene editing on human health are likely to take place through medical interventions. In agrifood systems, food safety can be a major issue in terms of human health considerations. While biofortification itself was largely regarded as beneficial and humanitarian, the introduction of foreign genes into plants, and some animals, often raised questions. There were fears about potential adverse effects from consumption of transgenic food products. The debates about the benefits of genetically engineered foods were taking place at a time when organic farming was being mooted by some as a viable alternative to high-tech farming. This was regarded by many as representing a conflict between a natural and a synthetic approach to agricultural production (Rausser, Simon and Ameden, 2000). However, there are, and have always been, negative effects of agriculture on human health, including occupational health, from direct use of agrochemicals to the indirect impact of agricultural practices on the environment. A necessary discussion is one of whether an intervention, in this case gene editing, represents an advance over current practices or not.

Potential food safety hazards of agricultural products, including those conventionally produced, includes both toxicity and allergenicity of elements in the food. Changes in the levels of toxins, allergens and nutrients, as well as mutations, can occur naturally. Gene-edited foods are no exception, and unintended effects and off-target mutation can occur (NASEM, 2016a). Genetically modified crops have been assessed on a comparative basis with their conventional or non-GM counterparts (Kok *et al.*, 2008) and as a broad category, marketed GM crops were determined to be as safe to eat as their conventionally bred comparators (NASEM, 2016a).

Allergenicity

SDN-3 can be used for the expression of novel proteins intentionally to create a transgenic organism. As with other transgenic crops, new or

crossed allergenicity associated with introduced novel proteins could potentially arise in SDN-3 applications. In contrast, for SDN-1/2 the allergyrelated potential risk might be a rise in endogenous allergens, an outcome that is also possible with mutants obtained using techniques such as radiation, chemical mutagenesis or somaclonal variation, especially if the host crop is a known source of food allergens.

Traditional allergenicity assessment for intentionally introduced proteins (transgenics and SDN-3) has relied on several methods, including bioinformatic analysis, looking for sequence homology to known allergens and by using a threshold homology (Fernandez et al., 2021). Such methods are better suited for detecting potential cross-reactivity with an existing allergy to other foods. Complementary methods, used in combination with bioinformatics to assess the possibility of *de novo* allergenicity of introduced proteins, include thermal and protease digestibility. Only in cases where the new protein comes from an organism known to cause allergies, or when there is a positive match in the bioinformatic analysis, is another study warranted using IgE binding of sera from people with known allergies to a protein (Su, Ezhuthachan and Ponda, 2020).

In the case of SDN-1/2 gene-edited crops for which the host is a known source of food allergens, allergenicity due to upregulation (increase) in the expression of allergens may require a complete genetic characterization of the unintended changes and quantitation of endogenous allergens in the whole food, in the same way as it should be required for mutants obtained using any other techniques where upregulation of allergens is also a similar possibility.

Toxicity

For SDN-1/2 gene editing, upregulation of endogenous toxicants is possible because it has occurred occasionally with new varieties obtained by spontaneous or induced mutation (for example in potato and celery). However, for other mutagenesis-based techniques, the breeder typically ignores the mutation leading to the new trait and the existence of potential off-target mutations. Conversely, gene editing offers more insight that might help breeders discard potentially risky mutants more reliably compared with previous techniques. For SDN3-based transgenesis, current methods applied to assess the potential toxicity of novel proteins in foods derived from recombinant-DNA organisms likely will continue to be applied. Countries have applied these robust and reliable methods following Codex Alimentarius Guidelines for three decades.

Composition analysis

Off-target edits and unintended DNA insertions with SDN-1 and SDN-2 modifications can occur and this should be considered for assessing the safety implications of food products (Lema, 2021). Although the intended modification might be safe and may even already be present in the food supply, unintended insertions or off-target edits might change the food's composition regarding nutrients, toxins or allergens. With the advent of cheaper and more efficient DNA sequencing, whole genome sequencing (WGS), in addition to bioinformatic approaches, has been suggested to detect and assess changes in gene-edited foods (NASEM, 2016a; Lema, 2021). For example, during and after the regulatory review of the polled gene-edited cattle, WGS detected the insertion of transgenic antibiotic markers (Young et al., 2020; Carlson et al., 2016). Hahn and Nekrasov (2019) suggested that the most important factor for reducing CRISPR-Cas off-targeting in plants is careful selection of target sequences, which can be helped by using various software tools.

There are several reviews on gene-edited foods done by regulatory agencies that present substantial equivalence data relevant to safety. For example, substance equivalence data were presented to the United States Food and Drug Administration (FDA) for a high-oleic acid soybean (edited using transcription activator-like effector nucleases, TALENs), for a reduced alpha-gal sugar pig (edited using older transposon methods), and for thermotolerant cattle (edited using CRISPR) (FDA, 2019; FDA, 2020; FDA, 2022). For each, it was not deemed necessary to conduct feeding trials on the entire gene-edited food using animals or humans, and although some differences were noted, employing compositional assessment, in comparison with conventional food counterparts they were determined not to be important for nutrition or safety. One exception was the transgene that was integrated into the GalSafe pig (a transgenic animal) for neomycin antibiotic resistance, which was determined to be a potential microbial food safety hazard. This required the

pigs to be reared without neomycin so evolution of bacterial resistance to that class of antibiotics would be reduced and human health would not be put at risk (FDA, 2020). For the thermotolerant cattle, during the FDA review, WGS generated evidence of unintended mutations in the genomic sequences from gene editing. However, through comparative bioinformatic analysis, these were determined not to result in changes to protein expression or impact the safety of the food relative to its conventional counterparts. The necessity for whole-food feeding studies to assess first generation GM crop safety has been contested (Devos et al., 2016; EuropaBio, 2018; Kuiper, Kok and Davies, 2013; Schiemann, Steinberg, and Salles, 2014), and improved guidelines for the conduct of such GM food studies have been developed (Schmidt et al., 2016). Alternatively, changes in endogenous toxins or allergens arising from unintended edits or epigenetic effects in gene-edited crops may be assessed using a component-by-component compositional approach, which requires prior knowledge of which compounds to test for.

Gene editing and the environment

Settled agriculture, including commercial fishing, has continuously changed the environment. As the global population increased and agriculture spread, much of the environment changed from its initial unmanaged state to one of being intensively managed. The transition from a natural environment to an agricultural environment has been taking place over millennia because the environment is the most valuable resource of humankind. Both extensive and intensive farming have had marked impacts on a range of ecosystems, and the effects have been exacerbated by changes in climate, particularly increasing temperatures and erratic rainfall patterns. Many agrifood systems have been degraded and others are no longer sustainable and are unable to feed those populations reliant on them. There are intrinsic concerns for the environment, with which humans have a personal relationship, and concerns that in many instances degradation and disappearance of key ecosystems will result in famine and migration.

While there are legitimate concerns about the introduction or removal of specific genes in organisms to increase production or ameliorate a situation, there is a long history of introduction of entire genomes into new environments.

Various countries have been plagued by the introduction of alien species that have wreaked serious environmental damage. Moreover, while crop plants seldom represent an environmental hazard because they are not adapted to an off-farm environment, the same is not true of domesticated livestock, which are often able to thrive off-farm and do considerable damage to the natural and managed environment. Some of the world's worst weeds are also introductions (May, 1981) and while the importance of such introductions is acknowledged, they usually do not attract the same level of attention and discussion as the introduction or deletion of fractions of entire crop genomes that are to be consumed as food.

Gene-edited organisms and products in food, agriculture and the environment raise equivalent concerns about environmental and ecosystem impacts as transgenic (for SDN-3) and conventionally bred (for SDN-1/2) crops (NASEM, 2016a; NASEM, 2017). Potential risks can stem from the introduced traits or through the editing processes (Eckerstorfer et al., 2021). Types of risk include, but are not limited to, toxicity to non-target species in ecosystems, increased weediness after genetic introgression, invasiveness of the gene-edited organism itself, and changes to water, land, and energy use that may accompany deployment of gene-edited organisms (NASEM, 2016a; NASEM, 2017). Factors influencing the assessment of the potential risk to ecosystems from gene-editing products will depend on what the product replaces, its management, the host environment and socioeconomic context. The magnitude of the risks from gene-edited products will depend on the product's characteristics, use and management in the environment, and the features of the ecosystem into which it is placed. Potential risks of gene-edited products, first generation GM products, and conventionally bred products inevitably vary on a case-by-case or product-by-product basis. Their relative risks cannot be placed in broad categories because the products are of numerous types (NASEM, 2016a; NASEM, 2017) However, in principle, it can be anticipated that SDN-1/2 products have the same risk scopes as earlier mutationbased breeding, including natural mutations, while SDN-3 has the same potential risk scope as earlier transgenesis-based breeding methods.

Environmental studies on gene-edited organisms in their intended sites of use are scarce because few gene-edited products have entered the market, and regulatory approval processes have

not required long-term environmental field trials. However, this is not necessarily an obstacle for policymaking or safety assessment, given the equivalence of final products with products of conventional and transgenic breeding. Therefore, instead of speculative literature on the potential impacts of gene-edited products in agriculture and the environment, it is instructive to consider the experience on transgenic products and conventional varieties generated using mutagenesis for anticipating the range of potential impacts. This approach has been supported by several scientific bodies, risks being dependent on the characteristics of the product, rather than the process by which it was produced, and that the general categories of risk will be the same in kind for gene editing, conventionally bred and transgenic products (NASEM, 2016a; NASEM, 2017).

Issues associated with gene-edited plants

Risks from the introgression of genes from genetically modified crops into wild relatives is an area of concern for both GM, gene-edited crops, conventionally bred varieties and varieties bred using mutagenesis. Generally, gene flow can reduce the differences between populations and decrease diversity within a population, thus broadly impacting biodiversity (Tsatsakis et al., 2017). Gene introgression into wild relatives may also pose more direct risks, depending on the introduced trait. For example, several varieties of spontaneous/ somaclonal/chemical mutagenesis, gene-edited and GM herbicide-tolerant crops were cleared from regulation in the United States of America and elsewhere (USDA, 2022). They included not only commodity and food crops, but also grasses able to cross pollinate with neighbouring wild relatives. Herbicide-resistant weeds have arisen from the use of GM and conventionally bred herbicidetolerant crops due to overuse of the companion herbicide, and herbicide-tolerant crops such as creeping bentgrass and rice have cross pollinated with wild relatives, transferring herbicide resistance genes, and increasing the potential for increased weediness under selective pressure (Zapiola and Mallory-Smith, 2017; Tsatsakis et al., 2017). Indirect agroecosystem impacts have stemmed from unintended migration of herbicide-tolerant GM grass pollen over long distances in the northwest United States of America, and the resulting herbicide-tolerant weeds have invaded irrigation systems and caused management problems for farmers (Rosen, 2018).

For several years after the introduction of herbicidetolerant crops, less toxic herbicides, and in some areas reduced application of herbicides, occurred. However, the level of toxicity, as measured by active ingredient from herbicide use with first generation herbicide-tolerant crops, was sometimes lower in comparison with similar production systems that did not rely on herbicidetolerant crops (NASEM, 2016a). Moreover, the adoption of herbicide-tolerant crops often accompanied the adoption of reduced tillage systems, with concomitant benefits to soil health (Frisvold, Boor and Reeves 2009). However, the evolution of herbicide-tolerant weeds following extensive use of GM and conventionally bred herbicide-tolerant crops, prompted the return to more toxic herbicides with potential negative health outcomes for farmers, in addition to more severe weed management challenges (Bonny, 2016). In contrast, the rate of development of herbicide tolerance has not changed substantially for GM crops, including maize, while it has for soybean and cotton (Kniss, 2018). Regardless of such inconsistencies, improved crop management and better communication of good management practices would help. Lessons learned from GM crops will help with management of gene-edited crops, many of which are likely to be herbicide tolerant (Bonny, 2016).

Invasiveness of gene-edited plants is of potential concern. For example, abiotic stress traits such as drought and heat tolerance, may theoretically confer a fitness advantage that could increase invasive potential in the environment, regardless of the trait engineered through gene editing, other mutagenesis techniques or transgenesis. Yet, this is not valid in most cases because domesticated plants are unlikely to become invasive as they cannot grow outside of a carefully managed farming environment (Smýkal et al., 2018), with the exception of few (Tsatsakis et al., 2017). However, some gene-edited crops in development, and cleared through United States of America regulation, including grasses, pennycress and canola (USDA, 2022), have wild relatives and can persist in the environment. For these, increased stress tolerance from gene editing may be a concern regarding invasiveness or weediness.

Issues associated with gene-edited animals

Invasiveness of gene-edited animals in special cases involving traits increasing their fitness (ability to survive, reproduce, feed and persist

in ecosystems) could theoretically pose risks to ecosystems should they escape or be deployed in unmanaged situations. However, there is considerable experience with the selection of new animal breeds having spontaneous mutations that did not confer increased invasive potential. For instance, the double muscle, slick and hornless traits in cattle, which are currently being obtained again through SDN-1/2 gene editing, have been selected in the past from spontaneous mutations and there is considerable experience that these mutations did not increase invasiveness.

In the case of SDN3-edited animals, prior experience with GM animals may be relevant. For example, there has been concern in the case of GM salmon modified to grow faster than non-modified salmon (Devlin et al., 2010; Devlin, Sundström and Leggatt, 2015), but the developer later showed that a faster growth rate constituted a fitness disadvantage (FDA, 2015). There is also the potential for hybridization of farmed GM salmon with wild salmon, should they escape from their controlled environments and enter nearby waters containing native salmon (Devlin, Sundström and Leggatt, 2015; Wringe et al., 2018). GM salmon have mainly been farmed at inland facilities, although some contained growth is occurring near shores where there are wild salmon populations (Tutton, 2021). CRISPR-based gene-editing methods are being explored for bioconfinement of farmed salmon so that they cannot reproduce should they escape from containment facilities (germ-cell free farmed salmon) (Wargelius et al., 2016).

More recently, transgenic pet fish containing green fluorescent protein (GFP) escaped into open bodies of water (Magalhães, Brito and Silva, 2022). The consequences of that escape have not been assessed, but the invader could directly affect local species by competing for food (Magalhães, Brito and Silva, 2022; Moutinho, 2022), although this possibility is unlikely to be much different from the escape of wild-type, non-fluorescent pet fish of the same species.

Overall, it is not possible to predict ecosystem impacts from introduction of gene-edited animals or the extent of introgression of their genes. However, this might not solely be an issue relevant to gene-edited animals – many corresponding traits can be derived by conventional breeding, but may require more time (Van Eenennaam *et al.*, 2021). There is the potential for escaped gene-edited animals to be innocuous or detrimental, and studies on anticipating risks are complex given the various ecological endpoints that need to be considered (Van Eenennaam *et al.*, 2021). Gene-edited animals may present potential environmental risks similarly to first generation transgenic animals, depending on the modified trait, the environmental context, and other related or non-related species in the ecosystem. Nevertheless, the reported cases of invasive species can be mainly traced back to an introduction via international trade and/or travel and not via gene editing or other breeding protocols (CBD, 2021).

Gene drive organisms are briefly discussed in Box 2.

Gene editing and animal welfare

Regarding non-human animals and the impacts of gene editing, views on ethical status are of particular importance, especially considerations of animal pain and suffering (Thompson and Hannah, 2008). One position is that prospective human benefits cannot justify harming animals, whereas other positions view animals as subjugated, being of extrinsic value and solely providing for the needs of humankind (de Graeff et al., 2019). Regardless, the amount of suffering of or relief provided to animals will vary depending on the gene-editing application and production methods used. It is, moreover, not solely a case of making a geneediting intervention, the research that leads to the intervention must be considered. The use of animals in research has long been controversial and centres on the extent to which animals register pain, the rights of animals and whether the rights of humans and the benefits that accrue from animal research outweigh the suffering induced during experimentation. Justifying animal research has always been contentious but were gene editing able to reduce or remove the pain suffered by animals during research, or to contribute to reducing the requirements for animal research, that would represent a positive contribution.

The early days of animal biotechnology often incorporated cloning steps, and deformities in offspring were relatively common. However, geneediting processes could represent an improvement in this regard. For example, in the case of the polled gene-edited cattle, only a single mild abnormality was associated with the offspring (Young *et al.*, 2020): the application of gene editing was designed to improve animal welfare by eliminating the need for painful dehorning of young cattle (Carlson et al., 2016). CRISPR prime editing was also successfully used in the Republic of Korea to correct a single mutation linked to hip dysplasia in two Labrador dogs (Kim et al., 2022), and they, like the fish (Magalhães, Brito and Silva, 2022), contain the added GFP gene. While, apparently successful, the procedure has attracted discussion about the ethics of the procedure and the unknowns, particularly unintended consequences. However, the method could have potential in correcting for genetically determined disorders in livestock. Another positive example of SDN for animal welfare involves the potential use of gene drives. Gene drives are being developed to eradicate invasive rodents by impacting their fertility, and this may reduce suffering to the rodents by substituting for chemical anti-coagulants that kill through internal bleeding (Leitschuh et al., 2018). With respect to animal welfare, Schultz-Bergin (2018) asked whether CRISPR is an ethical game-changer, concluding that it does have the potential to directly improve animal welfare, for example through introduction of disease resistance.

Gene editing, socioeconomic impact and distribution of benefits

Introduction of gene-editing technologies will have far-reaching implications for agrifood and social systems in terms of its potential for improving and securing production of food. The extent of the impact is, to date, speculative and it remains to be seen whether expectations can be lived up to. It is certain, however, that the benefits that accrue from gene-editing applications will be distributed among various parties, although not necessarily inclusively. World hunger, malnutrition and poverty are invariably located in areas where agrifood systems are under most pressure and where agriculture is most fragile and vulnerable. Many of these areas are within LMICs. Questions about whether small-scale producers are likely to benefit from introduction of gene-edited crops and livestock or whether it will be the richer, largescale farmers, are key to the discussion. Costs of production, ownership of technologies and materials, dependencies and market control are all part of the discussion. Within the limits set by the environment, it is possible that food production and food quality will be improved through application of gene-editing technologies, but if the costs outweigh the benefits, the technology may not be adopted.

Box 2 Gene drive organisms

Another application of SDNs termed gene drive, differing from gene editing, is currently being developed (NASEM, 2016b). Although gene drive organisms have yet to be deployed, a few are under development to decrease unwanted species in wild ecosystems. For example, to eliminate unwanted pest populations, invasive species and disease-carrying organisms. Gene drive systems, often based on CRISPR-Cas 9 and the introduction of cargo transgenes, allow an edited gene on one chromosome to copy itself into its partner chromosome during cell division and meiosis. Thus, inheritance with each generation is biased towards 100 percent, rather than 50 percent. Cargo genes, conferring any trait that can be genetically linked to an engineered gene drive system, can, with some fitness cost, spread through the population with the gene drive (Bier, 2022). Cargo genes can be designed that confer desirable traits, including disease resistance, or harmful traits causing population decline (for example, by killing females). In the latter case, theoretically, the release of few individuals with gene drives could cause an entire population to decline or collapse (given full population mixing and mating) (Esvelt and Gemmell, 2017). If the gene drive works, most offspring will inherit the engineered gene unless the gene drive is designed to be self-limiting (Champer, Buchman and Akbari, 2016; NASEM, 2016b). CRISPR transgenes must remain in the final product to propagate the gene drive.

Given short generation times and random mating across distances, the release of few individuals with gene drive systems designed for population suppression could, theoretically, cause substantial perturbations in a population. However, the gene drive scientific community is also working on gene drives that are geographically limited (that target genetic sequences associated with specific areas), self-limited, or threshold based, as well as ways to reverse the spread of gene drives by exploiting molecular mechanisms (reversal drives) (Esvelt et al., 2014; Bier, 2022; Chenurri, Adelman and Myles, 2022). In such cases, the organism is not designed to spread throughout the population but could be limited in terms of area or spread. Gene drives are being developed not only for eradicating agricultural pests (population suppression) (Romeis et al., 2020; NASEM, 2016b; Devos et al., 2022) but also to add beneficial genes, such as immunizing genes, to

protect valued populations (population modification) (NASEM, 2016b; Devos *et al.*, 2022).

Hayes et al. (2018) summarized the categories of potential hazard pathways and adverse ecological consequences from gene drive organisms at three levels - molecular, population, and ecosystem - as well as according to their impacts on target organism capacities to spread disease, survive, reproduce or spread, on non-target organism survival, and on ecosystem services. For example, potential risks from gene drive organisms for population suppression include the risks stemming from introgression of the suppression gene into desirable, related species in ecosystems (Romeis et al., 2020; Devos et al., 2022; Hayes et al., 2018). If so, the population of a desired species could collapse, which might cause additional ecosystem disruption. For example, gene drives in the fruit-fly Drosophila suzukii were developed to suppress the population and protect fruit crops. If the population is indiscriminately reduced, it may be necessary to assess if significant indirect foodweb effects could occur because of the intended large-scale reduction in population (Romeis et al., 2020). More direct effects could occur from the introgression of the suppression gene into related species that may be beneficial to ecosystems (Romeis et al., 2020). Hybridization or horizontal gene transfer (e.g. through transposable elements) could also have an impact if the hybridized species exhibited increased damage potential or if it had decreased fitness that led to a decline in important ecosystem services (e.g. via reduction in food source for predators of the hybrid) (Romeis et al., 2020).

Overall, impacts of open release gene drive organisms in agrifood systems, including whether the gene drive works as intended, are difficult to predict from laboratory or field-cage trials (NASEM, 2016b; Kuzma, 2019; Kuzma, 2020; Romeis *et al.*, 2020; Devos *et al.*, 2022). Scientific organizations and research communities have called for a stepwise, tiered process for field release of gene drive organisms and biosafety and biosecurity protocols to provide sufficient attention to risk issues prior to open release (Akbari *et al.*, 2015; James *et al.*, 2018; NASEM, 2016b). Some risks can potentially be mitigated using molecular or geographic methods (Bier, 2022; Min *et al.*, 2018; Kuzma, 2020).

Economic impact at farm household level

The economic impact of gene-editing technologies depends on how widely distributed the technologies become and which sectors of society will benefit from their deployment. Smallscale producers in LMICs, in general, might be expected to benefit relatively more than those farming more intensively in HICs (Wesseler, 2019). Intensive farming requires that pests and diseases are managed using a range of plant protection methods. A change in farming methods, possibly incorporating gene-edited products, would not be expected to increase crop yield, it would simply represent a substitution of one method for another (Wesseler, 2019), but could be more environmentally beneficial and reduce expenditure on pesticides, that is, reduce food costs. However, the relative benefits of gene editing to LMICs over HICs will only be determined on a case-bycase basis and will depend on which technologies are incentivized, developed and marketed, and their costs and accessibility. The benefits will also depend on the type of farming, for example, aquaculture farms, urban and vertical farming, and small-scale versus large-scale farming.

In LMICs, where fewer purchased inputs are used than in HICs, gene editing has the potential to increase yields substantially. One of the prime examples of the application of transgenic technologies is the introduction of insect-resistant cotton in China (Pray et al., 2001) and India (Qaim and Zilberman, 2003). If seeds improved using gene editing that provide resistance to pests and diseases become available in HICs and LMICs, small-scale producers in LMICs can be expected to get enhanced yields (Wesseler, 2019). A yield increase would be expected to boost farmhousehold income. Nevertheless, increases in vield often increase aggregate product supply, reducing output prices. The prices for seeds often increase as well, while other input costs might be lower, including those for pesticides and fertilizers. Moreover, the economic benefit from technological change might only be temporary, as has previously been the case (Wesseler, Jongeneel and Purnhagen, 2019).

Disease and pest-resistant crops are expected to reduce crop protection expenditures and related labour demand, but seed prices often increase (Klümper and Qaim, 2014). The demand for fertilizer at the farm level increases because of an expected increase in crop yields and profits. An increase in the importance of secondary pests was recorded with the control of the cotton-bollworm in China (Wesseler, Scatasta and Hadji Fall, 2011), while the wide use of Bt cotton resulted in the overall regional suppression of the cotton bollworm, not only benefitting Bt cotton farmers but also benefitting those not cultivating Bt cotton. Similar effects could be expected for pest control using gene-edited crops.

A change in labour demand stemming from geneedited crop deployment can be expected at two levels. The labour demand for crop protection, such as weeding and pesticide application, would be expected to decline, reducing female and child household labour. The decline in labour needed for weeding would benefit female household labour (Haggblade et al., 2017). The decline in labour used for pesticide applications has been linked with improvements in health (Mancini, 2006; Rola and Pingali, 1993). In the case of Bt crops, there is significant evidence for improved health outcomes for farmers from reduced chemical pesticide use (Kouser and Qaim, 2011) and there are spin-offs for improved environmental health. Herbicidetolerant crops produced through first generation GM technologies reduced the time farmers and farm workers spent on weed control, thus freeing up their resources of time and finance, in addition to providing direct economic gains (Brookes and Barfoot, 2018a; 2020). Furthermore, according to some estimates, both Bt and herbicide-tolerant crops led to the application reduced application of chemical pesticides from 1996 to 2016 in many instances (Brookes and Barfoot, 2018b; 2020). The expected increases in crop yield did result in an increase in labour for harvesting. Such increases are often for a short period of time, during peak season, and require hiring additional labour, with potentially positive effects on local labour markets.

Indirect health benefits can be expected as more food becomes increasingly available and as household incomes increase. Increases in staple crop consumption would have positive health effects, especially if containing higher levels of micronutrients and minerals such as beta-carotene, iron and zinc (HarvestPlus, 2017). An increase in micronutrient supply has been demonstrated to have long-lasting positive effects on human health (Fogel *et al.*, 1994).

The expected increase in better nutrition and higher income at the household level would have

positive social implications for those households. Women and children are the main beneficiaries of better food supply, strengthening their position within the household (Global Nutrition Report, 2021). However, cases have been reported where non-beneficiaries have attempted to undermine implementation of better food supply for women and children (Zingwe, Manja and Chirwa, 2021). Access to gene-edited crops could widen the economic gap between those who have access and those who do not (Klümper and Qaim, 2014). Importantly, for reaping the benefits, improved crops varieties need to fit into the farming systems, which is often overlooked (Schnurr and Dowd-Uribe, 2021). Furthermore, nutritional benefits can be substantial at household level (Van Der Straeten et al., 2020) and have often not been included explicitly in ex ante studies.

Economic impact at input market level

The potentially positive impact of gene-edited crops at the farm-household level depends on the quality of seeds and other inputs. Maintenance of seed quality has been a problem for seed-based plant pest and disease control options. The effectiveness of insect and herbicide resistance largely depends on seed quality. Markets for stealth seeds (Herring, 2009) and fake herbicides (Haggblade, Diarra and Traoré, 2022) were recorded in cases where the technologies were highly appreciated by farmers. Gene-edited crop seeds, in this case, could be expected to face the same problem as for any improved seed – the quality and control of seed systems and other inputs for crop production will be very important.

Improved seeds, in general, are more expensive than standard seeds. The additional costs need to be compensated for by higher revenues. Access to improved seeds might become a problem for cash-constrained farmers. Financial markets will be important to allow farmers to borrow so that inputs can be purchased. This not only applies to access to gene-edited crop seeds, but it is also important for other inputs such as fertilizers.

Differentiated markets

The application of gene editing to crop and animal production can, in principle, have effects on different markets. Seeds are often the target of gene-editing applications, which could be used in non-organic agricultural production and in organic production if not regarded as being transgenic. Purnhagen *et al.* (2018) argued that using gene

editing in agriculture can contribute to achieving the SDGs and, particularly if permitted for use in organic agriculture. If the technology were not to be allowed in organic agriculture, only for non-organic agriculture, the yield gap and the differences in gross margin would be expected to favour nonorganic agriculture and to strengthen the incentives for non-organic agriculture production. The Court of Justice of the European Union (CJEU), in June 2018, ruled that organisms developed by gene editing were to be GMOs (genetically modified organisms) and not exempted from regulatory oversight (Purnhagen et al., 2018). This implies that gene editing cannot be used in organic agriculture and that countries that export organic food products to the European Union must differentiate their markets if they choose a different legal definition. Tracking and tracing gene-edited products for export represents a challenge (Smith, Wesseler and Zilberman, 2021). The cultivation of gene-edited crops, as for cultivation of transgenic crops, might also generate discrimination against those cultivating the crop at the local level in the case of differentiated markets. Farm households in Germany are concerned about social discrimination if they cultivate transgenic crops (Venus et al., 2016). In those regions where gene-edited crops are legally treated like transgenic crops, such as in the European Union, similar responses can be expected. Kalaitzandonakes, Phillips and Wesseler (2016) include several examples from different parts of the world.

Societal factors and public acceptance

The first generation GM crops were associated primarily with intensive agriculture, which impacted farming, social and political systems and concentrated on conventional agriculture and larger corporations (e.g. Clapp and Ruder, 2020). Concerns have been raised about the lack of biotechnology applications for small-scale producers and developing countries (Stone, 2010) and increased corporatization of university and public-sector research in developed countries like the United States of America (Welsh and Glenna, 2006; Glenna et al., 2007). National policies have often favoured industrial agriculture, to which the first generation GM crops are linked, and those policies impacted social systems. For example, Fox and Haight (2010) documented the flow of subsidies in Mexico away from smallholders to large-scale producers in the wake of NAFTA (North American Free Trade Agreement). The impacts of agricultural policies in Mexico in the aftermath of NAFTA
led to 20 percent of Mexico's farmers leaving agriculture and depopulation of rural communities (Zahniser and Coyle, 2004). Although GM crops are not directly responsible for the impacts, they exert global influence (ISAAA, 2019). In 2020, the President of Mexico banned all imports and approvals of GM maize, citing the need to safeguard food security, sovereignty, native maize, the milpa system, Mexico's biocultural wealth, smallholder communities, and the gastronomic heritage and health of Mexicans (President of the United Mexican States, 2020). Prior to this decree, Mexico had been one of the world's largest importers of GM maize and soybean (USDA, 2021). It is unclear, however, how this decree applies to policies in Mexico regarding gene-edited crops because the policies are still evolving (Kuiken and Kuzma, 2021; Turnbull, Lillemo and Hvoslef-Eide, 2021).

To date, gene-edited crops are more diverse regarding varieties and traits in comparison with first generation GM crops, and so are the institutions developing them (i.e. more public organizations and smaller companies). Many larger multinational corporations are involved in gene-edited crop development and regulatory approval (Whelan, Gutti and Lema, 2020; George et al., 2022; USDA, 2022) and there is a continued focus on herbicide tolerance in commodity crops and sale of companion herbicides, mainly aimed at larger farming enterprises (Zhang et al., 2018a; Clapp and Ruder, 2019; USDA, 2022). However, there are signs that there is more interest in developing gene-edited crops with a much broader range of improved traits than solely herbicide and specific pest tolerances. Furthermore, there remain significant barriers to smaller developers and farmers posed by patents and ownership issues with CRISPR technologies and gene-edited seeds (de Wit, 2020). Although a range of traits to improve the environment and social good are on the horizon, gene-edited crops for enhanced sustainability face significant financial barriers for development and use (Jordan et al., 2022). Moreover, the cost of regulatory approvals, if geneedited crops are subject to the same regulatory requirements as GM crops, would also likely be prohibitive for small commodities.

Prior conflicts over cultural values, food and agriculture associated with first generation GM crops may also play out in the development of gene-edited crops despite greater diversity of actors and crop varieties. Public perception studies

are mixed as to whether the public and interested groups can distinguish among first generation transgenic approaches and gene editing in forming their attitudes and opinions. For example, in a recent public perception study comparing gene-edited with GM and conventional foods, respondents viewed CRISPR and GM food similarly and substantially less positively than conventional food (Shew et al., 2018). Other studies established that consumers may be more willing to accept cisgenic crops (genetic changes introduced from the same species, such as those produced by some gene-editing technologies) than transgenic crops, but less willing to accept cisgenic crops in comparison with conventionally bred crops (De Marchi et al., 2019; Edenbrandt, Gamborg and Thorsen, 2018). However, certain benefits associated with cisgenic and gene-edited foods can outweigh negative perceptions among consumers. For example, only a subset of consumers rejected cisgenic and transgenic crops under any circumstance (typically less than 20 percent), and other groups chose them based on health, safety and nutritional benefits, irrespective of whether they were cisgenic or transgenic (Yue, Zhao and Kuzma, 2015; Siegrist, 2008; De Marchi et al., 2019; Edenbrandt, Gamborg and Thorsen, 2018; Busch et al., 2022).

Specific-interest groups are likely to remain opposed to GM and gene-edited foods based on their value systems regarding agriculture, food, ecosystems and nature (Zilberman, Rausser and Wesseler, 2023). For example, there are ongoing tensions in rural communities between conventional agriculture and agroecological approaches to farming (Rissing, 2021). In some cases, the use of GM crops has led to divisions among conventional farmers and organic or non-GM farmers and conflicts over fears of cross contamination (seed, pollen and chemical drift from companion herbicides) and lost organic and international markets (Venus et al., 2016; Gupta, 2018; Paull, 2019). Depending on legalities, one group of farmers, organic or non-organic, must bear a higher level of responsibility than another but often without significant impact on economic efficiency (Beckmann, Soregaroli and Wesseler, 2010). The use of minimum distance requirements as part of coexistence policies discriminates against smaller farms (Beckmann, Soregaroli and Wesseler, 2010). Organic farmers in the United States of America bear responsibility for buffer zones to distance themselves from conventional and GM agriculture, testing for GM presence, and

assuring that their products are GM-free or that GM contamination is lower that than the specified threshold. Some have suggested that policies should compensate organic farmers if eventually they suffer losses (Paull, 2019; Azadi, Taube and Taheri, 2018). The issues are likely to persist for gene editing in some regions of the world. For example, in the United States of America, the National Organic Standards Board has excluded gene-edited products from being certified as organic (USDA, 2019). With growing consumer demand in the United States of America for organic and non-GM labelled foods (Castellari et al., 2018; Hartmann Group, 2018), tensions between organic and non-GM farmers and those industries that supply geneedited and GM crops are likely to persist.

Cultural concerns among Indigenous Peoples have also been documented with the use of first generation GM crops. For example, Native Hawaiians objected to the patenting and genetic modification of taro (kalo), a crop of significant religious and cultural significance (Gupta, 2018). Similarly, regarding quinoa, Andean farmers took issue with American agronomists over twenty years ago when they patented a male sterile line of a Bolivian cultivar of the crop (RAFI, 1997), considering it biopiracy. Indigenous Peoples may view gene editing similarly, with objections centring largely around sovereignty, ownership and preservation of natural heritage being violated by genetic modification, including gene editing. However, indigenous concerns have also intersected with economic and non-GM production concerns. In Yucatan, the local smallholder economy has relied on honey production, and Mayan producers had their markets to the European Union threatened by contamination with GM soybean pollen (Gómez González, 2016). Mayan communities formed alliances with other groups that were hostile to GM soybean, including organic farmers and NGOs (non-governmental organizations), to oppose cultivation of GM soybean (Gómez González, 2016).

There is considerable public concern about the potential environmental and human health risks of CRISPR-edited crops and animals, which are mainly fuelled by social, religious and ethical viewpoints (Ahmad *et al.*, 2021; Kato-Nitta *et al.*, 2021). Other concerns are due to issues relating to limited understanding of science, low trust in developers and regulations and inadequate communication about risks and benefits of gene-edited plants and

animals (Ahmad *et al.*, 2021). Despite purported marginal risks of gene-edited organisms to the environment, human health and the economy (Lassoued *et al.*, 2019), current biotechnology regulations and advocacy groups have mixed views on the adoption of gene editing, and this could have an impact on public perception and social acceptability (Helliwell, Hartley and Pearce, 2019).

Public attitudes to first generation GM crops have depended on a variety of factors that are likely to apply to gene-edited crops. Some survey results show that the less respondents knew about the technology the more they were opposed to it (Fernbach et al., 2019). However, other surveys of public attitudes to GM crops showed that consumer knowledge has a modest to no effect on public acceptance and that consumers with greater familiarity are often more concerned with GM foods, going against the "deficit model" (Rose et al., 2019). Thus, factors other than knowledge seem to be more important regarding public perception of GM crops. The factors include trust in scientists and governments to manage risk, the legitimacy of decision-making processes, respect for diverse cultural values and world views, and public ability to control exposure to risk or make choices about technological products (Siegrist, Connor and Keller, 2012; Yue, Zhao and Kuzma, 2015; Kuzma, 2017). Furthermore, less general surveys that address specific traits, crops and products indicate a differentiated picture. Presentation of the product, ordering of information, and other survey designs have an impact on the results (Huffman and McCluskey, 2020), as well as consideration of the specific benefits provided by GM crops (Yue et al., 2015).

Mandatory labelling, providing simple explanations on the use of genetic engineering, led to reduced opposition to the technology (Kolodinsky and Lusk, 2018). Yet, labelling could lead to avoidance of gene-edited crops by food companies, small-scale producers and trade-dependent LMICs. Therefore, labelling rules should be framed in a harmonized global system based on transparent science-based consideration of risks, in which new traits in food would be included in a label if they represented a fundamental change in the composition of the food; production method would not be a mandatory labelling requirement. Following the introduction of mandatory food disclosure labelling, an analysis of market behaviour and consumer purchases in the United States of America showed that sales of

genetically engineered soup products decreased by 5.9 percent, sales of non-GMO labelled products increased by 2.5. percent, and sales of organic products increased by 1.7 percent (Fan, Stevens and Thomas, 2022). Public perception and social acceptance can substantially impact how the food industry responds. The introduction of transgenic wheat in the United States of America and Canada was blocked by concerns expressed about access to European markets (Kalaitzandonakes, Phillips and Wesseler, 2016). A voluntary market has emerged for GM-free labelled food products in the United States of America and the European Union, taking heed of public demand (Castellari *et al.*, 2018).

Globally, perceptions of gene editing differ based on prevailing situations. McConnachie *et al.* (2019) established that 66 percent of respondents to a survey would consume products made from cattle polled using gene editing. The perception that farmers had an obligation to reduce animal pain and suffering through gene editing played a significant role in their willingness to consume gene-edited products from polled cattle (Smith, 2021).

Social acceptance of gene-edited organisms is also driven by how the perceived benefits meet the needs of communities and possibly by concerns over power changes, particularly those removing power from farmers. Woźniak, Tyczewska and Twardowski (2020) reported that the critical issue in acceptance of gene editing relates to its application and not the technology itself, and applications aimed at prevention or treatment of diseases, prevention of disabilities and organ transplantation received the highest support at the expense of gene-editing interventions aimed at improving crop and livestock production. For instance, Busch et al. (2022) reported that respondents in Canada, Japan, the United States of America, Germany and Italy viewed the application of gene editing for disease resistance in humans to be most favourable, followed by disease resistance in plants and lastly in animals. They viewed improvements in livestock for product quality and quantity as least desirable.

Some authors expect that social acceptance of gene-edited organisms necessitates strengthening seed systems through the operationalization of regulatory structures and upgrading stakeholder knowledge of genetic engineering, analysing the effects of the edited variety on biodiversity and soil nitrogen dynamics, and strengthening the technical and human capacities of the biosafety body (Nlend Nkott and Temple, 2021). However, much of the social science literature on risk perception and on attitudes to GM food shows that public acceptance is influenced by a range of factors, including perception of naturalness, trust, risk-benefit distribution, purpose of the product, cultural values and features of the technology such as whether it is controllable and fully understood (Siegrist, 2008; Siegrist, Connor and Keller, 2012; Frewer *et al.*, 2013; Scott *et al.*, 2018; Kuzma, 2017; Rose *et al.*, 2019).

Gene editing and fundamental ethical considerations

Ethics and transgenics have been extensively reviewed (Robinson, 1999: Ubalua, 2009; Gregorowius, Lindemann-Matthies and Huppenbauer, 2012) and while technologies have evolved, the ethical considerations discussed previously have largely remained similar. Ethical considerations concern all aspects of the potential impacts and consequences of the new technology, in terms of moral imperatives to make improvements over current situations related to human hunger, human health, the environment, societal impacts and distribution of benefits. These considerations are often referred to as extrinsic concerns, but there are also intrinsic concerns that must be taken account of. These address aspects of what is right and wrong, issues of theology and what constitutes naturalness and respect for nature. As with GM products, the opinions on products of gene editing are in many instances likely not to be based on scientifically established facts but on value judgements. This is also the case for risk assessment, which is not merely an objective technical matter, but depends on opinions and definitions.

Ethical aspects associated with the application of gene editing in agrifood systems span the fields of environmental ethics, bioethics, animal ethics, technological ethics and food ethics, as well as medical ethics if the product has applications to health and medicine in addition to food and agriculture (e.g. GalSafe pig). Different paradigms of ethical analysis that have been used to examine this include utilitarian approaches (weighing benefits versus costs, also known as consequentialist ethics), principle-based ethics (deontological), virtue-based ethics, care ethics and duty-based ethics. Most ethical issues associated with GM and gene-edited food and agricultural applications are not unique to the technology, applying to many technologies linked to agrifood systems (Thompson and Hannah, 2008).

It can be argued that failure to use technology to address a serious problem, such as one relating to food security, is morally wrong, but another view is that applying a technology is not morally justifiable if it involves undue risks, violating the principle of non-maleficence. Other concerns like autonomy, informed consent, rights of animals, privacy, equity and social justice, distribution of risks or benefits, and procedural justice are not typically considered in formal oversight systems or decision-making for GMOs. These ethical issues, as well as the societal issues, have been subordinated in governance systems for gene editing (Helliwell, Hartley and Pearce, 2019). There is an apparent paucity of policy spaces for public consideration of the ethical and normative questions associated with first generation GM and gene-edited crops (Thompson, 2003; Kuzma, 2021).

Ethical issues intersect with socioeconomic and political issues from social justice and commercial competition viewpoints. The empirical evidence shows that in many cases smaller farms benefitted from GM crops, most welfare gains did materialize down the supply chain and technology developers share was about 25 percent or less (Falck-Zepeda, Traxler and Nelson, 2000; Qaim, 2009). However, there are concerns over power and control of the food supply, the lack of market penetration of biotechnologies for poorer or smaller farmers, and the transfer of ownership and sovereignty to companies (Thompson and Hannah, 2008). Social justice concerns also relate to the ability of cultural groups to choose whether GM crops are used in their communities, such as the examples concerning Indigenous Peoples and their rejection of GM technologies in sacred, culturally significant, or economically important agricultural crops. Procedural justice suggests that those most affected by GM crops should have a voice and choice in decision-making about using them (Kuzma and Besley, 2008).

It has been indicated that although risk assessment is informed by science, it involves normative judgements, such as interpretation of uncertainty, deciding where safety thresholds lie, and determining which endpoints of risks or benefits are of most worthy of consideration (Thompson, 2003; Thompson, 2018; Kuzma, 2019). Therefore, it has been argued for having mechanisms include a more diverse sample of the public, experts and stakeholders in assessing engineered products for procedural justice, so that the normative commitments of a few powerful groups (often technology developers and regulators) do not dominate decision-making (Kuzma and Besley, 2008; Meghani, 2014; Meghani and Kuzma, 2011; Meghani and Kuzma, 2018; Kuzma, 2019). This also depends on who has the right to decide and that decision-making procedures derived from the constitution of the respective jurisdiction should not be overruled and undermine democracy (Purnhagen and Wesseler, 2020).

Uncertainty in determining the risks associated with GM food and agricultural products also represents a potential conflict between libertarian ethics and utilitarian ethics (Thompson and Hannah, 2008). The former proposes that individuals have inviolable rights to be shielded from harm caused by others, even if the risk is extremely low (which is also related to non-maleficence, a bioethics principle), whereas utilitarian ethics considers the benefit-risk balance to a population while allowing for potential risks to a few (Thompson and Hannah, 2008). Rights to know and choose via food labelling of gene-edited and GM products may reconcile the two ethical approaches by placing the responsibility on the consumer (Thompson and Hannah, 2008). Arguments in favour of GM and gene-edited food labelling have also been made using bioethical principle-based approaches (deontological) of autonomy and informed consent (Kuzma and Besley, 2008). Transparency is required in this case, yet currently, in the United States of America and several other countries, most gene-edited foods do not require labelling and little information about them is publicly available (Kuzma and Grieger, 2020).

More fundamental ethical considerations about animal gene editing are that it could violate the dignity of animals, prevent them from living according to their instincts, or affect their behaviour to the point that they lose their essence and purpose (de Graeff *et al.*, 2019). However, given the criticisms of modern animal husbandry, regardless of genetic engineering or gene editing, domesticated animal dignity has already been severely contravened. Kato-Nitta *et al.* (2021) reported that the Japanese were more concerned about gene-edited livestock than gene-edited vegetables. More respondents signalled acceptance of gene-edited vegetables but not gene-edited livestock, those with higher science literacy levels ranking gene-edited vegetables higher than those with lower levels. However, they remained antigene editing when it came to livestock. This could be because humans consider animals to be of intrinsic value, gene editing possibly negatively affecting their welfare if traits leading to disease are introduced. De-animalization would occur were natural traits to be knocked out, and humanization if non-human primates were altered to mimic humans (European Commission, 2021).

A representative EUROBAROMETER survey (European Commission, 2010) conducted among European Union citizens on moral delegation for decision-making asked respondents for their governance preferences using a 2x2 matrix on synthetic biology and animal cloning. Both are applications of modern biotechnology that largely benefit from the CRISPR-Cas technology (Doudna and Sternberg, 2017). Results showed most respondents preferred decisions to be made based on scientific evidence from experts. The results also showed that more than a third of the respondents preferred decisions to be made based on moral and ethical issues, but as these are often based on personal value judgements, there is likely to have been considerable disparity within that group.

At the most fundamental level, some ethical objections to GM and gene editing arise from moral positions on the intrinsic worth of unviolated nature (Bartkowski *et al.*, 2018), while what constitutes nature is not necessarily obvious or agreed on. It is not even universally accepted that naturalness is fundamentally good. There are various views (Ducarme and Couvet, 2020), but if natural excludes anything that humankind has had a hand in, this would rule out most things as being natural – humans are, after all, as much a part of the natural

world as other organisms. Moreover, except for anthropogenic climate change effects, there have always been natural disasters and serious diseases and genetic disorders, which dispel the concept of benevolent nature. For some, moral objections arise from humans assuming the role of a deity by intentionally changing the genomes of plants and animals and going against natural processes that purportedly maintain a balance within species and ecosystems. Counterarguments to this position note that gene editing may simply mimic natural processes (especially SDN-1 and SDN-2 in comparison with transgenesis). Hybridization is also far more common in nature than is generally realized. Furthermore, humans have interfered extensively in breeding systems for millennia, and more lately through conventional breeding (Bartkowski et al., 2018). In addition, the holistic argument that all organisms are part of a finely balanced environment, the disturbance of which is disrespectful, ultimately is reproving of all technological interventions, not just those in agriculture and not solely those concerned with gene editing.

If there is a moral obligation to provide the most deserving communities with gene-editing technologies and products, the new crops and livestock are unlikely to represent a panacea. Maybe the best that can be hoped for is that conflicts among welfare ethics, business ethics and environmental ethics can be resolved, and the adverse effects of current production practices can be reduced, and present-day inequities can be addressed. In this way progress can be made in transforming agrifood systems for the better. Through continued research and provision of information, public concerns about gene editing might be mitigated and those most in need of the benefits of the new technology might receive them.

4 Governance and regulation

SUMMARY

Arguably the most important governance aspect of the products generated by gene editing is sanitary and phytosanitary regulation. Sciencebiodiversity must be balanced against economic and social considerations. To date governments have not adopted a coordinated approach to regulating gene-edited products. Governments must focus on establishing a clear and rational regulatory approach, seeking international harmonization to the extent possible. Only after domestic regulatory protocols are established can policymakers adequately address other governance issues, such as trade impacts, fostering innovation, intellectual property at the multilateral level the diverse governance aspects may be dealt with in different specialized fora, and simultaneous discussions, when necessary, will be possible.

Sanitary and phytosanitary regulations

Regulators have considered how to manage geneedited products in agrifood systems for more than a decade. Because gene-editing techniques use recombinant-DNA techniques in direct or indirect ways, in most cases it is questioned whether the products should be regulated in the same way as transgenic organisms (and derived products).

Most national regulatory systems subject transgenic crops to regulations for genetically modified organisms (GMOs) or living modified organisms (LMOs). The leading multilateral references for these regulatory systems are:

- a) The Cartagena Protocol on Biosafety (CPB) under the Convention on Biological Diversity (CBD), which is based on the concept of LMOs.
- b) Specific Codex Alimentarius Guidelines, based on the concept of recombinant-DNA plant/ animal/microorganism.

However, gene editing can involve different types of intervention in the genome, from transgenesis and cisgenesis to simple mutations consisting of deletions or changes of a few nucleotides in the genomic DNA. Regulatory experts usually classify this array of techniques into SDN-1, SDN-2 and SDN-3 types.

It is generally agreed that SDN-3 interventions, especially when DNA from other species is used, are categorized as GMO, LMO, and recombinant-DNA organisms (Sprink et al., 2016). This results from insertion of foreign DNA into a host genome, which is recognized to generate a "novel combination of genetic material" (term used in the Cartagena Protocol LMO definition, which later inspired many GMO definitions in national regulations). However, at the other extreme of this classification, SDN-1 interventions do not involve, if properly executed, a DNA insertion. They damage the DNA at a target site, and then permit natural cellular repair. Such repair mechanisms can fail at a low frequency, resulting in nucleotide deletions or small random changes and insertions. Such an intervention is very similar, in its general mechanism and possible results, to breeding based on induced chemical or radiological mutagenesis.

Because of the differences in classification, there are differing views on appropriate regulatory treatment of the three gene-editing categories (Sprink et al., 2020). Some argue that SDN-1 derived products do not meet the legal definitions of an LMO/GMO, or the concept of a recombinant-DNA organism, because they lack insertion of foreign DNA into a genome. Conversely, others consider that SDN-1 products do meet the specific definitions used in their national definitions of GMOs; or focus instead on encompassing them under regulations designed for "modern biotechnology" because of the involvement of recombinant DNA at some juncture. A third approach is based on the techniques not having been developed when national biosafety laws and international agreements and guidance were elaborated. In this instance a regulatory update is required to accommodate the products (Eriksson et al., 2019). Qaim (2020) suggested that a traitbased regulatory approach would represent an improvement over product-based regulation of crops. This suggestion was reiterated by Gould et al. (2022).

Figure 2 represents a summary of many of the issues and organizations concerned with governance and regulation of gene-edited organisms and products.

A timeline of national approaches

This section summarizes development of the global regulatory situation for gene-edited organisms for agrifood systems over time, up to July 2022.

In 2013, Health **Canada** and the Canadian Food Inspection Agency published their first food safety and environmental assessments for a gene-edited crop, which was obtained using an ODM technique. The Canadian regulatory approach is based on the characteristics of the final product, regardless of how obtained. As with conventional breeding and recombinant DNA techniques, gene-editing techniques can be used to develop both novel and non-novel traits. Only those gene-edited products deemed to have a novel trait require pre-market safety assessments in Canada. This approach was finally made explicit through guidance published in 2022 by Health Canada.

A **New Zealand** research institute requested the Environmental Protection Authority (EPA) determine how gene-edited organisms were to be regulated. Initially, the New Zealand EPA compared the use of SDNs with chemical mutagenesis. Mutagenesis is included in a list of techniques excluded from regulation as GMOs. Therefore, the EPA interpreted that mutagenesis using SDNs was excluded. However, that decision was challenged in the High Court that same year. The court concluded that the list of exempted techniques in the regulation is of a closed kind and the applicable law would require modification to include gene editing.

In 2014, the **United States** Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) considered whether to allow sowing of gene-edited crops on a case-bycase basis. The current biosafety regulation is not based on a product/process definition; it is triggered by potential risk factors, such as DNA sequences from plant pests being in the final product.

Over time, USDA-APHIS has analysed numerous case-by-case consultations regarding gene-edited products. In most cases, the product was not considered to be governed by the regulatory section applying to genetically engineered plants. The criteria underlying the case-by-case decisions were recently refined and finally incorporated into the Code of Federal Regulations (7 CFR Part 340) in 2020. They are now embedded as a set of explicit regulatory exclusions comprising (a) genetic changes resulting from the cellular repair of a targeted DNA break in the absence of an externally provided repair template, (b) targeted single base pair substitutions, (c) the introduction or reconstruction of a gene already present in the plant's gene pool, and (d) other cases where the modifications are like those that could be achieved through conventional breeding. Conversely, the regulatory standing of gene-edited animals is different because the United States FDA proposed an oversight approach closely following its policy for genetically-engineered animals, which fall under their jurisdiction for "new animal drugs" and go through a mandatory pre-market approval process. The Environmental Protection Agency (EPA) is currently considering how to regulate gene-edited crops under their pesticide authorities if the gene-edited crop contains "plant-incorporated protectants". The EPA has asserted regulatory jurisdiction over GMO plants with incorporated pesticidal proteins or genetic changes.

In 2015, the **Argentina** Ministry of Agriculture, Livestock and Fisheries issued Resolution 176/2015, which introduced a procedure for classifying products from new breeding techniques



Figure 2 Issues and organizations concerned with governance and regulation of gene-edited organisms and products

(including gene-edited organisms) as GMOs or not. The procedure is based on the definition of an LMO from the Cartagena Protocol. According to this definition, an LMO (GMO in the Argentine domestic regulations) "possesses a novel combination of genetic material obtained through the use of modern biotechnology." This resolution does not create a new product category or special regulatory treatment. Subsequently, several geneedited plant and animal lines developed for food and agriculture were classified as non-GMO. In almost every case, the decision was taken because the resulting organism was not considered to possess a novel combination of genetic material. In 2018, Argentina presented a joint statement to the World Trade Organization (WTO) on behalf of a country coalition, which highlighted the potential benefits of applying gene editing to food and agriculture (G/SPS/GEN/1699). It also stated that governments should avoid arbitrary and

unjustifiable distinctions between gene-edited organisms and those obtained using other breeding methods.

In 2017, the Agriculture and Livestock Service (SAG) of **Chile** issued an official clarification on the applicability of its previous Resolution no. 1523/2001 for propagation material developed by new plant breeding techniques (including gene editing). The main attributes of the document were coincident with the Argentine case described. Since then, SAG has received applications to clarify the status of some gene-edited plant lines developed for food and agriculture, most of which have been classified as non-GMO.

The **Israel** Plant Protection Services Administration published a decision by the National Committee for Transgenic Plants, establishing that the progeny of gene-edited plants will not be subject to GMO regulations when foreign DNA sequences are not incorporated into the plant genome. This decision, however, only applied to field trials.

In 2018, the National Technical Biosafety Commission of **Brazil** (CTNBio) issued its Resolution 16/2018. The main features of the document are coincident with the Argentine and Chilean approaches, but the Brazilian legislation includes lists of techniques and genetic interventions that are not considered to produce a GMO. CTNBio has also received several petitions to clarify the regulatory standing of specific plant lines and animal breeds derived from gene editing, the results of which are routinely published in the official gazette.

Colombia notified the WTO of its Agricultural Institute (ICA) Resolution no. 29299/2018 "Setting out the applicable procedure for crops where any stages over the plant-breeding process incorporate innovative phyto-improvement techniques through modern biotechnology and the final product does not contain any foreign genetic material." Its text is like that of the Argentine Resolution 176/2015. Since then, ICA has processed several petitions for gene-edited rice and maize lines, which were ultimately classified as non-GMO.

The European Court of Justice (ECJ) ruled that mutagenesis induced by gene editing produces GMOs according to the GMO definition used in the European Union regulations. According to the ECJ ruling, all organisms obtained by mutagenesis (regardless of the technique used) are GMOs according to the European Union Directive 2001/18/EC, but those derived from mutagenesis techniques, which have conventionally been used repeatedly, and have a long safety record, are exempted from the regulation. During the years that preceded the ruling, several Member States of the European Union received applications and inquiries from developers regarding field trials with gene-edited crops. As a result, advisory bodies and competent authorities of some countries (including Belgium, Finland, Germany, Ireland, Netherlands, Spain, Sweden, and the United Kingdom of Great Britain and Northern Ireland) indicated that field trials with certain gene-edited products were not subject to the GMO legislation. While decisions on field trials are taken at the national level, the commercial authorization process for GMOs (including food safety assessment) is a centralized procedure. Authorization granted therein applies to

the entire European Union. Therefore, the European Commission asked the Member States to withhold from providing interpretations, awaiting clarification at the European Union level. That clarification arrived with the ECJ ruling. In 2022, the European Commission launched a public consultation on "Legislation for plants produced by certain new genomic techniques" to seek stakeholder views on a proposal for a legal framework for plants obtained using targeted mutagenesis and cisgenesis and their food and feed products and a road map was developed for improving the current legislation on gene editing. A proposal by the European Commission is expect in the second guarter of 2023 to be discussed by the European Council, the European Parliament and the European Commission, the so-termed trilogues.

The **Switzerland** Federal Council confirmed, in response to a parliamentary interpellation, that geneedited organisms fall under the definition of GMOs according to the national Gene Technology Act.

<u>In 2019</u>, **Australia** amended its GMO regulation Law to take account of gene editing. The amendment expanded the list of "organisms that are not GMOs" to include those (i.e. SDN-1).

In its Environmental Code regulations, **Ecuador** clarified that organisms not possessing recombinant or foreign DNA are excluded from GMO biosafety regulations.

Guatemala and Honduras signed their bilateral Resolution no. 60/2019, where both countries agreed to harmonize their GMO regulations, linked with broader policies establishing a common market. That resolution settled criteria for distinguishing which gene-edited products should be treated as GMOs and which as conventional new varieties in both countries. The criteria were later implemented domestically by the Honduran National Service of Agrifood Health and Safety (SENASA) in its regulation 8/2019 and by the Guatemala Ministry of Agriculture, Livestock, and Foodstuff through its Agreement no. 271/2019. The implementing regulations are based on a specific definition for "novel combination of genetic material", and the final product characteristics compared with conventional breeding products. They also pay special attention to harmonization with other countries.

Nigeria aamended its National Biosafety Management Agency (NBMA) Act to include emerging agricultural biotechnologies. The amendment defines gene editing as "a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism." Subsequently, in 2020, the NBMA published detailed guidelines for gene editing regulation. When the gene-edited product does not have a novel combination of genetic material, a non-GMO regulatory classification is applied.

The Ministry of Agriculture and Livestock of **Paragua**y published Resolution no. 565/2019, which approves a form for "Prior consultation for products obtained through new breeding techniques." It is very similar to its analogue in the Argentine regulation, while at the same time containing a list of techniques like the Brazilian regulation. The Paraguay National Commission on Agricultural and Forestry Biosafety is responsible for analysing applications using this form, although no case has been presented yet.

In the **Philippines**, the National Committee on Biosafety (NCBP) issued a resolution for the regulation of Plant Breeding Innovations (PBIs), including gene-edited plants, where, once again, products are regulated as GMO or not based on the concept of "novel combination of genetic material." The regulations define the latter as "a resultant genetic combination in a living organism that is not possible through conventional breeding." Consequently, the Department of Agriculture drafted its rules and procedures to determine the regulatory status of PBI products, which were subjected to stakeholder consultations.

In Japan, the Ministry of Environment (MoE) and the Ministry of Health, Labour and Welfare (MHLW) published procedures and guidelines to clarify when their GMO regulations apply to geneedited products. Later in 2020, the Ministry of Agriculture, Forestry, and Fisheries (MAFF) also published implementing guidelines on the same topic. The MoE criteria centre on determining if products fall outside the scope of the LMO definition in the applicable law, which is based on the Cartagena Protocol. It also clarifies that plants that (a) do not have integrations of "extracellularly processed nucleic acids", or (b) only incorporated genetic material that comes from the same or sexually compatible species, are both excluded. Conversely, MHLW criteria state that foods derived

from gene-edited plants presenting the same level of risk as those from conventional breeding are not subject to the GMO food safety assessment process. The MHLW criteria for identifying products that do not require a GMO safety assessment include (a) absence of foreign DNA in the final product and (b) changes induced by a site-directed enzyme that result in deletions, substitutions, or spontaneous insertion of one or more nucleotides. Regarding feeds derived from gene-edited plants, the MAFF guidelines are closely aligned with the approach taken by MHLW over foodstuffs. A locally developed gene-edited tomato with increased gamma-aminobutyric acid content (for health benefits) was the first product to receive confirmation of non-GMO status. Other products followed, including a gene-edited sea bream in which CRISPR technology was used to knock out the myostatin gene. It was also developed by a start-up incubated by a local university. Both the tomato and the fish became commercially available to the public in 2021.

In 2021, **South Africa** legally defined a GMO as "an organism, the genes or genetic material of which has been modified in a way that does not occur naturally through mating or natural recombination or both". Based on that definition, the Executive Council of the GMO Act (administered by the Department of Agriculture, Land Reform and Rural Development) concluded that the risk assessment framework for GMOs would also apply to NBTs (new breeding techniques) and modified its application forms accordingly to allow a tiered assessment approach.

In 2022 in **China**, the Ministry of Agriculture and Rural Affairs (MARA) issued guidelines for safety evaluation of gene-edited plants for agricultural use. The guidelines apply to gene-edited plants into which no exogenous genes are introduced, with differential treatments according to risk levels. For those gene-edited plants whose traits do no elicit food or environmental risks, the guidelines establish a simplified registration procedure with respect to transgenic plants.

The Ministry of Environment, Forest and Climate Change of **India** issued its Memorandum F12013/3/2020-CS-III exempting gene-edited plants falling into the categories of SDN-1 and SDN-2, which are free from introduction of exogenous DNA, from the rules that apply to genetically engineered organisms. This was done following recommendations from the Ministry of Science and Technology, and after requesting public comments to inform on future policies on gene editing. Previously, draft guidelines for regulation and risk assessment were released, comprising both agricultural and human health applications. The guidelines proposed a tiered approach to product groups based on risk. Minor DNA edits were identified as posing a low risk, while large or foreign DNA insertions were considered to pose higher risks. The document makes extensive reference to off-target effects.

Kenya's National Biosafety Authority (NBA) published its gene-editing guidelines, aimed at steering the approach to take while submitting and reviewing applications for research, trials and commercial release of gene-edited products. The main feature of the guidelines is the provision for early consultation to determine the regulatory pathway to be adopted, in view of the different potential outcomes of gene-editing techniques, depending on the case. Prior to publishing the guidelines, the NBA had reviewed several applications using new breeding techniques in contained facilities (biosafety laboratories and greenhouses).

In addition, many other countries worldwide are currently conducting policy-making processes to develop regulatory criteria for gene editing applied to agriculture. In some cases, the content of the processes is recorded in publicly available documents. Such is the case for The **Costa Rica** State Phytosanitary Service, which recently proposed a draft national legal framework for new plant breeding techniques. It comprises a procedure to define whether a crop derived using gene-editing techniques is an LMO or not, in line with other Latin American countries.

Food Standards Australia-New Zealand (FSANZ) is performing an ongoing review of how the binational Food Standards Code applies to foods derived from NBTs. This includes a proposal to amend the binational food code with definitions for "gene technology" and "new breeding techniques". **Norway** is currently following the European Union authorization procedures. However, after public surveys and parliamentary debates, the Norwegian Biotechnology Advisory Board presented recommendations for regulating GMOs, including exempting or expediting the safety assessment of gene-edited organisms. Although the **United** **Kingdom** officially left the European Union in 2020, all the relevant European Union regulations were retained. However, after a parliamentary debate on gene editing, the Department for Environment, Food and Rural Affairs set up a public consultation for updating the regulation of genetic technologies. The government response to the consultation results included an announcement to bring forward new legislation that would amend the GMO definition, excluding organisms having genetic changes that could occur naturally or be achieved through traditional breeding.

Multilateral instruments

Cartagena Protocol

The Cartagena Protocol on Biosafety is an international treaty governing the transboundary movements of LMOs. It is a supplementary agreement to the Convention on Biological Diversity, and it entered into force in 2003. It currently has 173 parties, most of which base national regulations on the language used in the Protocol.

The CPB contains provisions regarding the procedure for the first transboundary movement of an LMO into a country, including the risk assessment of its potential adverse effects on the conservation and sustainable use of biodiversity. It also considers risks to human health.

Two definitions in particular under the Protocol serve as a paramount reference to regulators on establishing if this treaty and related domestic legislations apply to gene-edited organisms:

- Living Modified Organism is any living organism that possesses a novel combination of genetic material obtained by modern biotechnology.
- 2. Modern biotechnology means the application of a) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells, or b) Fusion of cells beyond the taxonomic family that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.

Parties to the CPB and its parent agreement, the CBD, hold periodic meetings to negotiate

implementation aspects of the treaties. Gene editing began to be discussed in the concurrent meetings that took place in Sharm El-Sheik, Egypt, in 2018. Two decisions of the parties arising from those meetings explicitly refer to gene editing:

- Decision on horizon scanning (CBD): "{...} new technological developments in synthetic biology ... including ... concrete applications of genome editing if they relate to synthetic biology".
- Decision on Risk Assessment (CPB): "Calls for broad international cooperation {...} in assessing the potential adverse effects ... from LMOs produced through new developments in modern biotechnology, including LMOs developed through genome editing {...}".

The language in the decisions recognizes that some development of gene editing could be considered LMOs and/or synthetic biology.² However, it recognizes that some other applications may not.

Codex Alimentarius Guidelines

The *Codex Alimentarius* (Codex) is a collection of standards, guidelines and codes of practice for foodstuffs. It is elaborated by the Codex Alimentarius Commission (CAC), which is an intergovernmental body with more than 180 members. CAC is the central component of the Joint FAO/WHO Food Standards Programme, fully established by FAO and WHO in 1963 to protect consumer health and promote fair practices in food trade.

Codex comprises some general food guidelines and others that apply to specific foods, including "foods derived from modern biotechnology". For this purpose, Codex adopted the definition of Modern Biotechnology from the CPB. In contrast, the "Living Modified Organism" concept from the CPB is not used in Codex guidelines. Instead, the guidelines refer to "foods derived from recombinant-DNA plants, animals, or microorganisms", without providing a definition.

Codex guidelines for foods derived from modern biotechnology were published several years before

governments began considering the regulation of gene editing and have not been reopened to discussion afterwards. However, the guidelines are widely used worldwide and have proven robust and valid for new developments in modern biotechnology and recombinant-DNA organisms.

Codex guidelines that apply to foods derived from modern biotechnology include:

Guidelines on food safety assessment, providing overarching principles on the risk analysis of foods derived from modern biotechnology and detailed guidance for the safety assessment of foods derived from recombinant-DNA plants, animals, and microorganisms. Specifically, they are the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CXG 4+003) and the Guidelines for the Conduct of Food Safety Assessment of Foods Derived from recombinant-DNA Plants (CXG 45-2003), Animals (CXG 68-2008), and Microorganisms (CXG 46-2003). The three latter guidelines state that, while they were designed for foods derived from recombinant-DNA organisms, the approach they describe could generally be applied to foods derived from organisms that have been altered by other techniques.

Guidelines on labelling comprise the *Compilation* of *Codex Texts Relevant to Labelling of Foods Derived from Modern Biotechnology* (CAC/GL 76-2011), providing guidance from earlier Codex texts of broad application, which are also relevant to the labelling of foods derived from modern biotechnology.

Guidelines on analytic methods, include the Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods (CAC/GL 74-2010). These guidelines provide information on criteria for the validation of food analysis methods involving the detection, identification, and quantification of specific DNA sequences and specific proteins of interest that may be present in foods, including those foods containing materials derived from modern biotechnology. The guidelines apply to a wide range of biomarkers in foods, including the detection of foods derived from modern biotechnology and food speciation. It is clear now that the methods of analysis based on DNA sequences covered by the guidelines are applicable to detect foods derived from gene-edited

² Current operative definition of synthetic biology in CBD negotiations is "a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/ or modification of genetic materials, living organisms and biological systems."

organisms, from SDN-1, SDN-2 and SDN3 if the analyst has information on the exact change made to the DNA sequence of the organism.

Other general guidelines. There are many other Codex guidelines of general applicability that may apply to foods derived from gene-edited organisms, regardless of their regulatory classification. These include the Guidelines on the Judgement of Equivalence of Sanitary Measures Associated with Food Inspection and Certification Systems (CXG 53-2003), which is helpful when countries have different classification systems or regulatory approaches for gene-edited products. The Principles and Guidelines for the Exchange of Information between Importing and Exporting Countries to Support the Trade in Food (CXG 89-2016) may help to facilitate communication to clarify the presence of the products in international shipments. Finally, the Guidelines for use of Nutrition and Health Claims (CAC/GL 23-1997) may be used in connection with leading cases of gene-edited crops intended to provide health and nutritional benefits.

The International Plant Protection Convention

The International Plant Protection Convention (IPPC) provides a system of standards and procedures for identifying pests that threaten plant health, assessing their risk, and determining the strength of measures to be used against their introduction and spread. Under the IPPC, most countries have established regulatory organizations experienced in assessing and managing the risk of pests that threaten plant health. Albeit of broader scope, IPPC risk analysis and management systems are appropriate for assessing and managing, if necessary, the direct or indirect risks of pests to cultivated and wild flora and plant products that may be presented by LMOs and products of modern biotechnology.

The Organisation for Economic Co-operation and Development

The Organisation for Economic Co-operation and Development (OECD) and its member countries have been addressing issues related to biotechnology since 1982. Its *Working Party on Harmonisation in Biotechnology* and the *Working Party for the Safety of Novel Foods and Feeds* focus on environmental and food safety issues, respectively. Their guidance documents usually provide complementary and more detailed content on the issues covered more broadly by the multilateral fora mentioned earlier. Different OECD areas also cover other relevant governance issues. New topics often arise faster in the OECD than in the Cartagena Protocol or Codex, which was also the case for gene editing. The OECD has already organized a workshop focused on the agricultural applications of this technology and its procedures constitute a valuable source of information for regulators. Additionally, the OECD regularly publishes regulatory updates at the country level, compiling reports by its member countries.

Other key issues

Identification

A seemingly simple but fundamental issue has not been resolved for gene-edited products: a universal nomenclature. Currently, the same product is often presented under dissimilar (technical or commercial) names according to different governments, in technical publications and more with important implications for international trade (Punt and Wesseler, 2016).

For the transboundary movement of living organisms, derived products and food speciation, sworn statements, databases, and governmental exchanges are based on standardized scientific names (binomial nomenclature) when referring to the species that features in a product. For GM plants to date, the OECD Unique Identifier for Transgenic Plants is the internationally recognized denomination for regulatory and technical purposes. Therefore, for gene-edited organisms that are transgenic plants, the OECD identifiers are applied. However, some governments may consider an SDN-1 mutant not to be a transgenic plant, but still require that the developer use an internationally recognizable identification code. In this regard, it is interesting to consider the FAO/IAEA (International Atomic Energy Agency) database on plant mutants. This database currently registers mutants obtained using radiation, chemical mutagens, and the biotechnology-based mutagenesis technique of somaclonal variation.

Off-target changes and unintended insertions

Gene-editing techniques aim to target genomic locations precisely and effectively generate a desired sequence change. However, they can occasionally lead to unintended results, including off-target changes and unintentional DNA insertions (see section 2). Off-target changes in genomic DNA sequences may occur if the target sequence (or a similar one) is also present elsewhere in the genome. An off-target change in the DNA sequence of the host organism, in turn, may or may not lead to a significant difference in phenotype or safety. The off-target issue is new to SDN techniques. Therefore, it was not anticipated in existing international guidance, and scientific literature to date presents a plethora of diverging results that may confuse regulators in the absence of standardized criteria to address the topic.

On the other hand, unintentional DNA insertions result from nucleic acid fragments incorporating into the host cell, purposely or not, during a gene-editing procedure. Even gene-editing techniques conceived as "DNA-free" have resulted in the integration of foreign DNA inadvertently introduced in small amounts. The insertions can occur anywhere in the genome, not only off/on target sites.

The issue of searching for unintentional DNA insertions is not new in the safety assessment of transgenic and GM organisms. However, regarding gene-edited organisms, it may have an additional significance for certain countries when deciding if a particular gene-edited organism is to be regulated as a GMO or not (Eriksson *et al.*, 2019; Lema, 2021).

Property, access and benefit sharing

Governance of property, access and benefit distribution in connection with plant varieties, animal breeds, and microbial strains is governed by various international treaties.

Access and benefit sharing

Over recent decades, there has been intense general debate over the access to genetic resources and the sharing of benefits derived from their utilization. This includes not only the use of plant varieties, animal breeds, and microbial strains unmodified and as they were found in nature or the market. It also involves varieties obtained by any breeding method. Because gene editing enables direct editing of genes in elite breeding lines or commercial varieties and eliminates the need for backcrossing to introgress a trait from a non-elite or wild relative trait donor (Pixley *et al.*, 2022), it reduces the need for access to, and utilization of genetic resources in breeding, which are regulated by access and benefit-sharing frameworks.

Two paramount international agreements currently assist countries in the governance of access and benefit-sharing in agriculture. The *International*

Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity. The ITPGRFA objectives are the conservation and sustainable use of all designated plant genetic resources for food and agriculture, as well as promoting fair and equitable sharing of the benefits arising from their use.

The ITPGRFA entered into force in 2004. It aims to establish a global system to provide farmers, plant breeders and scientists with access to plant genetic materials, ensuring that recipients share benefits they derive from using those genetic materials with the countries of origin. The treaty prevents the recipients of genetic resources from claiming intellectual property rights over those resources in the form they were received and ensures that access to genetic resources already protected by international property rights is consistent with international and national laws. The ITPGRFA aims to enable a global pool of genetic resources from 64 crops, which account for 80 percent of all plant-derived foods, to be available to potential users among the 150 State parties to the treaty under standard terms and conditions that, on the one hand, facilitate access to samples of genetic material for research and breeding and, on the other, ensure the equitable sharing of the monetary and non-monetary benefits resulting from research and breeding.

The Nagoya Protocol is a supplementary agreement to the CBD. It aims at sharing the benefits arising from the utilization of genetic resources from all biodiversity fairly and equitably, contributing to the conservation and sustainable use of biodiversity. The Nagoya Protocol also covers traditional knowledge associated with genetic resources and the benefits arising from its utilization. It is important to highlight that "utilization" in this treaty is understood to include research and development on the genetic or biochemical composition of genetic resources, as well as subsequent applications and commercialization. Benefits may be monetary or non-monetary, such as royalties and the sharing of research results. The Protocol entered into force in 2014 and currently has 138 State parties. It aims to foster a transparent, fair and predictable legal framework for international access to local genetic resources. Its provisions aim to help ensure benefit-sharing when genetic

resources leave the country, provide incentives to conserve and sustainably use genetic resources, and enhance biodiversity's contribution to food security and human well-being.

The constituencies of these two agreements are currently discussing the access and benefit-sharing obligations using "digital sequence information" without recourse to the physical genetic materials.

Intellectual property

The World Intellectual Property Organization (WIPO) administers several treaties on intellectual property, including the Patent Law Treaty (PLT), which entered into force in 2005. Additionally, the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement), which entered into force in 1995 under the WTO, is also of great significance. However, these multilateral instruments do not provide standards for the patentability of specific technology fields. In this regard, certain biotechnology developments have been particularly challenging, facing different criteria depending on national context.

Factors that led to questioning patentability in several cases included inventions incorporated into self-replicating entities (such as seeds), patenting of DNA sequences (and other biological elements) that can be found in nature, the sufficiency of disclosure on processes not fully understood by biologists, replicability of processes incorporating a random factor (such as earlier mutagenesis methods), and outcomes perceived to be contrary to morality or regulations (such as those impacting on animal welfare). The patenting of gene-editing technology has also been criticized for having the potential to increase monopoly power, disrupting the market for animal and plant breeding (Then, Bauer-Panskus and Tippe, 2021).

New plant varieties are protected either by patents and/or *sui generis* systems, depending on each country's criteria. The multilateral agreements used as a reference for establishing national protection frameworks are the acts of the *International Union for the Protection of New Varieties of Plants* (UPOV). Some national regulations are updated to the (latest) 1991 Act, while others still have rules in line with the act prior to 1978. This discrepancy leads to a significantly different treatment of key issues like protection of essentially derived varieties and the impact of breeders' and farmers' rights on the subsequent use of a variety after the initial seed purchase (Rapela, 2019; Yu and Chung, 2021).

Gene-edited products considerations

To date, gene-editing applications for agrifood systems have not made a significant appearance in the meetings of the parties discussing the implementation of these treaties, and there are very few references in the specialized literature. However, new varieties/breeds/strains obtained using traditional breeding methods and GMOs have been extensively discussed, with numerous study cases and *ad hoc* national guidelines being produced.

Attending to scientific and (sanitary) regulatory considerations, gene-edited products may be considered either GMOs or, in some countries, equivalent to mutant breeds obtained by other means. Therefore, prima facie, gene editing may not result in opening a significant new chapter in the governance of intellectual property and genetic resources because gene-edited products could be accommodated by rules applied to preexisting product categories. For instance, the patentability or variety registration of an SDN-1 mutant maize line obtained by CRISPR could be the same as applied previously to a new variety obtained by radiation breeding. In addition, if the breeder mutated a pre-existing variety owned by another developer, the relative rights of the original developer and the mutation breeder could be solved as already customary in that specific country for essentially derived varieties (Graff and Sherkow, 2020). Moreover, suppose the base genetic resources used by the breeder fall under the access and benefit-sharing systems mentioned. In that case, the breeder may have obligations regarding disclosure of the resource origin, mutually agreed terms with the resource provider, and sharing of derived benefits. However, once again, there may be no significant difference in the obligations, or how they would be implemented, as if the mutation were obtained using radiation, chemicals, somaclonal variation, or spontaneous mutation. Similarly, a transgenic plant (and derived foods and other products) obtained through an SDN-3 gene-editing procedure may or may not be protected using the same criteria that a country had developed for transgenic organisms to date.

Patents and gene-editing methods

In contrast to the patenting of organisms and derived products, where gene editing is not

expected to introduce significant issues, the patenting of the gene-editing methods has already raised attention (Gehrke, 2019). This mainly focuses on the different players who struggle to own the technology, their license agreements, innovations to obtain new patents, etc. Some research organizations have already announced that they will, under certain conditions, make the patents they own freely available. This includes the Broad Institute (Graff and Sherkow, 2020) and Wageningen University (Sikkema, 2021). Many of the patents enter licencing agreements as with the rapid development in RDN technologies, developers often having to rely on patents owned by other organizations where cross-licensing of patents have become common (Schenkelaars et al., 2011). Moreover, in certain countries, the randomness of mutation breeding and of (transgenic) transformation events have been considered an obstacle to their protection by patents. However, SDN-1/2/3 can achieve the same results with less or no randomness, which may increase their acceptability by patent offices (Rapela, 2019; Graff and Sherkow, 2020).

Economic interventions

Innovation funding

Innovative breeding techniques, including gene editing, are recognized as an emerging area of bioeconomy research, for example to increase plant carbon sequestration and help mitigate climate change (Trigo *et al.*, 2021). Consequently, several governments have announced public funding to support the development of gene-edited products for food and agriculture. Synthetic biology is often considered in bioeconomy sources (Gomez San Juan and Bogdanski, 2021), and gene editing in various national bioeconomy strategies. However, an appropriate regulatory framework is more fundamental to fostering gene-editing innovations than the availability of governmental subsidies (Wesseler *et al.*, 2022). This is especially relevant for developing countries with little to invest, and for international agencies that may offer funding to foster gene-editing innovations (Smith *et al.*, 2021). Additionally, the importance of sustainability criteria in investments is growing rapidly. Among the bioeconomy indicators used by different sources, gene editing provides information on the quality and the safety of a bioeconomy product (Bracco *et al.*, 2019).

Trade interventions

In the past, trans-Atlantic asymmetries in the use and regulation of certain biotechnologies have been linked with actual or potential trade disruptions and disputes, as in the case of veterinary hormones, GMOs and animal clones (Smith, Wesseler and Zilberman, 2021). A similar situation may be developing with gene-edited organisms. Specifically, a developer may obtain approval for cultivation in a certain country, but not get approval for food import in another because of asynchronous approvals or differential regulatory treatment. Differential labelling rules may be also a significant asymmetry (Punt and Wesseler, 2016). Moreover, declarations have been presented to the WTO, in which certain governments pledge others to cooperate, to avoid situations that could lead to a different regulatory treatment across geographies (Pixley et al., 2022). Differential regulatory treatment can lead to potential trade disruptions, the mere possibility of which could hamper development of gene-edited products, as has already occurred with GMOs (Smith et al., 2021).

5 Roles of the private and public sectors and transformative partnerships

SUMMARY

Both the private and public sectors engage in agricultural research, although focus and incentives can differ. Private sector research is generally more directed to products that are marketable, and consequently profit-making, whereas public sector research is often less constrained and allows greater academic freedom. Issues of ownership of technologies and products can be problematic, and the motives of private enterprise have sometimes been questioned. However, many of the aspirations of public and private organizations are compatible and collaboration between the two sectors can be beneficial.

Gene-edited organisms and products have been researched in both the private and public sectors and some ground-breaking discoveries have been made in each (Tramper and Zhu, 2011). Frequently, the private sector is involved in the final stages of the development process, translating research into a marketable product (Barrett et al., 2022). This often relies, however, on work done in the public sector, including that done in universities and research institutes, and can engender publicprivate partnerships (Rausser, Simon and Ameden, 2000). This can take place at an informal level, as reported for CGIAR, where researchers customarily exchange germplasm among colleagues (Louafi and Welch, 2021), contributing to substantial gains from research (Alston, Pardey and Rao, 2022). Consequently, gene editing represents a potential transformative technological breakthrough, and it is important for all stakeholders, including local and international researchers, policymakers and the public, to understand the innovation trajectories, who finances them, who bears the risks and rewards of innovation, and for whom technologies are ultimately developed (Fajardo-Ortiz et al., 2022).

An important responsibility assigned to the public sector in many countries is oversight of safety protocols arising from developments in plant and animal breeding, shaping markets and shouldering the risks of early transformative research investment (Fajardo-Ortiz *et al.*, 2022). Numerous countries have agencies that assess food safety and biosafety aspects of new products. This requires collaboration with the private sector and sharing of information. The collaboration with the private sector and the role and tasks of the public and private sectors for ensuring food and biosafety has frequently been controversial.

Public sector

Much of the innovative work underpinning the advances made in modern biotechnology has been done by scientists working in the public sector, public sector funding being instrumental in important areas of research. Informal exchange among scientists in conferences, writing joint papers, and discussing and reviewing research of colleagues has contributed significantly to the development of gene-editing organisms and products (Doudna and Sternberg, 2017; Heimann, 2018). Those involved also reported on the importance of academic freedom, the significance of funding, and the possibilities to draw on human capital.

Early on, public sector researchers involved in developing gene-editing technologies recognized the societal challenges their research would face. To address the challenges, Jennifer Doudna, the Nobel laureate, organized meetings following the example that had been set by the Asilomar conferences (following the creation of synthetic DNA molecules using rDNA technology) where ethical and legal issues stemming from gene editing were discussed. The discussions not only included public sector scientists but also involved regulators, representatives from academies of science and others, and resulted in funding guidelines that public and private sector researchers comply with to receive public sector research funding. Examples include the funding requirements under the European Union Horizon Europe programme,³ the Dutch Top Sectoren funding,⁴ and United States Agency for International Development (USAID) funding.⁵ Private sector research funding is often sought, larger research projects needing to draw on support from a variety of sources. Ultimately, the funder setting the highest standards is the one the research project must comply with.

The public sector has established both an informal and a formal system governing the safety of gene-editing research (see section 4). The safety standards governing the application of rDNA technologies were summarized in the Blue Book of the OECD (OECD, 1986) and set the safety standards that are followed by many countries (Schiemann, 2006). The initiative supported by OECD was a follow-up to the Asilomar conference, where guidelines for laboratory work were established. The safety standards are regularly updated and cover the latest developments in modern biotechnology.

Nevertheless, the public sector research system and its incentive apparatus for professional advancement has resulted in deception in some cases, researchers having fabricated data (van den Belt and Keulartz, 2007). In other cases, ethical boundaries have been tested and breached (Mallapaty, 2022). Inevitably, public sector researchers have sometimes become victims of the controversies associated with GMO and gene-editing technologies (Dubock, 2014; Robinson, 2022).

Private sector

Private sector research has played an important role in modern biotechnology development. However, private sector research is targeted more towards product development than towards scientific advance. It is generally the case that private sector funding is more focussed than that of the public sector.

The private sector plays an important role in developing products for markets, with the main incentive to generate profitable products. For the products to be profitable however, as they have been for seeds of improved varieties for instance, prices and sales must be acceptable. One of the important benefits of gene editing and CRISPR-Cas systems is the substantial reduction in research costs for developing new products in comparison with using traditional, time-consuming, methods. Employing gene editing, the time needed to develop a new crop with improved traits is reduced considerably. Some scientists consider this to be a democratization of science because more researchers have the possibility to conduct research and develop products with new traits. Researchers in countries where budgets are tighter could benefit relatively more with decreasing returns to scale. Nevertheless, the democratization of science does not solely depend on simplifying gene-editing processes and discussion continues about the motives of big business.

Investment costs include not only the costs of conducting the research, but also the costs for getting a product on to the market. This has implications for the market structure of the plant breeding sector (Deconinck, 2020; Wesseler, Jongeneel and Purnhagen, 2019). Often larger plant breeding companies are only able to shoulder the investment costs, increasing their market share over time. The profits a company can make depend on the extent of the market: being able to market seeds to farmers of a country where there are large areas of uniform environment, and relatively many farmers, is much more attractive than attempting to provide to a smaller, niche clientele. It is moreover, from the private sector point of view, much more

³ https://research-and-innovation.ec.europa.eu/funding/fundingopportunities/funding-programmes-and-open-calls/horizon-europe_en

⁴ https://www.topsectoren.nl/

⁵ https://www.usaid.gov/work-usaid/find-a-funding-opportunity

economically sound to develop technologies, and provide products from their application, to major crops such as wheat, maize and rice, than to attempt the same for minor crops, which are, axiomatically, grown over smaller areas. The differences in market size and related investment costs for adopting gene-editing technologies also explain why not all plant breeding companies favour changes in legislation that would lower market entry costs (Wesseler, Jongeneel and Purnhagen, 2019).

Differences in the approval costs are often countryspecific and can have a substantial effect on investment incentives (Wesseler et al., 2022). This explains why the private sector has keen interest in the regulation of gene-edited products. If gene-edited crops are GMOs in the European Union, which they currently are, they must follow the approval processes for GMOs, and related labelling and tracking and tracing rules apply. Many authors have concluded that this will make cultivation of gene-edited crops almost impossible in the European Union and imports of gene-edited crops very expensive (Purnhagen and Wesseler, 2020; Nationale Akademie der Wissenschaften Leopoldina, Deutsche Forschungsgemeinschaft und Union der Deutschen Akademien der Wissenschaften, 2019). The European Union has recognized the problem and has set a process in motion for revising the regulations for gene-edited organisms (European Parliament, 2021). Several other countries have also adjusted, or are in the process of adjusting, their regulatory frameworks with the expectation of stimulating investments in gene editing (see section 4).

This investment perspective does not exclude the possibility that individuals and companies in the private sector are solely driven by profit motivation. Many private sector companies are concerned with delivering products that provide societal benefits, such as better nutrition or health, although private sector altruism has limits. Geneedited products that have reached the market are present in products such as seeds, food, animals and microbes (e.g. making vat-based protein and cheese). The development of such products, especially with respect to the regulatory process, is often very costly. To be able to recover the costs, the private sector often secures investments by protecting their work with patents. Patents allow the owner of the patent exclusive rights for a set period. This excludes others from using the patent-protected product without the consent

of the owner. The owner gains monopoly power over the product for a set time. Patents, however, are increasingly being replaced by trade secrets that rely on getting to market fast, with exclusive rights to the technology (Lassoued et al., 2021). Concerns have been raised that this will allow seed and pesticide companies to exploit farmers by charging high prices. Empirical studies investigating the distribution of rents from improved seeds in the United States of America show that farmers gained more than 50 percent of the rents while about 30 percent went to the technology provider, and the remaining part to consumers and others (Falck-Zepeda, Traxler and Nelson, 2000). Furthermore, pricing power was shown to be limited by the alternative technologies available for crop production. Farmers have the option to buy the improved seeds (Weaver and Wesseler, 2004). Such situations characterize countries where the institutional environment secures property rights and law enforcement is well established, preventing elites from exploiting their powers. It does not necessarily hold for all countries (Acemoglu and Robinson, 2019).

Some previous experiences regarding the role of private companies in developing seeds of high yielding crops could be informative for how geneediting technologies might impact farmers. Pray et al. (1991), with reference to private sector seeds for India, said that there is "widespread belief that small-farmer subsistence agriculture in developing countries cannot sustain a commercial private breeding industry for food crops." At that time, the private seed sector (comprising 17 major companies) spent as much on research and development as the Government of India, but the privately produced crop varieties were higher yielding than those produced by the public sector because the private sector research was more carefully directed. He suggested that artificially low prices of public sector seeds encouraged a false economy and that farmers were better off buying the more expensive, privately produced, seeds. He went on to explain how the benefits from private research were largely reaped by farmers despite the private companies only capturing six percent of the benefits of their own research. They were happy nonetheless because their internal rates of return were sufficiently high. This example illustrates that competition between the public and private sectors is not as straightforward as it might initially seem, and that private industry incentives can be compatible with small-scale farmer needs and circumstances.

Intellectual property considerations

A more general debate has emerged with respect to assigning property rights to seeds. The International Union for the Protection of New Varieties of Plants (UPOV) is an intergovernmental organization that supports the protection of plant breeders' rights. UPOV was established by the International Convention for the Protection of New Varieties of Plants (UPOV Convention: adopted on December 2, 1961). The UPOV convention does not cover patents, which were introduced at a later stage. Specific activities are patented and if used for seeds, the seeds are also protected by the patent. This has been guestioned. Is breeding a new plant an innovation that did not exist before, and hence patentable? One of the landmark decisions was that of the Harvard mouse, the first patented mammal. The mouse was developed by researchers at Harvard University with funding from DuPont. Harvard gave the company exclusive rights for commercializing any inventions resulting from the research. The patentability of the transgenic mouse set the precedent for patentability of transgenic plants and for private sector funding of public research via exclusive licensing of the results, such as the Novartis deal of UC Berkeley.

Some regions have more restrictive rules on patents based on moral reasoning. Article 53(a) of the European Patent Convention states that "inventions, the publication or exploitation of which would be contrary to *ordre public* or morality are excluded from patentability." This has set some limits on patents for seeds and crops (Gehrke, 2019).

If seeds are protected by patents, it can negatively impact farmers because they are not permitted to save seeds, or only a limited amount. Consequently, they must buy new seeds each year, especially if patented genetic use restriction technology, which stops harvested seeds germinating, is applied commercially (Kabir et al., 2022; Ohlgart, 2002; Lombardo, 2014). Important research is increasingly being done by large multinational corporations, which control usage through patents, and lately trade secrets, which are even more restrictive and could potentially limit the use of advanced technologies in agriculture (UNCTAD, 2021; OECD, 2001). However, given the costs associated with regulatory procedures, it is often only multinationals that can afford to commercialize products. Furthermore, there remain

significant barriers to smaller developers and farmers represented by patents and ownership surrounding CRISPR technologies and gene-edited seeds (de Wit, 2020). Consequently, opponents to patenting of plants argue that farmers will become more dependent on seed companies, which deprives farmers of the right to access their proprietary seed and the ability to not only select them but also to produce, store, use, exchange and sell them. Yet, these seeds are of great importance to farmers because they often represent the bedrock of smallholder seed systems (Agriculture Food, 2017; Goray and Bessa, 2019). However, farmers retain the right to continue using their ancestral seed stocks or buy seed from other sources. Those in favour of patent/variety protection of seeds argue that the lack of protection disincentivizes bringing better seed varieties to market, thus leaving farmers at a disadvantage in comparison with farmers in countries where the situation is improved because Intellectual Property (IP) is protected. In the case of GMOs, some private sector seed companies have not pursued IP litigation in LMICs and a number of clearinghouse mechanisms have been developed to facilitate access to IP-protected technologies for LMICs (Graff, Roland-Holst, and Zilberman, 2005). This line of reasoning is not limited to patents. Also, the protection of plant breeders' rights limits the reuse of seeds. Nevertheless, certified seeds are often of higher quality and represent an economic incentive to purchase new seed. Hence, this is a more general issue that is independent of the breeding technology applied.

Public-private and transformative partnerships

The links that have been established through research funding and property rights between public and private sector organizations have resulted in numerous transformative partnerships (Alston, Pardey and Rao, 2022). Such partnerships can contribute to transforming agrifood systems towards improved sustainability (Alston, Pardey and Rao, 2022; Trigo et al., 2021). In these transformative partnerships, the private and public sectors work together and use their comparative advantages to increase the efficiency of transforming agrifood systems. Examples include cyanaophycin-enriched transgenic tobacco plants (Huckauf et al., 2022), insect resistant Bt cowpea in Africa (GAIN, 2022), a virus-resistant potato with an industrial tuber starch quality (Glais, Bellstedt and Lacomme, 2017), microalgae-based production of industrially relevant mycosporine-like amino acids and replacing trans-fatty acids in oil seeds (Zambelli, 2020).

Private sector investment in agricultural and food research and development (R&D) is growing more rapidly and is proprietary, creating additional costs on farms compared with public R&D. Additionally, the formation of CGIAR led to an increased participation of the private sector in global agricultural and food R&D as a response to changes in the policy and practice of intellectual property or market structures throughout the food value chain, in addition to new innovations that have made research benefits more appropriable by private investors. More specifically, private sector spending on agricultural R&D increased from 32 percent in 1980 to 50.4 percent in 2015 (Alston, Pardey and Rao, 2022). However, much of the private sector spending on agricultural R&D is concentrated in high income countries (52.5 percent in 2015) and is less pronounced in the LMICs (private sector share of 12.3 percent in 2015). This can be attributed to the farming systems that are unattractive for private sector investment such as the small farms, low usage of purchased inputs and the limited post-farm food logistics, food processing, and demand for foodaway-from-home (Alston, Pardey and Rao, 2022). This has consequently limited the role of the private sector in agricultural R&D for the LMICs. The public sector often has a comparative advantage in conducting unconstrained research, while the private sector's comparative advantage is in more directed research and delivering the product to market. Examples of public-private partnership include the collaboration in the international research centres of CGIAR (Alston, Pardey and Rao, 2022). The overall expectation is that the partnerships generate more funding for addressing societal challenges as, for example, detailed in the SDGs (Di Sibio, 2022). Other transformative partnerships have developed that support public communication on gene editing. However, public and private sector partnerships have limitations because of differences in focus, especially when it comes to high-end technologies, high profit-margin areas and crops, and the perceived mistrust, lack of transparency and non-adherence to agreement among partners (Trotsenko and Slukin, 2020). Additionally, public-private-producer partnerships are increasingly becoming very important in areas of agricultural innovation that were once predominantly public- or private-sector domains

(Rausser, Simon and Ameden, 2000). For example, Australia's system of end-point royalties for wheat varieties, as a mechanism for implementing and enforcing plant breeder's rights, has led to the birth of public- private-sector partnerships because the seed companies that collect the royalties comprise of a mix of private, producer and public-sector partners (Alston and Gray, 2000).

The role of public-private partnerships in transforming agrifood systems through geneediting technologies may, however, be hampered by the discrepancies in regulatory frameworks. For instance, some countries in Latin America and the United States of America view gene-edited organisms as being distinct from GMOs, while others, including South Africa and the European Union, view them as being like GMOs and therefore subject to the same stringent regulations governing GMOs. This, in turn, limits development, release and adoption of gene-edited crop products in LMICs (Falck-Zepeda et al., 2022; Mayet, 2022). Additionally, many partnerships encounter financial constraints during implementation because of the insufficiency of market assessment and feasibility studies, which leads to longer payback periods and lower returns to investments. Ultimately, this challenges survival of many partnerships (FAO, 2016).

Identifying and managing conflicts of interest in public-private partnerships

Public and private partnerships face diverse problems that originate from differing incentive systems. There is potential for conflict between the public and the private sectors. More specifically, the public sector has a keen interest in development and adoption of technologies and maintains a very strong interest in controlling and ensuring the safety of new technologies. The private sector, however, has interests mainly centred on maximizing profits (Byiers et al., 2016). The private sector often views safety regulations as generating additional costs. The incentives of the private sector are to keep costs to a minimum, but this has not necessarily always been the case. The public sector advocates stringent ex ante safety regulations while the private sector would prefer to see less stringent safety regulations. From an economic perspective, both viewpoints must be considered. If only public sector regulatory activities were operational, a stringent ex ante regulatory system could affect the incentives of the private sector to invest, which could result in

reduced benefits. If safety regulations were left to the private sector only, the safety measures might not be optimal from a social perspective. Public sector regulators could face the problem of striking the optimal balance between *ex ante* regulations and *ex post* liabilities (Lema, 2019; Shleifer, 2010).

Regarding gene editing, it has been argued that the *ex post* liabilities for society are close to zero and that the technology is substantially overregulated, which reduces incentives for investment (e.g. Eriksson *et al.*, 2018; Wesseler *et al.*, 2022). This is an important issue also for public-private partnerships. The distribution of the risk between the public and the private sectors could result in costs being socialized and benefits privatized. Many public sector organizations that have developed patented gene-editing technologies have entered into licensing agreements with private sector companies (Contreras and Sherkow, 2017). Such

agreements may exclude others gaining access to the technologies. To avoid exclusion of patent users from LMICs, several patent owners made patents freely available (Wageningen University in 2021 and the BROAD Institute in 2017). These types of agreement are not without problems, especially when they result in patent infringements. The liability for possible damages can become a problem. Another question being raised is why public sector financed research results are patented and are not automatically freely available. The advantage of patenting public sector research is to maintain control over a technology and avoid privatization of the technology by the private sector (Scheinerman and Sherkow, 2021). Nevertheless, legal issues concerning patent ownership are to be expected, as illustrated by the struggle over CRISPR-Cas related patents. This could result in conflicts over property rights and licencing fees and their distribution.

6 The way forward

Agriculture is the cornerstone of human existence, but it is facing hitherto unmatched pressures. Hunger, malnutrition and environmental erosion are among the consequences of the pressures, and are increasing in magnitude as the pressures build. These issues represent a **problem** that science and society must address if all people are to be able to have regular access to sufficient high-quality food to lead active, healthy lives.

The **causes** of the current problems are not new, but they have grown in intensity and importance over recent years. Climate change is among the most potent of the forces that currently affect agrifood systems; severe and often unpredictable weather events taking a substantial toll. Excessive heat and drought, as well as heavy rainfall and inundation, make it impossible for agriculture to function. Such extreme weather events lead to erosion of agricultural land and force demographic changes, large numbers of people having to leave their homes, many in already marginal areas of production, because their sole means of livelihood vanish. The climate effects are compounded by the spread of disease, the recent COVID-19 pandemic being a particularly calamitous example. Conflict is also frequently an aggravating factor that stimulates migration, as well as being destructive in its own right.

The **challenge** for society, and scientists in particular, is to develop solutions to the problems that ease the burden on those most affected by the problems. Interventions must be made that reduce the severity of the effects of climate change, conflict and displacement, among other forces, but which do not, inadvertently, inflict more damage.

Past efforts to increase agricultural production and address issues of hunger, malnutrition and inequity have been many, but arguably the most impactful was the Green Revolution. This represented the application of knowledge of genetics to breeding more productive varieties of wheat and rice, in the main. The benefits derived from sowing the high-yielding varieties were numerous in terms of reducing hunger, promoting human health and raising living standards. However, as with the introduction of all new technologies, there were unintended effects, and not all the socioeconomic and environmental consequences of the Green Revolution were positive – it was not a panacea.

With advances made in genetics and molecular biology, it was suggested that the next revolution in agriculture might be the gene revolution (Davies, 2003), building on where the Green Revolution left off. While many of the tools furnished by research into molecular genetics assisted plant and animal breeders in their work, the production of transgenic organisms raised a series of questions about the risks and benefits to human health and the environment, in addition to fundamental concerns over what is intrinsically beneficent and acceptable and what is not. Much of the disapproval of genetic engineering emanated from unintended consequences of the technology, its perceived imprecision. However, more than three decades of research into the risks associated with genetically engineered crops indicates that they have been no riskier than conventionally bred crops (EASC, 2013; NASEM, 2016a).

Among the most recently developed technologies that can be applied in **future efforts** to improve plant and animal breeding is gene editing. This represents an advance over producing transgenics because it is inherently more precise and more versatile, and not prone to some of the errors associated with previous technologies, although in this regard it is not problem-free. Another advantage gene editing has over other technologies is that it is relatively rapid, which will be very important given the speed of climate change. Development and application of techniques such as CRISPR has already generated an enormous amount of information on the genetic workings of an array of plants, animals and microorganisms, and has also made it possible to breed new germlines of many agriculturally important organisms. However, despite its success and the promise it has shown, gene editing has come up against many of the barriers that previous technologies have faced.

Not only are the barriers ethical in nature, whether the benefits outweigh the risks, they also include the need to fit the products of gene editing into functional regulatory frameworks. In addition, for gene editing to make a substantial impression on world hunger, it must be applied to the major crops, those on which most of humankind relies for its nutrition. Wheat, rice and maize contribute nearly half of the world's calories. However, this creates a dilemma because the most vulnerable communities, in terms of pressures on agrifood systems, are those in marginal areas, often in LMICs, where agriculture is already in jeopardy. The problem will not solely be one of total food production, but one of improving resilience of agrifood systems that might not represent prime investments. Moreover, applications of gene editing to major crops, to enhance their resistance to various stresses, would have to be balanced against direct introduction of, for example, drought resistant minor crops such as amaranth and fonio, or even reintroduction of traditional crops. For example, in some areas of Africa, sorghum and pearl millet are making a reappearance because maize cultivation is increasingly difficult as rainfall becomes unpredictable and often insufficient. Under such conditions, when crop replacement is seen as the solution to a changing environment, gene editing is unlikely to be helpful regarding the crop that needs to be replaced. However, as conditions for agriculture deteriorate further, gene editing could contribute to ensuring that replacement crops, possibly old traditional crops, are better able to withstand the changing conditions by having the capacity to tolerate prevailing abiotic and biotic stresses. Moreover, as crops change, so do nutritional profiles, and gene editing for changed and improved micronutrient content could become an important consideration. This is particularly important given that plant breeders have often prioritized yield over nutritional content, resulting in high-yielding cereals sometimes being micronutrient deficient (DeFries et al., 2015). Because of this, calorie undernutrition has declined more slowly than micronutrient deficiency (Gödecke, Stein and Qaim, 2018).

Whichever way gene-editing technologies are used, and their products deployed, there will inevitably be trade-offs in terms of who benefits, much as occurred for the Green Revolution. As Doudna (2022) has said, "One risk that is often overlooked is the real possibility that some of the advances we make in genome editing will benefit a small fraction of society. With new technologies this is often the case at first, so we have to consciously work from the start to make new cures and agricultural tools that are accessible and affordable." Recent research on the economics of gene editing versus genetic modification indicates that gene editing is

far superior (Bullock, Wilson and Neadeau, 2021). There is a much higher probability of success in the discovery phase (25 versus 5 percent) and the lack of strict regulatory approval in many countries, combined with the relatively rapid development of products, speeds up commercialization. Other benefits of gene editing over genetic modification mean that potential market sizes for gene-edited crops are considerably smaller than for genetically modified crops (up to 96 percent), which makes it more attractive for development of crops and traits with lower area potentials and for niche features. Moreover, a decline in break-even areas means that some traits that would never be economically attractive for genetic modification are attractive for gene editing. These include many traits for allergenicities. Bullock, Wilson and Neadeau (2021) also suggested that for many traits, development and marketing would become more efficient and would stimulate closer relationships among all those involved in gene editing, including developers, providers and growers.

Another major issue is that adaptive traits must be targeted to have a significant impact, and it is inevitable that some specific traits in particular species will be more easily manipulated than others. It also must be recognized that such scientific interventions, as represented by gene editing, will not have any effect on world hunger, and evening up of other inequalities, in environments that are no longer able to support food production. This applies to terrestrial and aquatic ecosystems, many of which, because of the effects of unmanageable abiotic and biotic stresses, are irreparably damaged, at least in the short term. In brief, there are many barriers to progress in alleviating world hunger, not all of which are surmountable.

If gene editing is to help reduce world hunger, malnutrition and other inequities, it will be part of a consolidated effort that incorporates a range of other interventions. Gene editing will have to be a component in a partnership approach to problem solving, involving representatives from the public and private sectors, and, most importantly, representatives from the communities where the interventions are to be made. In this way, many of the problems previously encountered with attempted introduction of new technologies might be obviated. Coalitions formed among scientists, representatives from commerce, politicians and the public will ensure that all interested parties have a voice. Moreover, to ensure that smallholder agriculture benefits from gene editing, small-scale producer circumstances must be better appreciated than is currently often the case. They rely heavily on the work done by, and the products from, public institutions, including NARS. Small-scale producers are usually not foremost in the minds of representatives of the private sector and so public sector services must be bolstered to make sure that they do not miss out. Naturally, this would also mean that small-scale producer crops and livestock would be prioritized and researched by public sector bodies.

Innovative agricultural research remains a priority, and identification of priority agrifood systems and crops will become necessary to ensure that efforts are optimally directed and that there is minimal waste of resources. Because gene editing is a sophisticated technology, requiring relatively well-equipped laboratories, it might be most effective to set up regional research facilities and programmes. The scale and speed of changes to the world's weather patterns means that there is an urgency to address the most pressing problems in the most vulnerable areas, recognizing that some agrifood systems and environments are beyond rehabilitation. Low income households in Africa and Asia are likely to be most affected by climate change because they are most susceptible to changing prices and because large proportions of their populations are heavily dependent on agriculture for their livelihoods (Wheeler and von Braun, 2013). Levels of hunger in Africa are much higher than in other regions (FAO, 2022).

Coordination will be essential to ensure optimal impact of any interventions made in agrifood systems, requiring that communication channels are well maintained and fully functional. It is in this area that FAO, among other organizations, can play a leading role by stimulating discussion, providing information and hosting fora (Directorate-General for Research and Innovation of the European Commission *et. al*, 2022).

In summary, gene editing does not represent a stand-alone technology. It will be necessary to ensure that it is incorporated into currently used plant and animal breeding systems and that it is used in conjunction with improved husbandry practices. Its products should be available to those that need them most, and account should be taken of the crops and animals that are important to small-scale producers living in vulnerable environments. The use of gene editing should not be confined to or dominated by multinational corporations at the expense of those most in need of gene-editing technologies and products. Only through sustained research on the technical aspects of gene editing, and the associated benefits and risks, will its potential be fully realized. Previous radical changes to agrifood systems have not been without difficulties, but innovative applications of science and technology have invariably led to positive outcomes. Gene editing may represent an additional step towards the transformation of current agrifood systems that can withstand better the pressures they are currently facing and will face, possibly to an even greater extent, in the future.

References

- Acemoglu, D. & Robinson, J.A. 2019. The narrow corridor: states, societies, and the fate of liberty. New York, Penguin Press.
- Agriculture Food. 2017. THE RIGHT TO SEEDS: A fundamental right for small farmers! Paris, Coordination SUD. http://www.coordinationsud.org/ wp-content/uploads/23-Notes-C2A-N23-Defendingthe-rights-of-peasants.pdf
- Ahmad, A., Munawar, N., Khan, Z., Qusmani, A.T., Khan, S.H., Jamil, A., Ashraf, S. et al. 2021. An outlook on global regulatory landscape for genomeedited crops. *International Journal of Molecular Sciences*, 22(21): 11753. https://doi.org/10.3390/ ijms222111753
- Akbari, O.S., Bellen, H.J., Bier, E., Bullock, S.L., Burt, A., Church, G.M., Cook, K.R. *et al.* 2015. Safeguarding gene drive experiments in the laboratory. *Science*, 349(6251): 927–929. https://doi. org/10.1126/science.aac7932
- Al Amin, N., Ahmad, N., Wu, N., Pu, X., Ma, T., Du, Y., Bo, X. et al. 2019. CRISPR-Cas9 mediated targeted disruption of FAD2-2 microsomal omega-6 desaturase in soybean (Glycine max L.). BMC Biotechnology, 19(1): 1–10. https://doi.org/10.1186/S12896-019-0501-2
- Alston, J.M. & Gray, R. 2000. State trading versus export subsidies: The case of Canadian wheat. *Journal of Agricultural and Resource Economics*, 25: 51–67. http://www.jstor.org/stable/40987048
- Alston, J.M., Pardey, P.G. & Rao, X. 2022. Payoffs to half a century of CGIAR research. *American Journal* of *Economics*, 104(2): 502–529. https://onlinelibrary. wiley.com/doi/10.1111/ajae.12255
- Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A. et al. 2019. Search-andreplace genome editing without double-strand breaks or donor DNA. *Nature*, 576: 149–157. https://www. nature.com/articles/s41586-019-1711-4
- Anzalone, A.V., Koblan, L.W. & Liu, D.R. 2020. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nature Biotechnology*, 38: 824–844. https://www.nature. com/articles/s41587-020-0561-9
- Azadi, H., Taube, F. & Taheri, F. 2018. Co-existence of GM, conventional and organic crops in developing countries: main debates and concerns. *Critical Reviews in Food Science and Nutrition*, 58(16): 2677– 2688. https://doi.org/10.1080/10408398.2017.1322553
- Baloch, A.R., Franěk, R., Tichopád, T., Fučíková, M., Rodina, M. & Pšenička, M. 2019. Dnd1 knockout in sturgeons by CRISPR/Cas9 generates germ cell free host for surrogate production. *Animals*, 9(4): 174. https://doi.org/10.3390/ani9040174
- Barrett, C.B., Reardon, T., Swinnen, J. & Zilberman,
 D. 2022. Agri-food value chain revolutions in lowand middle-income countries. *Journal of Economic*

Literature (forthcoming). https://www.aeaweb.org/ articles?id=10.1257/jel.20201539&&from=f

- Bartkowski, B., Theesfeld, I., Pirscher, F. & Timaeus, J. 2018. Snipping around for food: Economic, ethical and policy implications of CRISPR/Cas genome editing. *Geoforum*, 96: 172–180. https://doi.org/10.1016/J. GEOFORUM.2018.07.017
- Beckmann, V., Soregaroli, C. & Wesseler, J. 2010. Exante regulation and ex-post liability under uncertainty and irreversibility: governing the coexistence of GM Crops. *Economics*, 4(1): 1. https://doi.org/10.5018/ economics-ejournal.ja.2010-9
- van den Belt, H. & Keulartz, F.W.J. 2007. Worldwide cultural differences in socio-ethical views in relation to biotechnology. *Cogem*: 343–354. https://cogem. net/app/uploads/2019/07/CGM2007-05-Worldwide-Cultural-Differences1-1.pdf
- Bharadwaj, P., Fenske, J., Kala, N. & Mirza, R.A. 2020. The green revolution and infant mortality in India. *Journal of Health Economics*, 71: 102314. https://doi. org/10.1016/j.jhealeco.2020.102314
- Bier, E. 2022. Gene drives gaining speed. *Nature Reviews Genetics*, 23(1): 5–22. https://doi. org/10.1038/s41576-021-00386-0
- Bonny, S. 2016. Genetically modified herbicide-tolerant crops, weeds, and herbicides: overview and impact. *Environmental Management*, 57(1): 31–48. https://doi.org/10.1007/s00267-015-0589-7
- Bracco, S., Tani, A., Çalicioglu, Ö., Gomez San Juan, M. & Bogdanski, A. 2019. Indicators to monitor and evaluate the sustainability of bioeconomy. Overview and a proposed way forward. Rome, FAO.
- Brinegar, K., K. Yetisen, A., Choi, S., Vallillo, E., Ruiz-Esparza, G.U., Prabhakar, A.M., Khademhosseini,
 A. & Yun, S.H. 2017. The commercialization of genome-editing technologies. *Critical Reviews in Biotechnology*, 37(7): 924–932. http://www.intelon. org/publications/BrinegarCRB2017.pdf
- Brookes, G. & Barfoot, P. 2018a. Farm income and production impacts of using GM crop technology 1996–2016. *GM Crops & Food*, 9(2): 59–89. https:// doi.org/10.1080/21645698.2018.1464866
- Brookes, G. & Barfoot, P. 2018b. Environmental impacts of genetically modified (GM) crop use 1996–2016: Impacts on pesticide use and carbon emissions. *GM Crops & Food*, 9(3): 109–139. https://doi.org/10.1080/2 1645698.2018.1476792
- Brookes, G. & Barfoot, P. 2020. Environmental impacts of genetically modified (GM) crop use 1996–2018: impacts on pesticide use and carbon emissions. *GM Crops & Food*, 11(4): 215–241. https://doi.org/10.1080/ 21645698.2020.1773198
- Bullock, D.W., Wilson, W.W. & Neadeau, J.F. 2019. Genetic Editing (GE) Versus Genetic Modification (GM) in the Research and Development of New Crop Varieties: An Economic Comparison. Agribusiness & Applied Economics Report 293186. North Dakota

48

State University, Department of Agribusiness and Applied Economics. https://ideas.repec.org/p/ags/ nddaae/293186.html

- Burkard, C., Opriessnig, T., Mileham, A.J., Stadejek, T., Ait-Ali, T., Lillico, S.G., Whitelaw, C.B.A. & Archibald, A.L. 2018. Pigs lacking the scavenger receptor cysteine-rich domain 5 of CD163 are resistant to porcine reproductive and respiratory syndrome virus 1 infection. *Journal of Virology*, 92(16). https://pubmed.ncbi.nlm.nih.gov/29925651/
- Busch, G., Ryan, E., von Keyserlingk, M.A.G.G. & Weary, D.M. 2022. Citizen views on genome editing: effects of species and purpose. *Agriculture and Human Values*, 39(1): 151–164. https://doi.org/10.1007/s10460-021-10235-9
- Büschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., Van Daelen, R. *et al.* 1997. The barley Mlo gene: a novel control element of plant pathogen resistance. *Cell*, 88(5): 695–705. https://doi. org/10.1016/S0092-8674(00)81912-1
- Byiers, B., Große-Puppendahl, S., Huyse, H., Rosengren, A. & Vaes, S. 2016. Principles for public-private partnerships-towards sustainability? Lessons from SAGCOT, healthcare in Lesotho, and better factories Cambodia. European Centre for Development Policy Management. https://www.academia.edu/es/67848033/ Principles_for_public_private_partnerships_towards_ sustainability_Lessons_from_SAGCOT_healthcare_in_ Lesotho_and_Better_Factories_Cambodia
- Carlson, D.F., Lancto, C.A., Zang, B., Kim, E.-S., Walton, M., Oldeschulte, D., Seabury, C., Sonstegard, T.S. & Fahrenkrug, S.C. 2016. Production of hornless dairy cattle from genome-edited cell lines. *Nature Biotechnology*, 34(5): 479–481. https://doi. org/10.1038/nbt.3560
- Casini, A., Olivieri, M., Petris, G., Montagna, C., Reginato, G., Maule, G., Lorenzin, F., Prandi, D., Romanel, A., Demichelis, F. et al. 2018. A highly specific SpCas9 variant is identified by in vivo screening in yeast. *Nature Biotechnology*. 36: 265–271. https:// www.ncbi.nlm.nih.gov/pmc/articles/PMC6066108/
- **Castellari, E., Soregaroli, C., Venus, T.J. & Wesseler,** J. 2018. Food processor and retailer non-GMO standards in the US and EU and the driving role of regulations. *Food Policy*, 78: 26–37. https://doi. org/10.1016/j.foodpol.2018.02.010
- **CBD**. 2021. Causes and impacts of invasive alien species. https://www.cbd.int/idb/2009/about/causes/
- Champer, J., Buchman, A. & Akbari, O.S. 2016. Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nature Reviews Genetics*, 17(3): 146–159. https://doi.org/10.1038/nrg.2015.34
- Chennuri, P.R., Adelman, Z.N. & Myles, K.M. 2022. Genetic approaches for controlling CRISPR-based autonomous homing gene drives. *Frontiers in Bioengineering and Biotechnology*, 10. https://doi. org/10.3389/fbioe.2022.897231

- Clapp, J. & Ruder, S.L. 2020. Precision technologies for agriculture: digital farming, gene-edited crops, and the politics of sustainability. *Global Environmental Politics*, 20(3): 49–69. https://doi.org/10.1162/GLEP_A_00566
- Clomburg, J.M., Crumbley, A.M. & Gonzalez, R. 2017. Industrial biomanufacturing: the future of chemical production. *Science*, 355(6320): aag0804. https:// pubmed.ncbi.nlm.nih.gov/28059717/
- Cong, L., Ran, F.A., Cox, D., Lin, S.L., Barretto, R., Habib, N., Hsu, P.D., Wu, X.B., Jiang, W.Y., Marraffini, L.A. *et al.* 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science*, 339: 819–823. https://europepmc.org/article/ MED/23287718
- Contreras, J.L. & Sherkow, J.S. 2017. CRISPR, surrogate licensing, and scientific discovery. *Science*, 355(6326): 698–700. https://doi.org/10.1126/science.aal4222
- Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F.N. & Leip, A. 2021. Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, 2(3): 198–209. https://doi.org/10.1038/s43016-021-00225-9
- Cyranoski, D. 2015. Gene-edited pigs to be sold as pets. *Nature*, 526(7571): 18. https://doi.org/10.1038/ NATURE.2015.18448
- Davies, W.P. 2003. An historical perspective from the Green Revolution to the Gene Revolution. *Nutrition Reviews*, 61(suppl_6): S124–S134. https://doi. org/10.1301/nr.2003.jun.S124-S134
- De Marchi, E., Cavaliere, A., Bacenetti, J., Milani, F., Pigliafreddo, S. & Banterle, A. 2019. Can consumer food choices contribute to reduce environmental impact? The case of cisgenic apples. *Science of The Total Environment*, 681: 155–162. https://doi. org/10.1016/J.SCITOTENV.2019.05.119
- Deckers, M., Vanneste, K., Winand, R., Hendrickx, M., Becker, P., De Keersmaecker, S.C.J., Deforce, D., Marie-Alice, F. & Roosens, N.H.C. 2020. Screening strategy targeting the presence of food enzymeproducing fungi in food enzyme preparations. *Food Control*, 117: 107295. https://doi.org/10.1016/J. FOODCONT.2020.107295
- Deconinck, K. 2020. Concentration in seed and biotech markets: extent, causes, and impacts. *Annual Review* of *Resource Economics* 12:129–47. https://doi. org/10.1146/annurev-resource-102319-100751
- DeFries, R., Fanzo, J., Remans, R., Palm, C., Wood, S. & Anderman, T.L. 2015. Metrics for land-scarce agriculture. *Science*, 349(6245): 238–240. https://doi. org/10.1126/science.aaa5766
- Demorest, Z.L., Coffman, A., Baltes, N.J., Stoddard, T.J., Clasen, B.M., Luo, S., Retterath, A. et al. 2016. Direct stacking of sequence-specific nucleaseinduced mutations to produce high oleic and low linolenic soybean oil. *BMC Plant Biology*, 16(1): 225. https://doi.org/10.1186/s12870-016-0906-1

References

- von Der Goltz, J., Dar, A., Fishman, R., Mueller, N.D., Barnwal, P. & McCord, G.C. 2020. Health impacts of the Green Revolution: Evidence from 600,000 births across the developing world. *Journal of Health Economics*, 74: 102373. https://doi.org/10.1016/j. jhealeco.2020.102373
- Deriano, L. & Roth, D.B. 2013. Modernizing the nonhomologous end-joining repertoire: alternative and classical NHEJ share the stage. *Annual Review of Genetics*, 47: 433–455. https://www.annualreviews. org/doi/10.1146/annurev-genet-110711-155540?url_ ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref. org&rfr_dat=cr_pub++0pubmed
- Devlin, R.H., Sakhrani, D., Biagi, C.A. & Eom, K.W. 2010. Occurrence of incomplete paternalchromosome retention in GH-transgenic coho salmon being assessed for reproductive containment by pressure-shock-induced triploidy. *Aquaculture*, 304(1–4): 66–78. https://doi.org/10.1016/J. AQUACULTURE.2010.03.023
- Devlin, R.H., Sundström, L.F. & Leggatt, R.A. 2015. Assessing ecological and evolutionary consequences of growth-accelerated genetically engineered fishes. *BioScience*, 65(7): 685–700. https://doi.org/10.1093/ BIOSCI/BIV068
- Devos, Y., Mumford, J.D., Bonsall, M.B., Camargo, A.M., Firbank, L.G., Glandorf, D.C.M., Nogué, F., Paraskevopoulos, K. & Wimmer, E.A. 2022. Potential use of gene drive modified insects against disease vectors, agricultural pests and invasive species poses new challenges for risk assessment. *Critical Reviews in Biotechnology*, 42(2): 254–270. https://doi.org/10.10 80/07388551.2021.1933891
- Devos, Y., Naegeli, H., Perry, J.N. & Waigmann, E. 2016. 90-day rodent feeding studies on whole GM food/ feed. *EMBO reports*, 17(7): 942–945. https://doi. org/10.15252/embr.201642739
- Directorate-General for Research and Innovation of the European Commission, Webb, P., Sonnino, R., Fraser, E. & Arnold T. 2022. Everyone at the table: Transforming food systems by connecting science, policy and society, Publications Office of the European Union, Luxembourg. https://data.europa.eu/ doi/10.2777/440690
- **Di Sibio, C.** 2022. How to harness the transformative potential of public-private partnerships. Cited 8 August 2022. https://www.greenbiz.com/article/how-harness-transformative-potential-public-private-partnerships
- Doman, J.L., Raguram, A., Newby, G.A. & Liu, D.R. 2020. Evaluation and minimization of Cas9independent off-target DNA editing by cytosine base editors. *Nature Biotechnology*, 38: 620–628. https:// pubmed.ncbi.nlm.nih.gov/32042165/
- Doudna, J. 2022. 10 years on, a spin-off use for CRISPR: Infectious disease testing. Big Think, The Well, An interview with CRISPR co-discoverer and Nobel Prizewinner Dr. Jennifer Doudna. https://bigthink.com/thewell/crispr-jennifer-doudna-interview/

- Doudna, J.A. & Sternberg, S.H. 2017. A crack in creation: gene editing and the unthinkable power to control evolution. HarperCollins. https://books.google.com/books/about/A_Crack_In_Creation. html?hl=de&id=F8WIDAAAOBAJ
- **Dubock, A.** 2014. The politics of Golden Rice. *GM Crops* & Food, 5(3): 210–222. https://doi.org/10.4161/216456 98.2014.967570
- Ducarme, F. & Couvet, D. 2020. What does 'nature' mean? *Palgrave Communications*, 6(1): 14. https://doi. org/10.1057/s41599-020-0390-y
- **EASC**. 2013. Planting the Future: Opportunities and Challenges for Using Crop Genetic Improvement Technologies for Sustainable Agriculture. Halle, European Academies Science Advisory Council.
- Eckerstorfer, M.F., Grabowski, M., Lener, M., Engelhard, M., Simon, S., Dolezel, M., Heissenberger, A. & Lüthi, C. 2021. Biosafety of genome editing applications in plant breeding: Considerations for a focused case-specific risk assessment in the EU. *BioTech*, 10(3): 10. https://doi. org/10.3390/biotech10030010
- Edenbrandt, A.K., Gamborg, C. & Thorsen, B.J. 2018. Consumers' preferences for bread: Transgenic, cisgenic, organic or pesticide-free? *Journal of Agricultural Economics*, 69(1): 121–141. https://doi. org/10.1111/1477-9552.12225
- Eriksson, D., Harwood, W., Hofvander, P., Jones, H., Rogowsky, P., Stöger, E. & Visser, R.G.F. 2018. A welcome proposal to amend the GMO legislation of the EU. *Trends in Biotechnology*, 36(11): 1100–1103. https://doi.org/10.1016/J.TIBTECH.2018.05.001
- Eriksson, D., Kershen, D., Nepomuceno, A., Pogson,
 B.J., Prieto, H., Purnhagen, K., Smyth, S., Wesseler,
 J. & Whelan, A. 2019. A comparison of the EU regulatory approach to directed mutagenesis with that of other jurisdictions, consequences for international trade and potential steps forward. *New Phytologist*, 222(4): 1673–1684. https://doi.org/10.1111/NPH.15627
- Esvelt, K.M. & Gemmell, N.J. 2017. Conservation demands safe gene drive. *PLOS Biology*, 15(11): e2003850. https://doi.org/10.1371/JOURNAL.PBIO.2003850
- Esvelt, K.M., Smidler, A.L., Catteruccia, F. & Church, G.M. 2014. Emerging Technology: Concerning RNA-guided gene drives for the alteration of wild populations. *eLife*, 3(July2014): 1–21. https://doi. org/10.7554/ELIFE.03401
- **EuropaBio**. 2018. Mandatory Feeding Studies for GM Crops Should Be Abolished Because They Contradict EU Science and Ethical Principles. https://www. europabio.org/sites/default/files/EuropaBio position on 90-day feeding studies-15.11.2018Final.pdf
- **European Commission**. 2010. *Eurobarometer 73.1 Biotechnology*. Brussels.
- **European Commission**. 2021. Study on the status of new genomic techniques under Union law and in light of the Court of Justice ruling in Case C-528/16. Brussels.

European Parliament. 2021. New genomic techniques. European Commission study and first reactions. https://www.europarl.europa.eu/RegData/etudes/ BRIE/2021/698760/EPRS_BRI(2021)698760_EN.pdf

Evenson, R.E. & Gollin, D. 2003. Assessing the impact of the Green Revolution, 1960 to 2000. *Science*, 300(5620): 758–762. https://doi.org/10.1126/ science.1078710

Fajardo-Ortiz, D., Hornbostel, S., Montenegro de Wit, M. & Shattuck, A. 2022. Funding CRISPR: Understanding the role of government and philanthropic institutions in supporting academic research within the CRISPR innovation system. *Quantitative Science Studies*, 3 (2): 443–456. https://doi.org/10.1162/qss_a_00187

Falck-Zepeda, J., Biermayr-Jenzano, P., Roca, M.M., Fuentes-Campos, E. & Kikulwe, E.M. 2022. Bioinnovations Genome-Edited Crops for Climate-Smart Food Systems. https://cgspace.cgiar.org/ bitstream/handle/10568/120347/book chapter. pdf?sequence=1&isAllowed=y

Falck-Zepeda, J.B., Traxler, G. & Nelson, R.G. 2000. Surplus distribution from the introduction of a biotechnology innovation. *American Journal of Agricultural Economics*, 82(2): 360–369. https://doi. org/10.1111/0002-9092.00031

Fan, L., Stevens, A.W. & Thomas, B. 2022. Consumer purchasing response to mandatory genetically engineered labeling. *Food Policy*, 110: 102296. https://www.semanticscholar.org/paper/Consumerpurchasing-response-to-mandatory-labeling-Fan-Steve ns/0c735d90187739469a2cf59b0bf1842af22b0b3f

FAO. 2016. Public-Private Partnerships for Agribusiness Development - A review of international experiences. Rome, FAO. https://www.fao.org/sustainable-foodvalue-chains/library/details/fr/c/417048/

FAO. 2018. The Future of Food and Agriculture. November. Rome, FAO. http://www.fao.org/3/I8429EN/i8429en.pdf

FAO. 2020. *The State of Food and Agriculture 2020*. Rome, FAO. https://doi.org/10.4060/CB1447EN

FAO. 2022. FAOSTAT Production Statistics. Rome, FAO.

FAO, IFAD, UNICEF, WFP & WHO. 2022. The State of Food Security and Nutrition in the World 2022: Repurposing food and agricultural policies to make healthy diets more affordable. The State of Food Security and Nutrition in the World (SOFI) 2022. Rome, Italy, FAO, IFAD, UNICEF, WFP, WHO. https:// doi.org/10.4060/cc0639en

FAO & ITPS. 2015. Status of the World's Soil Resources (SWSR) - Main Report. Rome, FAO. http://www.fao. org/3/i5199e/I5199E.pdf

Fasano, A. & Catassi, C. 2012. Celiac disease. *New* England Journal of Medicine, 367(25): 2419–2426. https://doi.org/10.1056/NEJMcp1113994

FDA. 2015. AquAdvantage® Salmon - Environmental Assessment. https://www.fda.gov/media/93817/ download FDA. 2019. Biotechnology Notification File No. 000164. https://www.fda.gov/media/120660/download

FDA. 2020. Freedom of information summary. Original new animal drug application. NADA 141-542. pPL657 rdna construct in domestic pigs. https:// animaldrugsatfda.fda.gov/adafda/app/search/public/ document/downloadFoi/10168

FDA. 2022. Risk Assessment Summary – V-006378 PRLR-SLICK cattle. FDA Makes Low-Risk Determination for Marketing of Products from Genome-Edited Beef Cattle After Safety Review. https://www.fda.gov/news-events/ press-announcements/fda-makes-low-riskdetermination-marketing-products-genomeedited-beef-cattle-after-safety-review?utm_ medium=email&utm_source=govdelivery https:// www.fda.gov/media/155706/download

Fernandez, A., Mills, E.N.C., Koning, F. & Moreno, F.J. 2021. Allergenicity assessment of novel food proteins: What should be improved? *Trends in Biotechnology*, 39(1): 4–8. https://doi.org/10.1016/J. TIBTECH.2020.05.011

Fernbach, P.M., Light, N., Scott, S.E., Inbar, Y. & Rozin, P. 2019. Extreme opponents of genetically modified foods know the least but think they know the most. *Nature Human Behaviour*, 3(3): 251–256. https://doi. org/10.1038/S41562-018-0520-3

Fogel, R.W., Acito, C.J., Adams, R.M., Becker, G.S., Cassel, C.K., Chavigny, K.A., Costa, D.L. et al. 1994. Economic Growth, Population Theory, and Physiology: The Bearing of Long-Term Processes on the Making of Economic Policy. https://doi.org/10.3386/W4638

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D. et al. 2011. Solutions for a cultivated planet. *Nature*, 478(7369): 337–342. https://doi.org/10.1038/nature10452

Fox, J. & Haight, L. 2010. Subsidizing inequality: Mexican corn policy since NAFTA. [Santa Cruz, Calif.], Woodrow Wilson International Center for Scholars, Centro de Investigación y Docencia Económicas, University of California, Santa Cruz. http://www. worldcat.org/oclc/701368631

Frewer, L.J., van der Lans, I.A., Fischer, A.R.H., Reinders, M.J., Menozzi, D., Zhang, X., van den Berg, I. & Zimmermann, K.L. 2013. Public perceptions of agri-food applications of genetic modification – A systematic review and meta-analysis. *Trends in Food Science & Technology*, 30(2): 142–152. https://doi.org/10.1016/J.TIFS.2013.01.003

Frisvold, G., Boor, A. & Reeves, J. 2009. Simultaneous diffusion of herbicide resistant cotton and conservation tillage. *AgBioForum*, 12(3 & 4): 249–257. https://www.semanticscholar.org/paper/Simultaneous-Diffusion-of-Herbicide-Resistant-and-Frisvold-Boor/0895c8fdbc0c52a47719d1c9084b2abc c6e95082

References

- Fu, Y., Foden, J.A., Khayter, C., Maeder, M.L., Reyon, D., Joung, J.K. & Sander, J.D. 2013. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nature Biotechnology* 31: 822–826. https://pubmed.ncbi.nlm.nih.gov/23792628/
- GAIN. 2022. BT Cowpea Approved for Environmental and Market Release. Report Number: GH2022-0012. USDA.
- Gao, C. 2021. Genome engineering for crop improvement and future agriculture. *Cell*, 184(6): 1621–1635. https://doi.org/10.1016/J.CELL.2021.01.005
- Gasiunas, G., Barrangou, R., Horvath, P. & Siksnys,
 V. 2012. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proceedings of the National Academy of Sciences*, 109: E2579-E2586. https:// pubmed.ncbi.nlm.nih.gov/22949671/
- Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I. & Liu, D.R. 2017. Programmable base editing of A.T to G.C in genomic DNA without DNA cleavage. *Nature*, 551: 464–471. https://pubmed.ncbi.nlm.nih.gov/29160308/
- **Gehrke, L.M.** 2019. Health law: Is gene editing patentable? *AMA Journal of Ethics*, 21: 1049–1055. https://journalofethics.ama-assn.org/article/gene-editing-patentable/2019-12
- George, D.R., Hornstein, E.D., Clower, C.A., Coomber, A.L., Dillard, D., Mugwanya, N., Pezzini, D.T. & Rozowski, C. 2022. Lessons for a SECURE future: Evaluating diversity in crop biotechnology across regulatory regimes. *Frontiers in Bioengineering* and Biotechnology, 0: 667. https://doi.org/10.3389/ FBIOE.2022.886765
- Ghodsi, R. & Nosrati, R. 2020. Effects of minor compounds of edible oils on human health. Current Nutrition & Food Science, 16(8): 1196–1208. https:// doi.org/10.2174/1573401316666200203121034
- Glais, L., Bellstedt, D. U. & Lacomme, C. 2017. Diversity, Characterisation and Classification of PVY. In: C. Lacomme, L. Glais, D.U. Bellstedt, B. Dupuis, A.V. Karasev & E. Jacquot, eds. *Potato Virus Y: Biodiversity, Pathogenicity, Epidemiology and Management.* Springer International Publishing AG, Chan. pp. 43–76. https://doi.org/10.1007/978-3-319-58860-5_3
- **Glandorf, D.C.M.** 2019. Re-evaluation of biosafety questions on genetically modified biocontrol bacteria. *European Journal of Plant Pathology*, 153(1): 243–251. https://doi.org/10.1007/S10658-018-1598-1
- **Glawe, D.A.** 2008. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. *Annual Review of Phytopathology*, 46(1): 27–51. https://doi.org/10.1146/annurev. phyto.46.081407.104740
- Glenna, L.L., Lacy, W.B., Welsh, R. & Biscotti, D. 2007. University administrators, agricultural biotechnology, and academic capitalism: Defining the public good to promote university-industry relationships. *The Sociological Quarterly*, 48(1): 141–163. https://doi. org/10.1111/j.1533-8525.2007.00074.x

- **Global Nutrition Report.** 2021. *The state of global nutrition*. Bristol, UK, Development Initiatives. https:// globalnutritionreport.org/reports/2021-global-nutritionreport/
- Gödecke, T., Stein, A.J. & Qaim, M. 2018. The global burden of chronic and hidden hunger: Trends and determinants. *Global Food Security*, 17: 21–29. https://doi.org/10.1016/j.gfs.2018.03.004
- Gollin, D., Hansen, C.W. & Wingender, A.M. 2021. Two blades of grass: The impact of the Green Revolution. *Journal of Political Economy*, 129(8): 2344–2384. https://doi.org/10.1086/714444
- Gómez González, I. 2016. A honey-sealed alliance: Mayan beekeepers in the Yucatan Peninsula versus transgenic soybeans in Mexico's last tropical forest. *Journal of Agrarian Change*, 16(4): 728–736. https:// doi.org/10.1111/JOAC.12160
- Gomez San Juan, M. & Bogdanski, A. 2021. How to mainstream sustainability and circularity into the bioeconomy? A compendium of bioeconomy good practices and policies. Rome, FAO. https://doi. org/10.4060/cb5798en
- Goray, C. & Bessa, A. 2019. The rights to seed in Europe: The United Nations declaration on the rights of peasants and other people working in rural areas and the protection of the right to seeds in Europe. https:// www.geneva-academy.ch/joomlatools-files/docmanfiles/The Right to Seeds in Europe.pdf
- Gosal, S.K., Kaur, J. & Kaur, J. 2020. Microbial Biotechnology: A Key to Sustainable Agriculture.
 In: M. Kumar, V. Kumar, & R. Prasad, eds. *Phyto-Microbiome in Stress Regulation*. pp. 219–243.
 Singapore, Springer. https://doi.org/10.1007/978-981-15-2576-6_11
- Gould, F., Amasino, R.M., Brossard, D., Buell, C.R., Dixon, R.A., Falck-Zepeda, J.B., Gallo, M.A. *et al.* 2022. Toward product-based regulation of crops. *Science*, 377(6610): 1051–1053. https://doi. org/10.1126/science.abo3034
- de Graeff, N., Jongsma, K.R., Johnston, J., Hartley, S. & Bredenoord, A.L. 2019. The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1772): 20180106. https://doi. org/10.1098/rstb.2018.0106
- Graff, G.D., Roland-Holst, D. & Zilberman D. 2005.
 Agricultural biotechnology and globalization: U.S.
 experience with public and private sector research. In:
 J. Wesseler, ed. *Environmental Costs and Benefits of Transgenic Crops*, 245–246. Wageningen UR Frontis Series Vol. 7, Springer, Dordrecht.
- Graff, G.D. & Sherkow, J.S. 2020. Models of technology transfer for genome-editing technologies. *Annual Review of Genomics and Human Genetics*, 21(1): 509–534. https://doi.org/10.1146/annurevgenom-121119-100145

- Gregorowius, D., Lindemann-Matthies, P. & Huppenbauer, M. 2012. Ethical discourse on the use of genetically modified crops: A review of academic publications in the fields of ecology and environmental ethics. *Journal of Agricultural and Environmental Ethics*, 25(3): 265–293. https://doi. org/10.1007/s10806-011-9330-6
- Gupta, C. 2018. Contested fields: an analysis of anti-GMO politics on Hawai'i Island. *Agriculture* and Human Values, 35(1): 181–192. https://doi. org/10.1007/s10460-017-9814-8
- Haggblade, S., Diarra, A. & Traoré, A. 2022. Regulating agricultural intensification: Lessons from West Africa's rapidly growing pesticide markets. *Development Policy Review*, 40(1): e12545. https://doi.org/10.1111/ DPR.12545
- Haggblade, S., Minten, B., Pray, C., Reardon, T. & Zilberman, D. 2017. The herbicide revolution in developing countries: Patterns, causes, and implications. *European Journal of Development Research*, 29(3): 533–559. https://doi.org/10.1057/ S41287-017-0090-7
- Hahn, F. & Nekrasov, V. 2019. CRISPR/Cas precision: do we need to worry about off-targeting in plants? *Plant Cell Reports*, 38(4): 437–441. https://doi.org/10.1007/ s00299-018-2355-9
- Hartman Group. 2018. Report: nearly half of consumers avoid GMOs; more are buying non-GMO products. https://www.hartman-group.com/acumenPdfs/ non-gmoreport_com-Report%20nearly%20half%20 of%20consumers%20avoid%20GMOs%20more%20 are%20buying%20non-GMO%20products.pdf
- HarvestPlus. 2017. Partnering to Scale Innovation. Annual Report, 2017.
- Hauschild, J., Petersen, B., Santiago, Y., Queisser, A.L., Carnwath, J.W., Lucas-Hahn, A., Zhang, L. et al. 2011. Efficient generation of a biallelic knockout in pigs using zinc-finger nucleases. *Proceedings of the National Academy of Sciences*, 108(29): 12013–12017. https://doi.org/10.1073/pnas.1106422108
- Hayes, K.R., Hosack, G.R., Dana, G.V., Foster, S.D., Ford, J.H., Thresher, R., Ickowicz, A. *et al.* 2018. Identifying and detecting potentially adverse ecological outcomes associated with the release of gene-drive modified organisms. *Journal of Responsible Innovation*, 5(sup1): S139–S158. https:// doi.org/10.1080/23299460.2017.1415585
- Heimann, J.M. 2018. Using Nature's Shuttle. Wageningen, NL, Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-880-3
- Helliwell, R., Hartley, S. & Pearce, W. 2019. NGO perspectives on the social and ethical dimensions of plant genome-editing. *Agriculture and Human Values*, 36(4): 779–791. https://doi.org/10.1007/s10460-019-09956-9
- Herring, R. 2009. Illicit Seeds: Epistemic Brokers and the Politics of Property in Genetic Engineering. APSA 2009 Toronto Meeting Paper. https://papers.ssrn.com/ Sol3/papers.cfm?abstract_id=1449228

- Hsu, P.D., Scott, D.A., Weinstein, J.A., Ran, F.A., Konermann, S., Agarwala, V., Li, Y., Fine, E.J., Wu, X., Shalem, O. *et al.* 2013. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nature Biotechnology*, 31: 827–832. https://pubmed.ncbi. nlm.nih.gov/23873081/
- Hu, J.H., Miller, S.M., Geurts, M.H., Tang, W.X., Chen, L.W., Sun, N., Zeina, C.M., Gao, X., Rees, H.A., Lin,
 Z. et al. 2018. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature*, 556: 57–63. https://www.nature.com/articles/nature26155
- Huckauf, J., Brandt, B., Dezar, C., Nausch, H., Hauerwaas, A., Weisenfeld, U., Elshiewy, O., Rua, M., Hugenholtz, J., Wesseler, J., Cingiz, K. & Broer, I. 2022. Sustainable production of the cyanophycin biopolymer in tobacco in the greenhouse and field. *Frontiers in Bioengineering and Biotechnology* 10:896863. https://doi.org/10.3389/fbioe.2022.896863
- Huffman, W.E. & McCluskey, J.J. 2020. New technology and conflicting information : assessing consumers' willingness-to-pay for new foods. Singapore, World Scientific Publishing.
- **IPBES.** 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. https://doi.org/10.5281/ZENODO.3832099
- IPCC. 2019. Summary for Policymakers Special Report on Climate Change and Land. https://www.ipcc.ch/ srccl/chapter/summary-for-policymakers/
- **ISAAA**. 2019. Executive Summary Biotech Crops Drive Socio-Economic Development and Sustainable Environment in the New Frontier.
- Iyer, V., Boroviak, K., Thomas, M., Doe, B., Riva, L., Ryder, E. & Adams, D.J. 2018. No unexpected CRISPR-Cas9 off-target activity revealed by trio sequencing of gene-edited mice. *PLOS Genetics*, 14. https://journals.plos.org/plosgenetics/ article?id=10.1371/journal.pgen.1007503
- Jacquier, A. & Dujon, B. 1985. An intron-encoded protein is active in a gene conversion process that spreads an intron into a mitochondrial gene. *Cell*, 41: 383–394. https://www.sciencedirect.com/science/article/abs/pii/ S0092867485800118
- James, S., Collins, F.H., Welkhoff, P.A., Emerson, C., J Godfray, H.C., Gottlieb, M., Greenwood,
 B. et al. 2018. Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: Recommendations of a scientific working group. *The American Journal of Tropical Medicine and Hygiene*, 98(6_Suppl): 1–49. https://doi.org/10.4269/ AJTMH.18-0083
- Jeong, Y.K., Song, B. & Bae, S. 2020. Current status and challenges of DNA base editing tools. *Molecular Therapy*, 28: 1938–1952. https://pubmed.ncbi.nlm.nih. gov/32763143/

References

Jin, S., Zong, Y., Gao, Q., Zhu, Z.X., Wang, Y.P., Qin, P., Liang, C.Z., Wang, D.W., Qiu, J.L., Zhang, F. et al. 2019. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science*, 364: 292–295. https://pubmed.ncbi.nlm.nih.gov/30819931/

Jin, S., Fei, H.Y., Zhu, Z.X., Luo, Y.F., Liu, J.X., Gao, S.H., Zhang, F., Chen, Y.H., Wang, Y.P. & Gao, C.X. 2020. Rationally designed APOBEC3B cytosine base editors with improved specificity. *Molecular Cell*, 79: 728–740. https://pubmed.ncbi.nlm.nih.gov/32721385/

Jin, S., Lin, Q.P., Luo, Y.F., Zhu, Z.X., Liu, G.W., Li, Y.J., Chen, K.L., Qiu, J.L. & Gao, C.X. 2021. Genomewide specificity of prime editors in plants. *Nature Biotechnology*, 39: 1292–1299. https://www.nature. com/articles/s41587-021-00891-x

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. & Charpentier, E. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337: 816–821. https://europepmc. org/article/MED/22745249

 Jordan, N.R., Kuzma, J., Ray, D.K., Foot, K., Snider, M., Miller, K., Wilensky-Lanford, E. & Amarteifio,
 G. 2022. Should gene editing be used to develop crops for continuous-living-cover agriculture? A multisector stakeholder assessment using a cooperative governance approach. *Frontiers in Bioengineering* and Biotechnology, 10. https://doi.org/10.3389/ fbioe.2022.843093

Jørgensen, I.H. 1992. Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica*, 63(1–2): 141–152. https://doi. org/10.1007/BF00023919

Kabir, R., Tayyab Mehmood, M., Asad, M., Iqbal, A., Muhammad Kashif, H., Yar Khan, R. & Author,
C. 2022. The use of terminal technology (Gurt) in producing new seed varieties and their impact on farmers. Annals of the Romanian Society for Cell Biology, 26(01): 799–812. https://www.researchgate. net/publication/359931276_The_use_of_Terminal_ Technology_Gurt_in_Producing_New_Seed_Varieties_ and_Their_Impact_on_Farmers

Kalaitzandonakes, N., Phillips, P. & Wesseler, J., eds. 2016. The Coexistence of Genetically Modified, Organic and Conventional Foods: Government Policies and Market Practices. New York, US, Springer Press. https://link.springer.com/content/ pdf/10.1007/978-1-4939-3727-1.pdf

Kato-Nitta, N., Inagaki, Y., Maeda, T. & Tachikawa, M. 2021. Effects of information on consumer attitudes towards gene-edited foods: a comparison between livestock and vegetables. *CABI Agriculture and Bioscience*, 2(1): 14. https://doi.org/10.1186/s43170-021-00029-8

Kim, H.J., Lee, H.J., Kim, H., Cho, S.W. & Kim, J.S. 2009. Targeted genome editing in human cells with zinc finger nucleases constructed via modular assembly. *Genome Research*, 19: 1279–1288. https:// genome.cshlp.org/content/19/7/1279 Kim, D., Lim, K., Kim, S.T., Yoon, S.H., Kim, K., Ryu, S.M. & Kim, J.S. 2017. Genome-wide target specificities of CRISPR RNA-guided programmable deaminases. *Nature Biotechnology*, 35: 797–797. https://www.nature.com/articles/nbt.3852

Kim, D.E., Lee, J.H., Ji, K.B., Lee, E.J., Li, C., Oh, H.J., Park, K.S. et al. 2022. Prime editor-mediated correction of a pathogenic mutation in purebred dogs. *Scientific Reports*, 12(1): 12905. https://doi. org/10.1038/s41598-022-17200-4

Klümper, W. & Qaim, M. 2014. A meta-analysis of the impacts of genetically modified crops. *PLOS ONE*, 9(11): e111629. https://doi.org/10.1371/journal. pone.0111629

Kniss, A.R. 2018. Genetically engineered herbicideresistant crops and herbicide-resistant weed evolution in the United States. Weed Science, 66(2): 260–273. https://doi.org/10.1017/WSC.2017.70

Kok, E.J., Keijer, J., Kleter, G.A. & Kuiper, H.A. 2008. Comparative safety assessment of plant-derived foods. *Regulatory Toxicology and Pharmacology*, 50(1): 98–113. https://doi.org/10.1016/J. YRTPH.2007.09.007

Kolodinsky, J. & Lusk, J.L. 2018. Mandatory labels can improve attitudes toward genetically engineered food. *Science Advances*, 4(6). https://doi.org/10.1126/sciadv. aaq1413

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A. & Liu, D.R. 2016. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 533: 420–424. https://www.nature. com/articles/nature17946

Koslová, A., Kučerová, D., Reinišová, M., Geryk, J., Trefil, P. & Hejnar, J. 2018. Genetic resistance to avian leukosis viruses induced by CRISPR/ Cas9 editing of specific receptor genes in chicken cells. *Viruses*, 10(11): 605. https://doi.org/10.3390/ v10110605

 Kostriken, R., Strathern, J.N., Klar, A.J.S., Hicks, J.B.
 & Heffron, F. 1983. A site-specific endonuclease essential for mating-type switching in Saccharomyces cerevisiae. *Cell*, 35: 167–174. https://pubmed.ncbi. nlm.nih.gov/6313222/

Kouser, S. & Qaim, M. 2011. Impact of Bt cotton on pesticide poisoning in smallholder agriculture: A panel data analysis. *Ecological Economics*, 70(11): 2105– 2113. https://doi.org/10.1016/j.ecolecon.2011.06.008

Kris-Etherton, P.M., Pearson, T.A., Wan, Y., Hargrove, R.L., Moriarty, K., Fishell, V. & Etherton, T.D. 1999. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *The American Journal of Clinical Nutrition*, 70(6): 1009–1015. https://doi.org/10.1093/ajcn/70.6.1009

Kuiken, T. & Kuzma, J. 2021. Genome Editing in Latin America: Regional Regulatory Overview. Washington, D.C., Inter-American Development Bank. https://doi. org/10.18235/0003410 Kuiper, H.A., Kok, E.J. & Davies, H.V. 2013. New EU legislation for risk assessment of GM food: No scientific justification for mandatory animal feeding trials. *Plant Biotechnology Journal*, 11(7): 781–784. https://doi.org/10.1111/pbi.12091

Kumar, D., Yadav, A., Ahmad, R., Dwivedi, U.N. & Yadav, K. 2022. CRISPR-based genome editing for nutrient enrichment in crops: A promising approach toward global food security. *Frontiers in Genetics*, 13. https://doi.org/10.3389/fgene.2022.932859

Kuzma, J. 2017. Society and Policy Makers' Responsibilities. In: G. Emilien, R. Weitkunat & F. Luedicke, eds. Consumer Perception of Product Risks and Benefits. Dordrecht, Springer.

Kuzma, J. 2019. Procedurally robust risk assessment framework for novel genetically engineered organisms and gene drives. *Regulation & Governance*, 15(4): 1144–1165. https://doi.org/10.1111/REGO.12245

Kuzma, J. 2020. Engineered Gene Drives: Ecological, Environmental, and Societal Concerns. In: A. Chaurasia, D.L. Hawksworth & M. Pessoa de Miranda, eds. *GMOs. Topics in Biodiversity and Conservation.* pp. 371–399. Springer, Cham. https:// doi.org/10.1007/978-3-030-53183-6_17

Kuzma, J. 2021. Deficits of Public Deliberation in U.S. Oversight for Gene Edited Organisms. *Hastings Center Report*, 51(S2): S25–S33. https://doi. org/10.1002/HAST.1317

Kuzma, J. & Besley, J.C. 2008. Ethics of risk analysis and regulatory review: From bio- to nanotechnology. *NanoEthics*, 2(2): 149–162. https://doi.org/10.1007/ s11569-008-0035-x

Kuzma, J. & Grieger, K. 2020. Community-led governance for gene-edited crops: A post-market certification process could promote transparency and trust. *Science*, 370(6519): 916–918. https://www. science.org/doi/10.1126/science.abd1512

Lassoued, R., Macall, D.M., Smyth, S.J., Phillips, P.W.B. & Hesseln, H. 2019. Risk and safety considerations of genome edited crops: Expert opinion. *Current Research in Biotechnology*, 1: 11–21. https://doi.org/10.1016/J.CRBIOT.2019.08.001

Lassoued, R., Macall, D.M., Smyth, S.J., Phillips, P.W.B. & Hesseln, H. 2021. Data challenges for future plant gene editing: expert opinion. *Transgenic Research*, 30: 765–780. https://doi.org/10.1007/ s11248-021-00264-9

Lee, J.K., Jeong, E., Lee, J., Jung, M., Shin, E., Kim, Y.H., Lee, K., Jung, I., Kim, D., Kim, S. *et al.* 2018. Directed evolution of CRISPR-Cas9 to increase its specificity. *Nature Communications*, 9. https://www. ncbi.nlm.nih.gov/pmc/articles/PMC6078992/

 Lee, S.S., Ding, N., Sun, Y.D., Yuan, T.L., Li, J., Yuan, O.C., Liu, L.Z., Yang, J., Wang, O., Kolomeisky,
 A.B. et al. 2020. Single C-to-T substitution using engineered APOBEC3G-nCas9 base editors with minimum genome- and transcriptome-wide off-target effects. *Science Advances*, 6. No. 29. https://www.science.org/doi/full/10.1126/sciadv.aba1773

Leitschuh, C.M., Kanavy, D., Backus, G.A., Valdez, R.X., Serr, M., Pitts, E.A., Threadgill, D. & Godwin, J. 2018. Developing gene drive technologies to eradicate invasive rodents from islands. *Journal of Responsible Innovation*, 5(sup1): S121–S138. https://doi.org/10.108 0/23299460.2017.1365232

Lema, M. 2019. Cómo integrar los aspectos regulatorios en un bioemprendimiento. In: P.A. Pelllegrini, ed. *Biotecnologia y Emprendimientos, Herramientas, Perspectivas y Desafíos*. Bernal, Argentina, UNQ.

Lema, M. 2021. Regulatory assessment of off-target changes and spurious DNA insertions in gene-edited organisms for agri-food use. *Journal of Regulatory Science*, 9(1). https://doi.org/10.21423/jrs-v09i1lema

Li, C., Zhang, R., Meng, X., Chen, S., Zong, Y., Lu, C., Qiu, J.-L. et al. 2020. Targeted, random mutagenesis of plant genes with dual cytosine and adenine base editors. *Nature Biotechnology*, 38(7): 875–882. https:// doi.org/10.1038/s41587-019-0393-7

Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., Li, J. & Gao, C. 2016. Gene replacements and insertions in rice by intron targeting using CRISPR– Cas9. *Nature Plants*, 2(10): 16139. https://doi. org/10.1038/nplants.2016.139

Li, J.Y., Manghwar, H., Sun, L., Wang, P.C., Wang, G.Y., Sheng, H.Y., Zhang, J., Liu, H., Qin, L., Rui, H.P. et al. 2018. Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9edited cotton plants. *Plant Biotechnology Journal*, 17: 858–868. https://onlinelibrary.wiley.com/doi/ full/10.1111/pbi.13020

Li, P, Estrada, J.L., Burlak, C., Montgomery, J., Butler, J.R., Santos, R.M., Wang, Z.Y. et al. 2015. Efficient generation of genetically distinct pigs in a single pregnancy using multiplexed single-guide RNA and carbohydrate selection. *Xenotransplantation*, 22(1): 20–31. https://doi.org/10.1111/xen.12131

Li, S., Lin, D., Zhang, Y., Deng, M., Chen, Y., Lv, B., Li, B. et al. 2022. Genome-edited powdery mildew resistance in wheat without growth penalties. *Nature*, 602(7897): 455–460. https://doi.org/10.1038/s41586-022-04395-9

Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., Dong, W., Gao, C. & Xu, C. 2018. Domestication of wild tomato is accelerated by genome editing. *Nature Biotechnology*, 36(12): 1160–1163. https://doi. org/10.1038/nbt.4273

Liang, P.P., Xie, X.W., Zhi, S.Y., Sun, H.W., Zhang, X.Y., Chen, Y., Chen, Y.X., Xiong, Y.Y., Ma, W.B., Liu, D. et al. 2019. Genome-wide profiling of adenine base editor specificity by EndoV-seq. Nature Communications, 10, No. 67. https://www.nature. com/articles/s41467-018-07988-z

References

 Liang, Z., Chen, K.L., Li, T.D., Zhang, Y., Wang, Y.P.,
 Zhao, Q., Liu, J.X., Zhang, H.W., Liu, C.M., Ran,
 Y.D. et al. 2017. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nature Communications*, 8. https://www. nature.com/articles/ncomms14261

Lillico, S.G., Proudfoot, C., Carlson, D.F., Stverakova, D., Neil, C., Blain, C., King, T.J. et al. 2013. Live pigs produced from genome edited zygotes. *Scientific Reports*, 3. https://doi.org/10.1038/srep02847

Liu, X., Qin, R., Li, J., Liao, S., Shan, T., Xu, R., Wu, D. & Wei, P. 2020. A CRISPR-Cas9-mediated domainspecific base-editing screen enables functional assessment of ACCase variants in rice. *Plant Biotechnology Journal*, 18(9): 1845–1847. https://doi. org/10.1111/PBI.13348

Liu, X., Wang, Y., Guo, W., Chang, B., Liu, J., Guo, Z., Quan, F. & Zhang, Y. 2013. Zinc-finger nickasemediated insertion of the lysostaphin gene into the beta-casein locus in cloned cows. *Nature Communications*, 4. https://doi.org/10.1038/ ncomms3565

Lombardo, L. 2014. Genetic use restriction technologies: a review. *Plant Biotechnology Journal*, 12(8): 995– 1005. https://doi.org/10.1111/PBI.12242

Louafi, S. & Welch, E. 2021. Improving the global exchange of germplasm for crop breeding. In: *taylorfrancis.com*. pp. 81–100. https://doi. org/10.19103/AS.2020.0085.20

Magalhães, A.L.B., Brito, M.F.G. & Silva, L.G.M. 2022. The fluorescent introduction has begun in the southern hemisphere: presence and life-history strategies of the transgenic zebrafish Danio rerio (Cypriniformes: Danionidae) in Brazil. *Studies on Neotropical Fauna and Environment*: 1–13. https://doi. org/10.1080/01650521.2021.2024054

Mallapaty, S. 2022. How to protect the first "CRISPR babies" prompts ethical debate. *Nature*, 603(7900): 213–214. https://doi.org/10.1038/D41586-022-00512-W

Mancini, F. 2006. Impact of integrated pest management farmer field schools on health, farming systems, the environment, and livelihoods of cotton growers in Southern India. Wageningen, NL, Wageningen University. PhD Thesis.

Matres, J.M., Hilscher, J., Datta, A., Armario-Nájera, V., Baysal, C., He, W., Huang, X. *et al.* 2021. Genome editing in cereal crops: an overview. *Transgenic Research*, 30(4): 461–498. https://doi.org/10.1007/ s11248-021-00259-6

May, R.M. 1981. The world's worst weeds. *Nature*, 290(5802): 85–86. https://doi.org/10.1038/290085a0

Mayet, M. 2022. The battle over regulation of new breeding techniques in South Africa. https://www.acbio.org.za/battle-over-regulation-new-breeding-techniques-south-africa

McConnachie, E., Hötzel, M.J., Robbins, J.A., Shriver, A., Weary, D.M. & Von Keyserlingk, M.A.G. 2019. Public attitudes towards genetically modified polled cattle. *PLOS ONE*, 14(5): e0216542. https://doi. org/10.1371/JOURNAL.PONE.0216542

Meghani, Z. 2014. Risk Assessment of genetically modified food and neoliberalism: An argument for democratizing the regulatory review protocol of the Food and Drug Administration. *Journal of Agricultural and Environmental Ethics*, 27(6): 967–989. https://doi. org/10.1007/s10806-014-9511-1

Meghani, Z. & Kuzma, J. 2011. The "Revolving Door" between regulatory agencies and industry: A problem that requires reconceptualizing objectivity. *Journal of Agricultural and Environmental Ethics*, 24(6): 575–599. https://doi.org/10.1007/s10806-010-9287-x

Meghani, Z. & Kuzma, J. 2018. Regulating animals with gene drive systems: lessons from the regulatory assessment of a genetically engineered mosquito. *Journal of Responsible Innovation*, 5(sup1): S203– S222. https://doi.org/10.1080/23299460.2017.1407912

Min, J., Smidler, A.L., Najjar, D. & Esvelt, K.M. 2018. Harnessing gene drive. *Journal of Responsible Innovation*, 5(sup1): S40–S65. https://doi.org/10.1080/ 23299460.2017.1415586

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., Danka, B. & Le Conte,
Y. 2020. Honey bee survival mechanisms against the parasite Varroa destructor: a systematic review of phenotypic and genomic research efforts. *International Journal for Parasitology*, 50(6–7): 433–447. https://doi.org/10.1016/j.ijpara.2020.03.005

 Montaner-Tarbes, S., del Portillo, H.A., Montoya, M.
 & Fraile, L. 2019. Key gaps in the knowledge of the porcine respiratory reproductive syndrome virus (PRRSV). *Frontiers in Veterinary Science*, 6(FEB): 38. https://doi.org/10.3389/fvets.2019.00038

Moutinho, S. 2022. Transgenic fish invades Brazilian streams. *Science*, 375(6582): 704–705. https://doi.org/10.1126/SCIENCE.ADA1331

Mussolino, C., Morbitzer, R., Lutge, F., Dannemann, N., Lahaye, T. & Cathomen, T. 2011. A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity. *Nucleic Acids Research*, 39: 9283–9293. https://www.ncbi.nlm.nih. gov/pmc/articles/PMC3241638/

NASEM. 2016a. Genetically Engineered Crops: Experiences and Prospects. Washington D.C., National Academies Press. https://doi. org/10.17226/23395

NASEM. 2016b. Gene Drives on the Horizon. Washington, D.C., National Academies Press. https:// doi.org/10.17226/23405

NASEM. 2017. Preparing for Future Products of Biotechnology. Washington, D.C., National Academies Press. https://doi.org/10.17226/24605

- Nationale Akademie der Wissenschaften Leopoldina, Deutsche Forschungsgemeinschaft und Union der Deutschen Akademien der Wissenschaften. 2019. Towards a scientifically justified, differentiated regulation of genome edited plants in the EU. Halle (Saale).
- Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D. & Kamoun, S. 2017. Rapid generation of a transgenefree powdery mildew resistant tomato by genome deletion. *Scientific Reports*, 7(1): 482. https://doi. org/10.1038/s41598-017-00578-x
- Nlend Nkott, A.L. & Temple, L. 2021. Societal acceptability conditions of genome editing for upland rice in Madagascar. *Technological Forecasting and Social Change*, 167: 120720. https://doi.org/10.1016/J. TECHFORE.2021.120720
- Nonaka, S., Arai, C., Takayama, M., Matsukura, C. & Ezura, H. 2017. Efficient increase of y-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Scientific Reports*, 7(1): 7057. https:// doi.org/10.1038/s41598-017-06400-y
- **OECD**. 1986. Recombinant DNA Safety Considerations (Safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA techniques). Prepared by Ad hoc Group of government experts created by that Committee in July 1983, with the assistance of the OECD. Paris, Organisation for Economic Co-operation and Development, 1986.
- **OECD**. 2001. Adoption of technologies for sustainable farming systems Wageningen Workshop Proceedings. https://www.oecd.org/greengrowth/sustainableagriculture/2739771.pdf
- Ohama, M., Washio, Y., Kishimoto, K., Kinoshita, M. & Kato, K. 2020. Growth performance of myostatin knockout red sea bream Pagrus major juveniles produced by genome editing with CRISPR/Cas9. *Aquaculture*, 529: 735672. https://doi.org/10.1016/J. AQUACULTURE.2020.735672
- **Ohlgart, S.M.** 2002. The terminator gene: intellectual property rights vs. the farmers' common law right to save seed. *Drake Journal of Agricultural Law*, 7(2). https://aglawjournal.wp.drake.edu/wp-content/uploads/sites/66/2016/09/agVoI07No2-Ohlgart.pdf
- **OIE**. 2021. Porcine reproductive and respiratory syndrome (infection with porcine reproductive and respiratory syndrome virus. In: *OIE Terrestrial Manual 2021*. Chapter 3.9.6.
- Oliva, R., Ji, C., Atienza-Grande, G., Huguet-Tapia, J.C., Perez-Quintero, A., Li, T., Eom, J.-S. et al. 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nature Biotechnology*, 37(11): 1344–1350. https://doi.org/10.1038/s41587-019-0267-z
- Pattanayak, V., Lin, S., Guilinger, J.P., Ma, E., Doudna, J.A. & Liu, D.R. 2013. High-throughput profiling of off-target DNA cleavage reveals RNA-programmed

Cas9 nuclease specificity. *Nature Biotechnology*, 31: 839–843. https://pubmed.ncbi.nlm.nih.gov/23934178/

- Paull, J. 2019. Contamination of farms by genetically modified organisms (GMOs): Options for compensation. *Journal of Organics*, 6(1). https:// www.academia.edu/40200835/Contamination_of_ Farms_by_Genetically_Modified_Organisms_GMOs_ Options_for_Compensation
- Pingali, P.L. 2012. Green Revolution: Impacts, limits, and the path ahead. *Proceedings of the National Academy* of Sciences, 109(31): 12302–12308. https://doi. org/10.1073/pnas.0912953109
- Pixley, K.V., Falck-Zepeda, J.B., Paarlberg, R.L., Phillips, P.W.B., Slamet-Loedin, I.H., Dhugga, K.S., Campos, H. & Gutterson, N. 2022. Genome-edited crops for improved food security of smallholder farmers. *Nature Genetics*, 54(4): 364–367. https://doi. org/10.1038/s41588-022-01046-7
- **Ponting, C.** 2007. A Green History of the World: The Environment and the Collapse of Great Civilizations. New York, NY, Penguin Books.
- Pray, C., Ma, D., Huang, J. & Qiao, F. 2001. Impact of Bt Cotton in China. World Development, 29(5): 813–825. https://doi.org/10.1016/S0305-750X(01)00010-9
- Pray, C.E., Ribeiro, S., Mueller, R.A.E. & Rao, P.P. 1991. Private research and public benefit: The private seed industry for sorghum and pearl millet in India. *Research Policy*, 20(4): 315–324. https://doi. org/10.1016/0048-7333(91)90092-5
- President of the United Mexican States. 2020. Mexico Decree Banning Glyphosate and GE Corn. https://www.dof.gob.mx/nota_detalle. php?codigo=5609365&fecha=31/12/2020
- Proudfoot, C., Carlson, D.F., Huddart, R., Long, C.R., Pryor, J.H., King, T.J., Lillico, S.G. et al. 2015. Genome edited sheep and cattle. *Transgenic Research*, 24(1): 147–153. https://doi.org/10.1007/ s11248-014-9832-x
- Punt, M. & Wesseler, J. 2016. Legal but costly: An analysis of the EU GM regulation in the light of the WTO trade dispute between the EU and the US. World Economy, 39(1):158–169. https://doi. org/10.1111/twec.12353
- Purnhagen, K. & Wesseler, J. 2020. EU Regulation of new plant breeding technologies and their possible economic implications for the EU and beyond. *Applied Economic Perspectives and Policy*, 43(4): 1621–1637. https://doi.org/10.1002/aepp.13084
- Purnhagen, K.P., Kok, E., Kleter, G., Schebesta, H., Visser, R.G.F. & Wesseler, J. 2018. The European Union court's advocate general's opinion and new plant breeding techniques. *Nature Biotechnology*, 36(7): 573–575. https://doi.org/10.1038/nbt.4174
- Qaim, M. 2009. The economics of genetically modified crops. Annual Review of Resource Economics, 1(1): 665–694. https://doi.org/10.1146/annurev. resource.050708.144203

References

- **Qaim, M.** 2016. *Genetically Modified Crops and Agricultural Development*. Palgrave Macmillan US. https://www.springerprofessional.de/geneticallymodified-crops-and-agricultural-development/10085686
- **Qaim, M.** 2020. Role of new plant breeding technologies for food security and sustainable agricultural development. *Applied Economic Perspectives and Policy*, 42(2): 129–150. https://doi.org/10.1002/ aepp.13044
- Qaim, M. & Zilberman, D. 2003. Yield effects of genetically modified crops in developing countries. *Science*, 299(5608): 900–902. https://doi.org/10.1126/ SCIENCE.1080609
- Raffan, S., Sparks, C., Huttly, A., Hyde, L., Martignago, D., Mead, A., Hanley, S.J. *et al.* 2021. Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene TaASN2. *Plant Biotechnology Journal*, 19(8): 1602–1613. https://doi. org/10.1111/PBI.13573
- **RAFI**. 1997. Bolivian farmers demand researchers drop patent on Andean food crop. Press release:18 June 1997. https://www.etcgroup.org/content/bolivianfarmers-demand-researchers-drop-patent-andeanfood-crop
- Rapela, M.A. 2019. The Regulatory Tangle. In: *Fostering Innovation for Agriculture 4.0*. pp. 17–52. Cham, Springer International Publishing. https://doi. org/10.1007/978-3-030-32493-3_2
- Rausser, G., Simon, L. & Ameden, H. 2000. Public– private alliances in biotechnology: Can they narrow the knowledge gaps between rich and poor? *Food Policy*, 25(4): 499–513. https://doi.org/10.1016/S0306-9192(00)00018-X
- Rees, H.A., Komor, A.C., Yeh, W.H., Caetano-Lopes, J., Warman, M., Edge, A.S.B. & Liu, D.R. 2017. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. *Nature Communications*, 8, No. 15790. https://www.nature.com/articles/ncomms15790/
- Richter, M.F., Zhao, K.T., Eton, E., Lapinaite, A., Newby, G.A., Thuronyi, B.W., Wilson, C., Koblan, L.W., Zeng, J., Bauer, D.E. *et al.* 2020. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. *Nature Biotechnology*, 38: 883–891. https://www.nature.com/ articles/s41587-020-0453-z
- **Rissing, A.** 2021. "We feed the world": the political ecology of the Corn Belt's driving narrative. *Journal* of *Political Ecology*, 28(1): 471–487. https://doi. org/10.2458/JPE.2959
- Robinson, C. 2022. Behind the smoke screen: Vested interests of EU scientists lobbying for GMO deregulation. The Greens/EFA in the European Parliament. https://www.greens-efa.eu/files/assets/ docs/greens_gmo_scientists_mainreport_final_2.pdf

- Robinson, J. 1999. Ethics and transgenic crops: a review. *Electronic Journal of Biotechnology*, 2(2): 5–6. http://www.ejbiotechnology.info/index.php/ ejbiotechnology/article/view/v2n2-3/821
- Rodríguez-Leal, D., Lemmon, Z.H., Man, J., Bartlett, M.E. & Lippman, Z.B. 2017. Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, 171(2): 470–480.e8. https://doi. org/10.1016/j.cell.2017.08.030
- Rola, A.C. & Pingali, P.L. 1993. Pesticides, rice productivity, and farmers' health: an economic assessment. Manila: IRRI. https://books.google. com/books/about/Pesticides_Rice_Productivity_and_ Farmers.html?hl=de&id=ayjWoNqOe4UC
- Romeis, J., Collatz, J., Glandorf, D.C.M. & Bonsall, M.B. 2020. The value of existing regulatory frameworks for the environmental risk assessment of agricultural pest control using gene drives. *Environmental Science & Policy*, 108: 19–36. https:// doi.org/10.1016/J.ENVSCI.2020.02.016
- Rose, K.M., Howell, E.L., Su, L.Y.F., Xenos, M.A., Brossard, D. & Scheufele, D.A. 2019. Distinguishing scientific knowledge: The impact of different measures of knowledge on genetically modified food attitudes. *Public Understanding of Science*, 28(4): 449–467. https://doi.org/10.1177/0963662518824837
- Rosen, J. 2018. GMO grass is creeping across Oregon (little weed, big problem) — High Country News – Know the West. Cited 10 August 2022. https://www. hcn.org/issues/50.11/plants-genetically-modifiedgrass-creeps-across-eastern-oregon
- Sánchez-León, S., Gil-Humanes, J., Ozuna, C.V., Giménez, M.J., Sousa, C., Voytas, D.F. & Barro, F. 2017. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnology Journal*, 16(4): 902–910. https://doi.org/10.1111/PBI.12837
- Sander, J.D. & Joung, J.K. 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*, 32: 347–355. https://www.nature. com/articles/nbt.2842
- Scheepmaker, J.W.A., Hogervorst, P.A.M. & Glandorf, D.C.M. 2016. Future introductions of genetically modified microbial biocontrol agents in the EU. Are current EU legislation and risk assessment fit for purpose? RIVM Letter Report 2016-0057
- Scheinerman, N. & Sherkow, J.S. 2021. Governance choices of genome editing patents. *Frontiers in Political Science*, 3: 745898. https://doi.org/10.3389/ fpos.2021.745898
- Schenkelaars, P., de Vriend, H., Kalaitzandonakes, N., Magnier, A. & Miller, D. 2011. Drivers of Consolidation in the Seed Industry and its Consequences for Innovation. Report commissioned by COGEM. https://www.lisconsult.nl/files/docs/ consolidation_seed_industry.pdf
- Schiemann, J. 2006. The OECD Blue Book on recombinant DNA safety considerations: it's influence
on ISBR and EFSA activities. *Environmental Biosafety Research*, 5(4): 233–235. https://doi.org/10.1051/ ebr:2007014

- Schiemann, J., Steinberg, P. & Salles, B. 2014. Facilitating a transparent and tailored scientific discussion about the added value of animal feeding trials as well as in vitro and in silico approaches with whole food/feed for the risk assessment of genetically modified plants. *Archives of Toxicology*, 88(12): 2067– 2069. https://doi.org/10.1007/s00204-014-1375-7
- Schmidt, K., Döhring, J., Kohl, C., Pla, M., Kok, E.J., Glandorf, D.C.M., Custers, R. et al. 2016. Proposed criteria for the evaluation of the scientific quality of mandatory rat and mouse feeding trials with whole food/feed derived from genetically modified plants. *Archives of Toxicology*, 90(9): 2287–2291. https://doi. org/10.1007/s00204-016-1762-3
- Schnurr, M.A. & Dowd-Uribe, B. 2021. Anticipating farmer outcomes of three genetically modified staple crops in sub-Saharan Africa: Insights from farming systems research. *Journal of Rural Studies*, 88: 377–387. https://www.sciencedirect.com/science/ article/abs/pii/S0743016721002266
- Schultz-Bergin, M. 2018. Is CRISPR an ethical game changer? *Journal of Agricultural and Environmental Ethics*, 31(2): 219–238. https://doi.org/10.1007/ s10806-018-9721-z
- Scott, S.E., Inbar, Y., Wirz, C.D., Brossard, D. & Rozin, P. 2018. An overview of attitudes toward genetically engineered food. *Annual Review of Nutrition*, 38(1): 459–479. https://doi.org/10.1146/annurevnutr-071715-051223
- Shan, Q.W., Wang, Y.P., Li, J., Zhang, Y., Chen, K.L., Liang, Z., Zhang, K., Liu, J.X., Xi, J.J., Qiu, J.L. et al. 2013. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature Biotechnology*, 31: 686–688. https://www.nature.com/articles/nbt.2650
- Shew, A.M., Nalley, L.L., Snell, H.A., Nayga, R.M. & Dixon, B.L. 2018. CRISPR versus GMOs: Public acceptance and valuation. *Global Food Security*, 19: 71–80. https://doi.org/10.1016/J.GFS.2018.10.005
- Shi, J., Gao, H., Wang, H., Lafitte, H.R., Archibald, R.L., Yang, M., Hakimi, S.M., Mo, H. & Habben, J.E. 2017. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnology Journal*, 15(2): 207–216. https://doi.org/10.1111/PBI.12603
- Shleifer, A. 2010. Efficient regulation. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.1532802
- Siegrist, M. 2008. Factors influencing public acceptance of innovative food technologies and products. *Trends in Food Science & Technology*, 19(11): 603–608. https://doi.org/10.1016/j.tifs.2008.01.017
- Siegrist, M., Connor, M. & Keller, C. 2012. Trust, confidence, procedural fairness, outcome fairness, moral conviction, and the acceptance of GM field

experiments. *Risk Analysis*, 32(8): 1394–1403. https://doi.org/10.1111/J.1539-6924.2011.01739.X

- Sikkema, A. 2021. WUR makes CRISPR-Cas patents available for free – Resource online. Cited 15 August 2022. https://www.resource-online.nl/index. php/2021/09/07/wur-makes-crispr-cas-patentsavailable-for-free/?lang=en
- Smith, K. 2021. The Public's Perceptions of Genome Editing in Agriculture: A Review. Oklahoma State University. https://shareok.org/bitstream/ handle/11244/332577/oksd_smith_HT_2021. pdf?sequence=1&isAllowed=y
- Smith, V., Wesseler, J.H.H. & Zilberman, D. 2021. New plant breeding technologies: An assessment of the political economy of the regulatory environment and implications for sustainability. *Sustainability*, 13(7): 3687. https://doi.org/10.3390/su13073687
- Smýkal, P., Nelson, M., Berger, J. & Von Wettberg, E. 2018. The impact of genetic changes during crop domestication. *Agronomy*, 8(7): 119. https://doi. org/10.3390/agronomy8070119
- Sprink, T., Eriksson, D., Schiemann, J. & Hartung, F. 2016. Regulatory hurdles for genome editing: process- vs. product-based approaches in different regulatory contexts. *Plant Cell Reports*, 35(7): 1493– 1506. https://doi.org/10.1007/S00299-016-1990-2
- Sprink, T., Wilhelm, R.A., Spök, A., Robienski, J., Schleissing, S. & Schiemann, J.H., eds. 2020. Plant Genome Editing – Policies and Governance. Frontiers Research Topics. Frontiers Media SA. https://doi. org/10.3389/978-2-88963-670-9
- Stenseth, N. Chr., Andersson, L. & Hoekstra, H.E. 2022. Gregor Johann Mendel and the development of modern evolutionary biology. *Proceedings of the National Academy of Sciences*, 119(30). https://doi. org/10.1073/pnas.2201327119
- Stevenson, J.R., Villoria, N., Byerlee, D., Kelley, T. & Maredia, M. 2013. Green Revolution research saved an estimated 18 to 27 million hectares from being brought into agricultural production. *Proceedings of the National Academy of Sciences*, 110(21): 8363– 8368. https://doi.org/10.1073/pnas.1208065110
- Stone, G.D. 2010. The anthropology of genetically modified crops. *Annual Review of Anthropology*, 39(1): 381–400. https://doi.org/10.1146/annurev. anthro.012809.105058
- Su, S., Ezhuthachan, I.D. & Ponda, P. 2020. Genetically modified foods and food allergy. *Journal of Food Allergy*, 2(1): 111–114. https://doi.org/10.2500/ jfa.2020.2.200012
- Tan, W., Carlson, D.F., Lancto, C.A., Garbe, J.R., Webster, D.A., Hackett, P.B. & Fahrenkrug, S.C.
 2013. Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proceedings* of the National Academy of Sciences, 110(41): 16526– 16531. https://doi.org/10.1073/pnas.1310478110

- Tan, Y., Du, H., Wu, X., Liu, Y., Jiang, M., Song, S., Wu,
 L. & Shu, Q. 2020. Gene editing: an instrument for practical application of gene biology to plant breeding. *Journal of Zhejiang University-SCIENCE B*, 21(6): 460–473. https://doi.org/10.1631/jzus.B1900633
- Tang, X., Liu, G.Q., Zhou, J.P., Ren, Q.R., You, Q., Tian, L., Xin, X.H., Zhong, Z.H., Liu, B.L., Zheng, X.L. et al. 2018. A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. Genome Biology 19. https://genomebiology.biomedcentral.com/ articles/10.1186/s13059-018-1458-5
- **The Roslin Institute**. 2020. PRRS Resistant Pigs. Cited 14 April 2022. https://www.ed.ac.uk/roslin/facilitiesresources/larif/case-studies/industry-partners
- The Roslin Institute. 2021. Agreement targets PRRS-resistant gene-edited pigs. Cited 15 August 2022. https://www.thepigsite.com/news/2021/09/ agreement-targets-prrs-resistant-gene-edited-pigs
- Then, C., Bauer-Panskus, A. & Tippe, R. 2021. New GE and food plants: The disruptive impact of patents on breeders, food production and society. Munich, Testbiotech e.V. https://www.testbiotech.org/en/ content/new-ge-and-food-plants-disruptive-impactpatents-breeders-food-production-and-society
- Thompson, D. & Benjamin, M. 2021. UPDATE: Progress toward PRRSv-resistant pigs. https://www.canr.msu. edu/news/update-progress-toward-prrsv-resistant-pigs
- Thompson, PB. 2003. Value judgments and risk comparisons. The case of genetically engineered crops. *Plant Physiology*, 132(1): 10–16. https://doi.org/10.1104/PP.103.022095
- Thompson, P.B. 2018. The roles of ethics in gene drive research and governance. *Journal of Responsible Innovation*, 5(sup1): S159–S179. https://doi.org/10.108 0/23299460.2017.1415587
- Thompson, P.B. & Hannah, W. 2008. Food and agricultural biotechnology: A summary and analysis of ethical concerns. In: *Food Biotechnology*. pp. 229–264. Vol. 111. Berlin, Heidelberg, Springer Berlin Heidelberg. https://doi.org/10.1007/10_2008_100
- Tome, K.G.N. 2021. First Commercialized Gene-edited Plant Developed Using TALENs. Cited 22 September 2022. https://www.isaaa.org/blog/entry/default. asp?BlogDate=1/13/2021
- Tramper, J. & Zhu, Y. 2011. Modern biotechnology: for better or for worse? *Modern Biotechnology*: 273–274. https://www.researchgate.net/publication/285066111_ Modern_Biotechnology_for_Better_or_for_Worse
- Tran, M.T., Doan, D.T.H., Kim, J., Song, Y.J., Sung, Y.W., Das, S., Kim, E.J. et al. 2021. CRISPR/Cas9-based precise excision of SIHyPRP1 domain(s) to obtain salt stress-tolerant tomato. *Plant Cell Reports*, 40(6): 999–1011. https://doi.org/10.1007/S00299-020-02622-Z
- Trigo, E., Chavarria, H., Pray, C., Smyth, S.J., Torroba, A., Wesseler, J., Zilberman, D. & Martinez, J.F. 2021. The Bioeconomy and Food Systems Transformation.

Food Systems Summit Brief prepared by Research Partners of the Scientific Group for the Food Systems Summit February 17, 2021. Center for Development Research (ZEF) in cooperation with the Scientific Group for the UN Food System Summit 2021. https:// doi.org/10.48565/SCFSS2021-W513

- Trotsenko, O. & Slukin, S. 2020. Public-private partnerships for agricultural innovation: opportunities and challenges in world practice. *E3S Web of Conferences*, 176: 06004. https://doi.org/10.1051/ E3SCONF/202017606004
- Tsatsakis, A.M., Nawaz, M.A., Kouretas, D., Balias, G., Savolainen, K., Tutelyan, V.A., Golokhvast, K.S. et al. 2017. Environmental impacts of genetically modified plants: A review. Environmental Research, 156: 818–833. https://doi.org/10.1016/J. ENVRES.2017.03.011
- Turnbull, C., Lillemo, M. & Hvoslef-Eide, T.A.K. 2021. Global regulation of genetically modified crops amid the gene edited crop boom – A review. *Frontiers in Plant Science*, 12: 258. https://pubmed.ncbi.nlm.nih. gov/33719302/
- Tutton, M. 2021. AquaBounty's GM salmon "available for sale" in Canada, says CFIA | CBC News. Cited 21 August 2022. https://www.cbc.ca/news/canada/ prince-edward-island/pei-aquabounty-salmon-salecp-1.6210851
- Ubalua, A.O. 2009. Transgenic plants: successes and controversies. *Biotechnology and Molecular Biology Reviews*, 4(6): 118–127. https://academicjournals.org/ article/article1380104928_Ubalua.pdf
- **UNCTAD**. 2021. Technology and innovation report 2021: catching technological waves, innovation with equity: xxvi, 170 pp.
- **UNEP**. 2019. Global Environment Outlook GEO-6: Healthy Planet, Healthy People. https://wedocs.unep. org/handle/20.500.11822/

27539

- Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S. & Gregory, P.D. 2010. Genome editing with engineered zinc finger nucleases. *Nature Reviews Genetics*, 11: 636–646. https://www.nature.com/articles/nrg2842
- USDA. 2019. National Organic Standards Board Materials/GMO Subcommittee Proposal Excluded Methods Determinations October 2019. August 13, 2019. https://www.ams.usda.gov/sites/default/files/ media/MSExcludedMethodsProposaFall2019.pdf
- **USDA**. 2021. United States Department of Agriculture Foreign Agricultural Service. Mexico Agricultural Biotechnology Annual Report Number: MX2021-003.
- USDA. 2022. United States Department of Agriculture. Regulated Article Letters of Inquiry. https://www. aphis.usda.gov/aphis/ourfocus/biotechnology/ami-regulated/regulated_article_letters_of_inquiry/ regulated_article_letters_of_inquiry

Van Der Straeten, D., Bhullar, N.K., De Steur, H. et al. 2020. Multiplying the efficiency and impact of biofortification through metabolic engineering. *Nature Communications*, 11: 5203. https://doi.org/10.1038/ s41467-020-19020-4

Van Eenennaam, A.L., De Figueiredo Silva, F, Trott, J.F. & Zilberman, D. 2021. Genetic engineering of livestock: The opportunity cost of regulatory delay. Annual Review of Animal Biosciences, 9(1): 453–478. https://doi.org/10.1146/annurev-animal-061220-023052

Venus, T.J., Dillen, K., Punt, M.J. & Wesseler, J.H.H. 2016. The costs of coexistence measures for genetically modified maize in Germany. *Journal of Agricultural Economics*, 68(2): 407–426. https://doi. org/10.1111/1477-9552.12178

Voytas, D.F. 2013. Plant genome engineering with sequence-specific nucleases. *Annual Review of Plant Biology*, 64: 327–350. https://www.annualreviews. org/doi/abs/10.1146/annurev-arplant-042811-105552

Waltz, E. 2021. GABA-enriched tomato is first CRISPRedited food to enter market. *Nature Biotechnology*, 40(1): 9–11. https://doi.org/10.1038/d41587-021-00026-2

Wan, D.Y., Guo, Y., Cheng, Y., Hu, Y., Xiao, S., Wang, Y. & Wen, Y.Q. 2020. CRISPR/Cas9-mediated mutagenesis of VvMLO3 results in enhanced resistance to powdery mildew in grapevine (Vitis vinifera). *Horticulture Research*, 7(1): 116. https://pubmed.ncbi. nlm.nih.gov/32821399/

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., Liu, Y.-G. & Zhao, K. 2016. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PLOS ONE*, 11(4): e0154027. https://doi.org/10.1371/journal.pone.0154027

Wang, Q., Nian, J., Xie, X., Yu, H., Zhang, J., Bai, J., Dong, G. et al. 2018. Genetic variations in ARE1 mediate grain yield by modulating nitrogen utilization in rice. *Nature Communications*, 9(1): 735. https://doi. org/10.1038/s41467-017-02781-w

Wang, W., Pan, Q., Tian, B., He, F., Chen, Y., Bai, G., Akhunova, A., Trick, H.N. & Akhunov, E. 2019. Gene editing of the wheat homologs of TONNEAU1recruiting motif encoding gene affects grain shape and weight in wheat. *The Plant Journal*, 100(2): 251–264. https://doi.org/10.1111/TPJ.14440

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C. & Qiu, J.-L. 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, 32(9): 947–951. https://doi. org/10.1038/nbt.2969

Wargelius, A., Leininger, S., Skaftnesmo, K.O., Kleppe, L., Andersson, E., Taranger, G.L., Schulz, R.W. & Edvardsen, R.B. 2016. Dnd knockout ablates germ cells and demonstrates germ cell independent sex differentiation in Atlantic salmon. *Scientific Reports*, 6(1): 21284. https://doi.org/10.1038/srep21284 Weaver, R.D. & Wessler, J. 2004. Monopolistic pricing power for transgenic crops when technology adopters face irreversible benefits and costs. *Applied Economics Letters*, 11(15): 969–973. https://doi. org/10.1080/1350485042000285814

Welsh, R. & Glenna, L. 2006. Considering the role of the university in conducting research on agribiotechnologies. *Social Studies of Science*, 36(6): 929–942. https://doi.org/10.1177/0306312706060062

Wesseler, J. 2019. Perspective: regulation of pest and disease control strategies and why (many) economists are concerned. *Pest Management Science*, 75(3): 578–582. https://doi.org/10.1002/ ps.5204

Wesseler, J., Jongeneel, R. & Purnhagen, K. 2019. Bioeconomy Economics and Policies. pp. 7–16. Palgrave Macmillan, Cham. https://doi. org/10.1007/978-3-030-28634-7_2

Wesseler, J., Kleter, G., Meulenbroek, M. & Purnhagen, K.P. 2022. EU regulation of genetically modified microorganisms in light of new policy developments: Possible implications for EU bioeconomy investments. Applied Economic Perspectives and Policy. https://doi.org/10.1002/aepp.13259

Wesseler, J., Scatasta, S. & Hadji Fall, E. 2011. Chapter 7, The Environmental Benefits and Costs of Genetically Modified (GM) Crops. In: *Frontiers of Economics and Globalization*. pp. 173–199. Vol. 10. Emerald Group Publishing Ltd. https://doi.org/10.1108/ S1574-8715(2011)0000010012

West, S.C. 2003. Molecular views of recombination proteins and their control. *Nature Reviews Molecular Cell Biology*, 4: 435–445. https://www.nature.com/ articles/nrm1127

WFP. 2020. 2020 – Global Report on Food Crises. https:// www.wfp.org/publications/2020-global-report-foodcrises

Wheeler, T. & von Braun, J. 2013. Climate change impacts on global food security. *Science*, 341(6145): 508–513. https://doi.org/10.1126/science.1239402

Whelan, A.I., Gutti, P. & Lema, M.A. 2020. Gene editing regulation and innovation economics. Frontiers in Bioengineering and Biotechnology, 8: 303. https:// pubmed.ncbi.nlm.nih.gov/32363186/

Whitworth, K.M., Rowland, R.R.R., Ewen, C.L., Trible, B.R., Kerrigan, M.A., Cino-Ozuna, A.G., Samuel, M.S. et al. 2016. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nature Biotechnology, 34(1): 20–22. https://doi. org/10.1038/nbt.3434

Willi, M., Smith, H.E., Wang, C.C., Liu, C.Y. & Hennighausen, L. 2018. Mutation frequency is not increased in CRISPR-Cas9-edited mice. *Nature Methods*, 15: 756–758. https://www.nature.com/ articles/s41592-018-0148-2

- de Wit, M.M. 2020. Democratizing CRISPR? Stories, practices, and politics of science and governance on the agricultural gene editing frontier. *Elementa*, 8. https://food.berkeley.edu/wp-content/uploads/2020/07/2020_Montenegro-de-wit_Democratizing-CRISPR_-Stories-practices-and-politics-of-science-and-governance-on-the-agricultural-gene-editing-frontier.pdf
- World Resources Institute. 2019. Creating a Sustainable Food Future: A Menu of Solutions. World Resources Institute. https://www.wri.org/research/creatingsustainable-food-future
- Woźniak, E., Tyczewska, A. & Twardowski, T. 2020. A shift towards biotechnology: Social opinion in the EU. *Trends in Biotechnology*, 39(3): 214–218. https://doi. org/10.1016/j.tibtech.2020.08.001
- Wringe, B.F., Jeffery, N.W., Stanley, R.R.E., Hamilton, L.C., Anderson, E.C., Fleming, I.A., Grant, C. et al. 2018. Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. Communications Biology, 1(1): 108. https:// doi.org/10.1038/s42003-018-0112-9
- Wu, H., Zhang, Y., Wang, Y., Zhang, Y., Yang, M., Lv, J. & Liu, J. 2015. TALE nickase-mediated SP110 knockin endows cattle with increased resistance to tuberculosis. *Proceedings of the National Academy* of Sciences, 112(13): E1530–E1539. https://pubmed. ncbi.nlm.nih.gov/25733846/
- Xing, S., Chen, K., Zhu, H., Zhang, R., Zhang, H., Li, B. & Gao, C. 2020. Fine-tuning sugar content in strawberry. *Genome Biology*, 21(1): 1–14. https:// pubmed.ncbi.nlm.nih.gov/32883370/
- Young, A.E., Mansour, T.A., McNabb, B.R., Owen, J.R., Trott, J.F., Brown, C.T. & Van Eenennaam, A.L. 2020. Genomic and phenotypic analyses of six offspring of a genome-edited hornless bull. *Nature Biotechnology*, 38(2): 225–232. https://doi.org/10.1038/s41587-019-0266-0
- Yu, H., Lin, T., Meng, X., Du, H., Zhang, J., Liu, G., Chen, M. et al. 2021. A route to de novo domestication of wild allotetraploid rice. *Cell*, 184(5): 1156-1170.e14. https://pubmed.ncbi.nlm.nih. gov/33539781/
- Yu, Y., Leete, T.C., Born, D.A., Young, L., Barrera, L.A., Lee, S.J., Rees, H.A., Ciaramella, G. & Gaudelli, N.M. 2020. Cytosine base editors with minimized unguided DNA and RNA off-target events and high on-target activity. *Nature Communications* 11. https:// www.nature.com/articles/s41467-020-15887-5
- Yu, J.-K. & Chung, Y.-S. 2021. Plant variety protection: Current practices and insights. *Genes*, 12(8): 1127. https://doi.org/10.3390/genes12081127
- Yue, C., Zhao, S., Cummings, C. & Kuzma, J. 2015. Investigating factors influencing consumer willingness to buy GM food and nano-food. *Journal of Nanoparticle Research*, 17(7): 1–19. https://pubmed. ncbi.nlm.nih.gov/34440301/

- Yue, C., Zhao, S. & Kuzma, J. 2015. Heterogeneous consumer preferences for nanotechnology and genetic-modification technology in food products. *Journal of Agricultural Economics*, 66(2): 308–328. https://doi.org/10.1111/1477-9552.12090
- Zahniser, S. & Coyle, W.T. 2004. USDA ERS U.S.-Mexico Corn Trade During the NAFTA Era: New Twists to an Old Story. Washington, DC, USDA Economic Research Service. https://www.ers.usda.gov/ publications/pub-details/?pubid=36452
- Zambelli, A. 2020. Current status of high oleic seed oils in food processing. *Journal of the American Oil Chemists' Society*, 98: 129–137. https://doi. org/10.1002/aocs.12450
- Zapiola, M.L. & Mallory-Smith, C.A. 2017. Pollenmediated gene flow from transgenic perennial creeping bentgrass and hybridization at the landscape level. *PLOS ONE*, 12(3): e0173308. https://doi. org/10.1371/JOURNAL.PONE.0173308
- Zhang, H.W., Si, X.M., Ji, X., Fan, R., Liu, J.X., Chen, K.L., Wang, D.W. & Gao, C.X. 2018b. Genome editing of upstream open reading frames enables translational control in plants. *Nature Biotechnology*, 36: 894–898. https://www.nature.com/articles/ nbt.4202
- Zhang, J., Zhang, H., Li, S., Li, J., Yan, L. & Xia, L. 2021. Increasing yield potential through manipulating of an ARE1 ortholog related to nitrogen use efficiency in wheat by CRISPR/Cas9. *Journal of Integrative Plant Biology*, 63(9): 1649–1663. https://pubmed.ncbi.nlm. nih.gov/34270164/
- Zhang, R., Sun, C., Zhu, J., Zhang, R. & Li, W. 2020. Increased European heat waves in recent decades in response to shrinking Arctic sea ice and Eurasian snow cover. *npj Climate and Atmospheric Science*, 3(1): 1–9. https://doi.org/10.1038/s41612-020-0110-8
- Zhang, Y., Massel, K., Godwin, I.D. & Gao, C. 2018a. Applications and potential of genome editing in crop improvement. *Genome Biology*, 19(1): 210. https:// doi.org/10.1186/s13059-018-1586-y
- Zhu, X., Richael, C., Chamberlain, P., Busse, J.S., Bussan, A.J., Jiang, J. & Bethke, P.C. 2014.
 Vacuolar invertase gene silencing in potato (Solanum tuberosum L.) improves processing quality by decreasing the frequency of sugar-end defects. *PLOS ONE*, 9(4): e93381. https://doi.org/10.1371/JOURNAL.
 PONE.0093381
- Zilberman, D., Rausser, G. & Wesseler, J. 2023. The Future of Agriculture. In: C. Berck, J. Perloff & D. Zilberman, eds. Sustainable resource development in the 21st century: Essays in memory of Peter Berck. New York, US, Springer (in print).
- Zingwe, D.E., Manja, L.P. & Chirwa, E.W. 2021. The effects of engendered intra-household power dynamics on household food security and nutrition in Malawi. *Journal of Gender Studies*: 1–19. https://doi. org/10.1080/09589236.2021.1940110

Appendices

Gene editing

Gene editing refers to using molecular tools to edit the genome of a host cell at precise locations to make targeted changes. Gene editing can involve foreign gene or genetic sequence incorporation, but foreign genes are not always incorporated at the target site and they can be removed from the final product. Some gene-edited products are analogous to those obtained by mutation breeding. The advantage of gene editing is its precision over other methods of genetic manipulation.

Principles of gene editing

Gene-editing approaches, such as mega nucleases, zinc finger nucleases (ZFNs), and TALENs, rely on intricate and specific protein-DNA interactions to target protein effectors for desired DNA sequences. Although effective at targeting a specific site, it is difficult to rapidly and simply reprogramme targeting of protein domains to new genomic sites of interest. The discovery and engineering of clustered regularly interspaced short palindromic repeats (CRISPR) has simplified the process (Doudna and Sternberg, 2017).

Gene editing relies upon two distinct processes. One, to be able to target any DNA sequence of interest in living cells, and two, to be able to edit the DNA. The DNA of all eukaryotic systems is stored securely in the nucleus. Therefore, to be able to edit, a DNA-targeting protein module must be able to enter the nucleus of a living cell, search through the tightly packaged DNA and bind to a specific targeting region. Subsequently, the genome editing machinery must perform a modification at that targeted region of DNA. Endogenous cellular DNA replication and repair converts the change into a permanent editing event.

Gene-editing technologies for use in agriculture have been categorized into three site-directed nuclease methods, termed SDN-1, SDN-2, or SDN-3. The three methods share the common feature of initiating genome editing through creation of a DNA double-strand break (DSB) at a targeted site in the genome of a living cell. SDN-1 relies on the cell's endogenous repair machinery to repair the DSB non-specifically, which often results in the creation of DNA insertions or deletions around the cut site. These small DNA base additions or subtractions can result in a frame-shift in the coding

sequence of a targeted protein, which would in essence knock-out the protein and eliminate protein expression, or a genomic regulatory region. SDN-2 utilizes a foreign donor DNA or RNA template to encode a precise edit around the DNA cut site. This donor template encodes homology with the endogenous genomic sequence and a desired edit, which gets incorporated into the cell's own genome through DNA repair. However, the generation of precise SDN-2 editing is inefficient and would usually result in SDN-1 outcomes: DNA insertions and deletions. SDN-3 also uses a foreign donor DNA but relies on the insertion of this DNA sequence into the cut site. Unlike SDN-2, which typically confers small precise DNA changes, SDN-3 can be a large DNA fragment insertion such as a promoter and gene. Similarly to SDN-2, SDN-3 is also inefficient and most outcomes are small DNA insertions or deletions. Newer precision genome editing technologies such as base editing and prime editing can enable precise genome edits without the formation of a DNA double-strand DNA intermediate. This suggests the need for additional genome-edited categories in the future.

Protein-based gene-editing systems

Mega nucleases, ZFNs and TALENs are biological tools that can be used for genome editing. Mega nucleases (also known as homing endonucleases) are large protein complexes that are programmed to specifically recognize a particular DNA sequence (Kostriken *et al.*, 1983; Jacquier and Dujon, 1985). These proteins rely on a complex network of interactions between the protein and the targeted DNA sequence.

Zinc finger proteins are small protein modules that can recognize a particular three DNA base sequence. A chain of zinc fingers can be fused together to enable user-defined targeting of the entire protein to a genomic DNA sequence. Researchers have fused these zinc finger proteins with other proteins that can manipulate DNA. Fokl is a bacterial restriction endonuclease found in nature that is composed of a DNA binding domain and a DNA cleaving domain. Researchers have harnessed just the DNA cleavage domain and fused this with zinc-finger modules to permit targeted cleavage of DNA. Such fusion complexes, ZFNs, function in human, animal and plant cells (Urnov *et al.*, 2010). Transcription activator-like (TAL) effectors are small bacterial proteins found in plant pathogens that bind to the DNA of plant host cells and engage in infection events. Each individual TAL effector can bind to a particular DNA base (adenine, thymine, guanine, or cytosine). Similarly to ZFNs, researchers have fused Fokl cleavage domains to TALEs (transcription activator-like effectors) to generate TALE nucleases (TALENs), which are fully protein-encoded programmable genome editing technologies (Sander and Joung, 2014).

CRISPR systems

The discovery of clustered regularly interspaced short palindromic repeat (CRISPR) arrays originates from researchers seeking to uncover the function of this stretch of DNA array in the bacterial genome. CRISPR arrays encoded in a bacterium's own genome are transcribed to RNA and processed within the cell into individual short stretches each of which is programmed to reflect a particular sequence. These short CRISPR RNAs then associate with Cas proteins, which program the Cas protein to recognize a DNA sequence encoded by the RNA. The targeting sequence in the CRISPR RNA can be easily replaced and programmed with a user-defined sequence and that this replacement completely alters and controls the genomic sequence a Cas protein targets (Jinek et al., 2012; Gasiunas et al., 2012; Cong et al., 2013). This is the first time in which the re-targeting of a protein complex can be easily dictated by replacing a nucleic acid sequence, unlike previous approaches, which all required complex and laborious protein engineering. It is also because of ease in programming that there is gene editing based on the CRISPR-Cas system.

Once the guide RNA is programmed to recognize a particular sequence of DNA, it enters the nucleus of a cell and can find the targeted sequence. Upon recognition, the Cas protein unwinds the DNA double helix and base pairs its guide RNA (gRNA) sequence with the complementary strand of DNA while releasing the other strand of DNA as a single-stranded DNA (ssDNA) R-loop region. Following Cas binding, the protein complex uses its nuclease domains to cut both strands of the DNA, thus initiating the process of editing the DNA of a living cell.

DNA repair affecting outcomes

Meganucleases, ZFNs, TALENs and Cas proteins all result in the creation of a DNA DSB at a stretch of DNA sequence in the genome of living cells that is specifically recognized and targeted for editing, either by a protein domain or a guide RNA. The generation of a DSB marks a significant perturbation in a living cell's growth condition. Therefore, on generating a DSB, the cell's endogenous DNA repair machinery will rapidly recognize the break and seek to repair the break without error (West, 2003). If the break is not repaired, the cell ultimately dies. The DNA repair machinery can result in an error in DNA repair, which typically arises in the form of small DNA insertion or deletion (known as indels) at the cut site (Deriano and Roth, 2013). This repair process is known as non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ). These random, small DNA indels surrounding a target site may result in frame shifting the coding sequence of a protein. This type of editing is known as a genetic knockout, in which the outcome of the genome editing event is the complete elimination of a protein's expression in a cell.

Although powerful, gene editing to generate indels is limited to applications in which a user seeks to knock out a protein. However, often there is a desire to alter the coding sequence of a protein rather than completely knock out its activity. In such cases, there is a need to be able to generate precise genome editing events rather than rely on spontaneous and random DNA repair. Homology directed repair (HDR) is another DNA repair process in which a cell's endogenous DNA repair machinery recruits a DNA donor template to use as a reference for repairing a DNA cut (West, 2003). In this case, a user seeking to perform the desired genome edit can encode the edit in a foreign donor template and co-deliver this template with a nuclease domain that can perform a targeted cut on the DNA. Following the cell's HDR repair machinery using this template as a donor, the programmed edit on the donor can be transferred into the cell's own DNA sequence, thus resulting in a permanent editing alteration of the cell's genome. Although programmable and precise, this type of editing event is rare and occurs much less frequently than the NHEJ/MMEJ pathway in which indels are produced surrounding the DNA cut site (Deriano and Roth, 2013; Cong et al., 2013; Shan et al., 2013; Voytas, 2013).

There is still a great need for gene-editing technologies that can efficiently change the sequence of DNA in a programmable and predictable approach. The following two sections, namely base editing and prime editing, discuss new technologies that address this limitation and can very precisely edit the DNA of living cells.

Base editing

Base editing relies on the target search and DNA binding activity of Cas proteins. However, rather than cutting both strands of the DNA, base editors are comprised of an additional DNA-modifying protein element fused to the Cas protein. These extra domains are deaminase proteins that can specifically perform a chemical transformation known as a deamination reaction on single-stranded DNA. Because Cas proteins unwind the double helix upon binding and expose a short single-strand DNA segment, this area can serve as a substrate for the locally tethered deaminase protein, thus enabling a site-specific chemical modification of individual DNA bases on a single strand.

The first class of base editors engineered are known as cytosine base editors (CBE) (Komor *et al.*, 2016). CBEs utilize cytosine deaminases as key enzymes in the editing protein. These enzymes can convert cytosine bases specifically in single-stranded DNA into a uracil base. Following endogenous cellular repair, this uracil base intermediate is resolved into a thymine base, ultimately resulting in a single C•G-to-T•A base edit.

The second class of base editors are adenine base editors (ABE) (Gaudelli *et al.*, 2017). ABEs leverage the fact that a deamination chemical transformation on adenine bases results in an intermediate, inosine, which is recognized by endogenous cellular polymerases as guanine. However, unlike CBEs, there is no naturally occurring deaminase enzyme that specifically recognizes adenine bases in singlestranded DNA. Therefore, researchers established a directed protein evolution platform to evolve a new enzyme capable of performing this desired reaction on DNA in living cells (Gaudelli *et al.*, 2017; Richter *et al.*, 2020).

Both CBEs and ABEs can edit DNA precisely and efficiently. In contrast with Cas-mediated HDR editing of individual bases, these base editors can edit desired base residues without resulting in undesired editing outcomes such as indel mutations. Although CBEs and ABEs are extremely useful in creating C•G-to-T•A or A•Tto-G•C base conversions respectively, there exist many other types of genome edits, such as other base conversions and programmable insertions and deletions, which require newer precisionediting techniques. The next section, prime editing, discusses a recently developed geneediting approach that addresses these remaining limitations.

Prime editing

Prime editing was developed recently to address the unmet biotechnological need for realizing a range of genome editing outcomes (Anzalone *et al.*, 2019). Prime editors fundamentally rely on a Cas protein to dictate a particular region of DNA in living cells for editing. Prime editors are encoded with a foreign nucleic acid template. The prime editing guide RNA (pegRNA) comprises an extension of the Cas protein guide RNA. On the pegRNA, a primer binding site can be specifically encoded, which is complementary to the released singlestranded R-loop from the Cas protein nick, and a template region encoding a particular desired DNA editing event.

In addition to the Cas protein, prime editors comprise a reverse transcriptase protein domain, which can use RNA as a template to extend DNA. Therefore, once the prime editor nicks one strand of DNA, it releases a single-stranded DNA primer region, which is complementary to and can base pair with the nearby pegRNA. This intermediate is recognized by the fused reverse transcriptase, thus initiating DNA polymerization directly on the endogenous genome using the user-encoded RNA as a template. Following subsequent DNA replication and repair, the newly synthesized DNA sequence is permanently encoded into the living cell's genome. Since the type of genome edit desired can be directly encoded into the pegRNA, prime editing offers the ability to perform versatile edits with high efficiency and precision.

Specificity

During the development and application of the original ZFN and TALEN genome editing systems, scientists discovered that these editing tools sometimes have off-target effects, which refers to events in which the editing system can sometimes target genomic sites other than the targeted locus in the genome of a cell (Kim *et al.*, 2009; Mussolino *et al.*, 2011). Off-target effects dictated by the location and type of edit will affect the specificity, which is critical for realizing the full potential of gene-editing systems in all applications.

During the process of CRISPR-Cas editing, the guide RNA forms a complex with a Cas protein, which activates the complex to scan through the genome to identify the targeted site of interest. If the Cas protein inadvertently binds to and edits other sites in the genome, not dictated by the guide RNA sequence, these events are known as an off-target edit.

The off-target effects of gene-editing tools can be largely divided into two categories based on their fundamental mechanism. The first class is characterized as guide RNA-dependent off-target editing (Anzalone *et al.*, 2020; Gao, 2021; Jeong, Song and Bae, 2020). During the development and application of CRISPR-Cas systems for eukaryotic genome editing, many studies found that Cas proteins can still bind to regions of DNA even when there is a 1–2 base mismatch between the desired target sequence and off-target binding.

The second class is known as guide RNAindependent off-target editing, which encompasses changes in genomic locations other than the target location caused by overexpression of genome editing agents, and that these locations do not resemble the desired on-target editing sequence (Anzalone *et al.*, 2020; Gao, 2021; Jeong, Song and Bae, 2020).

During initial characterizations of CRISPR-Cas binding to a target site, experiments showed that the extent of guide-dependent off-target editing is related to the position and sequence of mismatch sequences between the targeted sequence and off-target sequence. Extensive studies have developed approaches that are able to massively profile Cas protein-dependent offtarget editing based on the mismatch identities (Fu *et al.*, 2013; Hsu *et al.*, 2013; Pattanayak *et al.*, 2013). Furthermore, unbiased methods based on isolating sites of Cas protein cleavage genomewide enable high-throughput profiling of a Cas protein's editing precision. Through extensive protein engineering and evolution efforts, many

studies note the possibility of altering specific residues in the Cas protein to decrease the propensity of Cas-dependent off-target editing (e.g. Casini et al., 2018; Lee et al., 2018; Hu et al., 2018). Furthermore, researchers have demonstrated a correlation between the mode of delivery vs. the off-target potential of genome editing agents (e.g. Liang et al., 2017; Zhang et al., 2018b; Rees et al., 2017). The persistent expression of gene-editing tools in living cells increases the proportion of offtarget editing, which can be explained by the fact that after the editing tool performs the desired edit, any further expression can only result in undesired editing outcomes (Rees et al., 2017). Therefore, delivery approaches that limit the half-life of the editing protein in living cells can substantially decrease off-target editing effects. These include efforts reliant on the transient expression of the editing reagent, especially when delivered as RNA or protein complexes, because these get degraded rapidly (Rees et al., 2017).

Researchers next evaluated whether Cas proteins would randomly cut genomic DNA within the genome independently of the guiding role of sgRNA, thereby causing random DNA breaks. The evaluation of off-target effects based on individual genome sequencing data in mice, cotton and rice has successively found that the number of insertion or deletion mutations in cells treated with CRISPR-Cas editing tools is not different when compared with control groups treated without the Cas protein, therefore concluding that the CRISPR-Cas system does not increase any genome-wide guide RNA-independent off-target editing (lyer et al., 2018; Li et al., 2018; Tang et al., 2018; Willi et al., 2018). This demonstrates that Cas proteins only edit at sites in which the guide RNA can bind to the double helix and that these editing tools do not uncontrollably and spontaneously generate doublestrand breaks throughout the genome.

Precision gene-editing tools such as base editors also face the need for thorough evaluation any guide-dependent and guide-independent off-target editing. During the development of the cytosine base editor, it was demonstrated that Cas protein off-target binding sites were also off-target sites for CBEs if a cytosine is positioned within the binding region (Kim *et al.*, 2017). However, initial evaluations of the adenine base editor found that ABEs are much more precise and generate far less guide RNA-dependent off-target editing compared with Cas nuclease cleavage or CBEs (Liang *et al.*, 2019).

Researchers began to evaluate guide-independent off-target editing of base editors using genomewide sequencing in mouse embryos and rice (Jin et al., 2019). The initial studies all demonstrated that CBEs generated off-target editing events sporadically throughout a cell's genome, while ABEs were more specific and resulted in minimal guide-independent off-target editing. This effect was further explained by the fact that the deaminase domain of base editors can react with single-stranded DNA regions in living cells, such as areas of active transcription or DNA replication. To decrease the propensity of off-target editing effects, variants of the deaminase domains were engineered to maintain high levels of precise on-target editing while minimizing any off-target editing effects (Jin et al., 2020; Doman et al., 2020.; Yu et al., 2020; Lee et al., 2020).

Prime editing systems were also thoroughly evaluated for their propensity to cause off-target editing. Because the guide RNA used in the prime editing system is a pegRNA, which is different

from the base editing system, the off-target effects of the prime editing system can be divided into two main categories, one of which is pegRNAdependent off-target editing, which was low in cells possibly because of multiple distinct DNA hybridization events required to enable active prime editing outcomes (Anzalone et al., 2020). The three unique DNA hybridization events refer to the principles governing prime editing require the binding of the guide RNA sequence to the targeted DNA strand, the binding of a primer binding site to the nicked flap in the genomic DNA, and the complementation of newly edited DNA with endogenous DNA sequences. Recently, a comprehensive evaluation of pegRNA-dependent and pegRNA-independent off-target editing was performed using rice as a model system (Jin et al., 2021). It was noted that prime editors are extremely specific and precise in their editing outcomes. Furthermore, there is no perturbation on the cell's endogenous state and there does no significant perturbation to the cell's overall gene regulatory system (Jin et al., 2021). These properties suggest that prime editing is superior in generating accurate, precise and safe gene-editing events in living cells.

Appendix B.1a

Case study - Powdery mildew resistance

Powdery mildew is one of the most common plant pathogens worldwide. The yields of many cereal crops, including barley, wheat and oats, are affected by powdery mildew infection. Some horticultural crops are also susceptible to powdery mildew, such as grapes, strawberries, cucumbers, tomatoes and peppers (Glawe, 2008). A recessive powdery mildew resistance gene (mlo, an allele of the MLO locus) was first identified in barley over 70 years ago and has been successfully used on a commercial scale for decades, and continues to provide effective resistance (Büschges et al., 1997). The resistance in barley was mutation induced. Similar resistances have not been identified in other cereal crops. However, the advent of genome editing technologies provides an opportunity for researchers to generate powdery mildew resistance directly in other species.

Bread wheat has three distinct copies of the powdery mildew locus, which if knocked out using gene-editing techniques can promote a similar resistance effect as evident in barley. Initial studies in 2014 demonstrated that TALEN gene editing was able to knock out all three copies of *MLO* (Wang *et al.*, 2014), conditioning broad-spectrum, durable resistance to powdery mildew disease. Because bread wheat has a more complex genome than barley, the probability of generating powdery mildew resistance through natural variation and mutation breeding is extremely low. Gene-editing technologies therefore represent the only realistic chance of rapidly obtaining powdery mildew resistant bread wheat. Following this success, similar efforts were directed at tomatoes and grapes, with the rapid creation of new resistant varieties (Nekrasov *et al.*, 2017; Wan *et al.*, 2020).

Although the initial bread wheat variety with three knocked out copies of *MLO* exhibited durable powdery mildew resistance, the gene editing resulted in some undesirable pleiotropic growth defects and the variety was low yielding. Recently however, researchers described an improved gene-editing approach (Li *et al.*, 2022). In total, four genetic perturbations mediated by CRISPR-Cas9 enabled rapid creation of new bread wheat varieties exhibiting desired phenotypes. This work highlights the potential of gene editing to accelerate bread wheat breeding by directly performing targeted edits in elite varieties without recourse to extensive and laborious crossing.

Appendix B.1b Case study – PRRSV-resistant pigs

Porcine reproductive and respiratory syndrome (PRRS) is an endemic disease that severely affects domestic pigs. The disease triggers breathing problems and deaths in young animals, increases susceptibility to secondary bacterial infection, and can cause pregnant sows to lose their litters. The causal agent is the porcine reproductive and respiratory syndrome virus (PRRSV). The syndrome was first identified in the United States of America in 1987, and the virus was detected in Netherlands in 1991 (OIE, 2021).

Unfortunately, vaccines have not successfully stopped the spread of PRRSV, which is endemic in most pig-producing countries and causes considerable economic damage (The Roslin Institute, 2021). PRRSV shows complex interactions with the immune system and a high mutation rate, making the development and implementation of control strategies a significant challenge (Montaner-Tarbes et al., 2019). The virus results in annual losses in excess of £1.3 billion to the European pig industry and over £500 million to the United States of America pig industry (The Roslin Institute, 2020). The direct and indirect impacts of PRRS make it the most economically significant pig disease in North America, Europe and Asia (Whitworth et al., 2016). Even 30 years after the PRRSV was first detected in the United States of America, there are no effective vaccines or drugs to address the problem.

However, in 2016, animal science researchers at the University of Missouri and Kansas State University made progress in the fight against PRRS using the CRISPR-Cas9 system. Pigs were produced that are resistant to infection by PRRSV. Pigs have the CD163 gene that encodes a protein

on the surface of lung defence cells (macrophages) critical for the PRRS virus to enter the lung cell and cause an infection. Using the CRISPR-Cas9 system, the pig's genome was modified at a single point, stopping production of the section of the CD163 protein required for PRRSV to cause an infection. The effectiveness was tested by placing geneedited pigs in pens with control pigs (animals that still produced CD163) and challenging all animals with a standardized dose of PRRSV. The control pigs got the disease and showed clinical and tissue level signs of the virus associated with acute PRRS infection. None of the gene-edited/CD163 "knockout" pigs showed signs of illness, and tissue sampling demonstrated that they were free of the virus. Even the foetus of knockout dams was protected from PRRSV, showing that resistance is heritable (Thompson and Benjamin, 2021).

The research on gene-edited/CD163 pigs continues and could impact the fight against the virus. The Roslin Institute also produced pigs resistant to PRRSV. A recent study shows that their geneedited pigs are healthy under standard husbandry conditions and maintain the biological function of the CD163 protein while being resistant to PRRSV infection (Burkard et al., 2018). However, it will likely take several more years before gene-edited/ CD163 pigs can be used in commercial operations. Numerous additional hurdles will have to be overcome, and the technology must pass through appropriate regulatory channels in the United States of America, China and other countries. The case of PRRSV-resistant pigs emphasizes the potential of gene editing for achieving disease resistance in animals in cases where progress has been slow and costly.

Appendix B.1c Case study – CRISPR-Cas9 and fish

Sea bream (*madai*) accounts for ten percent of the total value of Japanese aquaculture and is therefore very important. In 2021 a gene-edited sea bream was approved for commercial use in Japan, representing the first gene-edited animal food. CRISPR-Cas9 was used to knock out the gene for myostatin production (*Pm-mstn*), which normally suppresses muscle growth. The resulting fish has a proportionally larger edible part, 20–60 percent more edible yield, and a 14 percent improved feed utilization efficiency. Ohama *et al.* (2020) reported a 16 percent increase in skeletal muscle in fish that had had the *Pm-mstn* gene knocked out. It was suggested that gene editing can speed up fish breeding considerably.

A second gene-edited fish species was developed in 2021 in Japan and approved for commercial production. A tiger puffer (*22-seiki fugu*) had four leptin receptor genes knocked out with CRISPR. Those genes control appetite and when removed result in a fish with enhanced appetite and weight gain. Gene-edited fish are about twice as heavy at the same growth stage as non-edited fish.

In Japan, gene-edited products do not have to adhere to the same regulations as genetically modified products that contain foreign genes.

CRISPR-Cas9 has also been used to gene edit other fish species. Baloch *et al.* (2019) worked with sturgeon, a critically endangered fish species due to overfishing for caviar and interference in their natural habitats. With life spans exceeding 100 years and sexual maturity only being reached at 20–25 years, they are difficult to work with. However, the related sterlet reproduces quickly and can function as a surrogate for sturgeons. All germ cells in developing embryos derive from primordial germ cells. The *dnd1* gene conditions formation and migration of primordial germ cells. Knockedout embryos devoid of primordial germ cells were successfully used as sterile hosts for surrogate sturgeon production.

