GUIDELINES ON THE REGULATION OF GENOME-EDITED PLANTS IN ZAMBIA

In accordance with the Biosafety Act No. 10 of 2007

PREFACE

Genome-editing is an advancement in modern biotechnology offering a suite of tools for trait improvement that enables cost- and time-effective targeted and precise alterations of the genomes of plants. Unlike transgenic plants, which are produced by transferring genes from a species outside of the plant's gene pool, the production of genome edited plants may not necessarily involve the transfer of genes with species outside of the plant's gene pool. Thus, the resulting product of genome editing may or may not have a novel combination of genetic material. When there is a novel combination of genetic material it is considered to be a genetically modified organism.

Considering the increased investment in research for genome-editing globally to address challenges being faced in the agriculture sector and also being mindful that Zambia is part of the global community, it is imperative that a guideline is put in place to ensure measures are taken before the adoption of the technology.

This guideline provides a summary of commonly used techniques in genome editing; highlights biosafety legal and institutional framework including processes that are followed in reviewing applications on genome editing in Zambia; and a flow chart with step-by-step procedures for determining which genome-edited products are regulated as GMOs plants and those that will be regulated as conventional.

The guideline is by no means exhaustive; it will be updated from time to time as new scientific and regulatory knowledge becomes available. It is my hope and belief that key stakeholders will find the guideline helpful in understanding its usage.

Mr Webster D. Shabusale

CHAIRPERSON FOR THE NATIONAL BIOSAFETY AUTHORITY BOARD

ACKNOWLEDGEMENTS

The National Biosafety Authority through the Ministry responsible for biosafety and biotechnology wishes to express its sincere gratitude to all institutions and individuals who were actively involved in the drafting, review and validation of this guideline. Particularly the Africa Union Development Agency, Ministry of Fisheries and Livestock, Ministry of Agriculture, Zambia Agriculture Research Institute, Seed Control and Certification Institute, University of Zambia, Mulungushi University

Mr. Webster D. Shabusale

CHAIRPERSON FOR THE NATIONAL BIOSAFETY AUTHORITY BOARD

TABLE OF CONTENTS

LIST OF ACRONYMS

CPB	Cartagena Protocol on Biosafety
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic Acid
GM	Genetically Modified
GMO	Genetically Modified Organism
NBA	National Biosafety Authority
NBTs	New Breeding Techniques
ODM	Oligonucleotide-Directed Mutagenesis
rDNA	Recombinant DNA
RNA	Ribonucleic Acid
SDN	Site –Directed Nucleases
TALENs	Transcriptional Activator Like Effector Nucleases
ZEMA	Zambia Environmental Management Authority
ZFNs	Zinc Finger Nucleases

DEFINITION OF TERMS

Applicant	A person who submits an application for a license or permit under the Act.
Biosafety	A set of measures, policies and procedures used or established for assessing, preventing, monitoring and managing any risk associated with genetically modified organisms to human and animal health and safety and to the environment.
Biosafety Act No.10 of 2007	An Act to regulate the research, development, application, import, export, transit, contained use, release or placing on the market of any genetically modified organism whether intended for use as a pharmaceutical, food, feed or processing, or a product of a genetically modified organism.
Biotechnology	In this context biotechnology means the application of *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (rDNA) and direct injection of nucleic acid into cells or organelles, or fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection.
CRISPR-Cas	Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR associated protein. A type of side-directed nuclease based on a naturally occurring endonuclease enzyme (Cas) and the guide RNA molecule complementary to the targeted sequence and directing Cas to introduce a DNA break.
Contained use	Any activity in which organisms are genetically modified or in which genetically modified organisms are cultured, stored, used, transported, destroyed or disposed of and for which physical barriers or a combination of physical, chemical or biological barriers are used to limit contact thereof with

	the environment.
Conventional counterpart	A related organism, its components and/or products for which there is experience of established safety based on common use or prior assessment.
Donor organism	The organism from which the genetic sequence is derived for transfer to the recipient organism
Environment	The natural or man-made surroundings at any place, comprising air, water, land, natural resources, animals, buildings and other constructions.
Foreign DNA	In the context of this guideline, the term refers to DNA from sexually non-compatible species through the use of modern biotechnology.
Genetically Modified Organism	An organism whose genes or genetic material has been altered in a way that does not occur naturally through mating or natural recombination.
Genome Editing	A technique in which the deoxyribonucleic acid (DNA) is inserted, modified, replaced, or deleted in the genome of a living organism at predetermined locations.
Intentional Introduction into the Environment	Any deliberate use of genetically modified organisms other than contained use.
Meganuclease	A type of site-directed nuclease based on a naturally occurring endonuclease enzyme (Meganuclease) which recognizes and cleaves DNA sequence targets, typically from 12 to 40 bp.

Oligonucleotide Directed Mutagenesis	A type of genome editing technology to introduce targeted nucleotide replacements guided by oligonucleotide molecules. ODM does not involve a Site -Directed endonuclease enzyme.
Site Directed Nuclease	DNA-cutting enzymes that take advantage of the targeted DNA break and the host cells endogenous DNA repair mechanisms to induce specific changes at the site of the DNA break that are of economic importance. The change can either be a small deletion, a substitution, or the addition of several nucleotides. Such targeted edits result in a new and desired characteristic, such as enhanced nutrient uptake or decreased production of allergens.
Transcription Activator-Like Effector Nuclease	A site-directed endonuclease based on a naturally occurring endonuclease enzyme which combines a customizable array of protein modules, found in bacterial proteins called transcription activator-like effectors, where each recognizes a single DNA base and the catalytic domain of a DNA cutting enzyme called *Fok*I
Transgenesis	Introduction of one or more DNA sequences from sexually incompatible species through biotechnology
Zinc Finger Nuclease	A site-directed nuclease based on a naturally occurring endonuclease enzyme composed of a zinc finger part and a nuclease part. The zinc finger protein binds accurately on a specific DNA location on each side where the nucleases perform their function in pairs.
Plant products	Any material derived by processing, or howsoever, from any genetically modified organism

1.0. INTRODUCTION

1.1 Background

Agriculture is a critical sector in the Zambian economy for providing jobs and livelihood for more than 70 percent of the population (International Monitoring Fund, 2023). However, its contribution to GDP has shrunk from 9 percent in 2012 to 3.3 percent in 2022 (Africa Development Bank, 2022). Improving food security is one of the national objectives of the agricultural sector, however, climate change, pests and diseases, and soil degradation, droughts, flash floods, and climate variability have negatively affected productivity over the years. Currently, Zambia uses conventional methods of plant improvement in order to develop high-yielding plants which have desired traits. Breeding techniques have been incorporating new advances in plant science but so far in order to create variability and selection of the best varieties breeders have to deal with inefficient and time-consuming processes. Genome editing is a new set of tools that expand the breeder's toolbox and offers new opportunities to advance plant breeding with a higher degree of precision and efficiency.

The adoption of genome editing techniques in Zambia could contribute to the achievement of increased agricultural productivity, commercialisation, industrialisation and urbanisation. The government recognises that there are several opportunities for enhancing agricultural production and productivity to catalyze the realization of Zambia's aspiration of inclusive wealth creation and self-reliance, including: an enabling and supportive policy environment and availability of developed technologies that are ready for scaling up. This is in line with the National Biotechnology and Biosafety Policy of 2003 which recognizes the potential and crucial role biotechnology can play in Zambia's socio-economic development in combating food and nutrition insecurity including reducing poverty.

The application of genome editing must be in accordance with the Biosafety Act. Zambia became a Party to the Cartagena Protocol on Biosafety, which seeks to create an enabling environment for the environmentally sound application of biotechnology, making it possible to derive maximum benefit from the potential that biotechnology has to offer, while minimising the possible risks to the environment and to human health. However, in spite of having the biosafety legal framework, the country noted gaps in dealing with genome-edited products that necessitated the development

of this guideline. Therefore, this guideline outlines the procedures that should be followed when making a decision on whether a genome-edited product and process should be regulated under the Biosafety Act or not.

1.2. Institutional and Legal Framework

In Zambia the Biotechnology and Biosafety Policy provides direction while the Biosafety Act and subsequent regulations makes the Regulatory System. This regulatory framework provides for effective handling of biotechnology programs and activities.

The Act was enacted to provide for the safe management of biotechnological activities and it is administered by the Minister responsible for biosafety and other public personnel appointed under the Act. The Biosafety Regulations give effect to the Act and provide more technical information on the administration of the NBA, which coordinates and implements the legislation.

In addition to the biosafety legislation, there are other pieces of legislation that have a bearing on biotechnology and biosafety including the Science and Technology Act; the Plant Varieties and Seed Act (Cap 236); the Environment Management Act No. 12 of 2011; the Consumer Protection Act No. 24 2010; and the Standards Act No. 4 of 2017.

1.3 Legal Authority

The Legal authority for these guidelines is derived from section five of the Biosafety Act No. 10 of 2007 which provides that the Authority shall issue guidelines to be followed by applicants in the conduct of biotechnology activities.

1.4 Scope

This guideline shall apply to plants and plant product whose genome has been altered using genome editing techniques resulting in either a GMO or organisms that are not distinguishable from those developed from conventional breeding or natural selection. The guideline shall provide clarity on whether a specific genome edited product should be regulated or not. Users of this guideline may include applicants who submit an application to the NBA for approval for a product that has been altered using genome editing techniques, regulators and reviewers who have to determine which genome editing processes and products are subject to regulations and other relevant stakeholders.

1.5 Objectives of the Guideline

The main objective of this guideline is to provide to applicants, regulators, reviewers and stakeholders on the criteria for determining the regulatory options of genome- editing and products that should, or should not be regulated. Specifically, the guideline seeks to:

a) provide procedures for determining the regulatory status
b) set out the guiding principles and core information for making decisions.

2.0 GENOME EDITING

Genome editing is targeted methods to introduce new traits in organisms using various techniques which induce breaks in the DNA that can be repaired by endogenous mechanisms and lead to a range of changes at a targeted locus within the genome. This may be achieved by deleting, replacing, editing organisms' own DNA or inserting a DNA sequence in the organisms genetic material

These technologies act like molecular scissors, cutting the DNA at a specific spot. The genome editing methodologies are mediated by the following techniques: Oligonucleotide-Directed Mutagenesis (ODM) and Site Directed Nuclease (SDN) whose processes are described below.

a) Oligonucleotide Directed Mutagenesis (ODM) technique

This involves targeted nucleotide changes guided by synthetic Oligonucleotides, resulting in specific genetic variations such as single nucleotide polymorphisms (SNPs).

b) Site-Directed Nuclease technologies

Utilises Nucleases to create DNA breaks near target sequences to create defined target sequence, enabling a range of modifications through cellular repair mechanisms. Examples of SDN include the following:

i). Meganuclease Technology

Meganuclease technology is a variant of site-directed nuclease technology that uses naturally occurring restriction enzymes isolated from bacteria and yeasts that recognize and cleave DNA sequence targets, typically from 12 to 40bp. More accuracy is achieved with mega nucleases as only very specific, rare fragments are targets.

ii). Zinc Finger Nuclease Technology

This is a technology that uses proteins composed of a zinc finger part and a nuclease part. The zinc finger protein binds accurately on a specific DNA location on each side where the nucleases perform their function in pairs. Zinc finger sequence can be adjusted such that the nucleases can cut any sequence in the plant.

iii). Transcription Activator-Like Effector Nuclease Technology

Transcription Activator -Like Effect Nuclease is a type of technology that uses site-directed nucleases that utilizes customisable array of protein modules, found in bacterial proteins called transcription activator-like effectors, that each recognize a single DNA base and the catalytic domain of a DNA cutting enzyme called *Fok*I.

iv). Clustered Regularly Interspaced Short Palindromic Repeats/Cas Technology

This ia a m ore precise form of site-directed nuclease technology based on CRISPR/Cas bacterial defense system against viruses. The nuclease is coupled to an RNA molecule which then binds to a specific DNA site. With this technology, scientists can elicit simple DNA changes in specific genes.

In agriculture, genome editing is a relatively new and rapidly developing area of innovation which has the potential to improve agricultural productivity, nutrition and develop climate-smart crops. Genome editing techniques may alter the genome of an organism in a way that results in a new combination of genetic material or results in organisms that are not genetically distinguishable from those developed from conventional breeding and natural selection. For this reason, there is ongoing debate globally on how to regulate genome -edited products. Many countries are choosing a tiered approach in their regulation of genome-edited products. This is dependent on the presence of recombinant DNA in the process and the final product. For example, if rDNA remains in the final product, it is regulated as a GMO. Where genome editing does not lead to the retention of foreign genetic material in the final product, it is not regulated as a GMO under the biosafety regulatory framework.

Unlike genetically modified (GM) crops, which are produced by introducing genes from a distant species to another species (transgenic), genome edited crops do not normally involve the transfer of genes from species outside the plants' gene pool.

3.0 REGULATORY CONSIDERATIONS FOR GENOME EDITING TECHNIQUES

The NBA shall determine which stage of genome editing processes and products to be regulated. Guidance will be provided on a case-by-case basis and using stepwise procedures as provided in Table 1, Figure 1 and the Genome editing Initial Screening Form appendix 1.

Table 1: Categories for regulation of genome editing techniques and derived products

Category	Considerations/scenarios
Regulated under the Biosafety Act	i). All cases of insertions (for foreign /DNA/*Novel combinations and/or regulatory elements, i.e., from a sexually non-compatible species outside of the plant's gene pool) in the final product). By definition, according to the Biosafety Act No. 10 of 2007, these are classified as GMOs and are regulated under the Biosafety Act No. 10 of 2007 ii). In cases where R&D phase starts with a GMO, NBA will regulate genome edited organisms up to the stage where foreign DNA component is removed/segregated
Not regulated under the Biosafety Act	i). All genome editing modifications that are done by inserting genes from sexually compatible species within the plant gene pool and where regulatory elements (promoters and terminators) are also from a sexually compatible species within the plants gene pool. ii). Processed products whose inserted foreign DNA sequences cannot be detected. iii). Genome editing outcomes similar to those that could occur in nature or through use of conventional breeding techniques iv). NBA will NOT regulate genome edited organism beyond the stage where the foreign DNA component is removed/segregated.

*** Note that Novel combinations for the Cartagena Protocol refers to Foreign DNA and insertions.**

3.1 Initial Screening Procedure

All applicants intending to conduct research, develop products, or place on the market products developed through genome editing shall observe the following procedure.

i). An applicant is required to fill a form and submit information detailing the experimental processes and end product of the activity to the National Biosafety Authority Registrar.

ii) **Application Review**: Upon receipt of the application, the NBA Registrar will assign responsible officers to review the submission for completeness and accuracy. The Registrar will provide feedback to the applicant within seven (7) working days of receiving the application. If any gaps or discrepancies are identified, the application will be returned to the applicant for clarification or completion.

ii). If the application is complete and accurate, the NBA will consider the application and determine whether there is integration of foreign DNA and therefore whether it should be regulated under the Biosafety Act.

iii). The decision following the early consultation by the NBA will be communicated to the applicant within 14 working days.

The National Biosafety Authority Registrar will communicate with the applicant,

These guidelines shall be reviewed from time to time based on availability of new scientific information. NBA, therefore, reserves the right to alter its decision if new scientific information previously unknown becomes available.

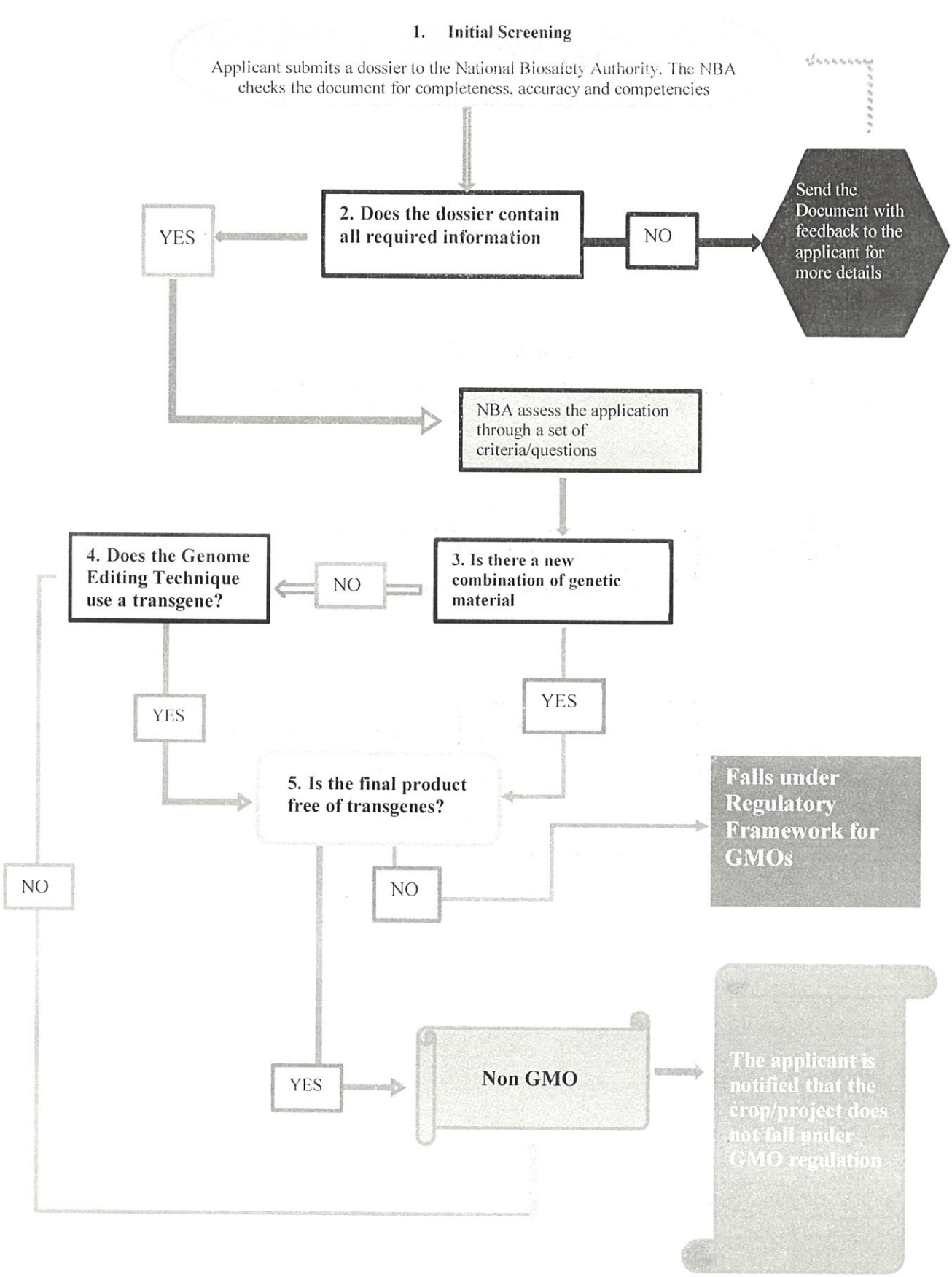

Fig. 1 Schematic diagram on the regulatory process (Adapted from Argentina regulatory process https://doi.org/10.1787/d835e540-en).

REFERENCES

1. Biosafety (Genetically Modified Organisms) Regulations 2007, Zambia Government.
2. Biosafety Act No. 10 of 2007, Zambia Government.
3. Boluwade, E. and Smith, G., 2021. Government of Nigeria approved National Biosafety Guideline on Gene Editing. Available at: https://www.aatf-africa.org/wp-content/uploads/2021/02/Government-of-Nigeria-approved-National-Biosafety-Guideline-on-Gene-Editing_Lagos_Nigeria_02-08-2021.pdf [Accessed 6 August 2024].
4. Cartagena Protocol on Biosafety, 2000. Available at: http://www.biodiv.org/ [Accessed 6 August 2024].
5. Entine, J., Felipe, M.S.S., Groenewald, J.G., Kershen, D.L., Lema, M., McHughen, A., Nepomuceno, A.L., Ohsawa, R., Ordonio, R.L., Parrott, W.A., Quemada, H., Ramage, C., Stuart, I.S., Smyth, J. and Wray-Cahen, D., 2021. Transgenic research. *Transgenic Research*, 30, pp.551-584.
6. Giertz, Å., Caballero, J., Dileva, M., Galperin, D. and Johnson, T., 2015. Managing agricultural risk for growth and food security in Malawi. *Agriculture Global Practice Note-October 2015*. World Bank Group.
7. OECD, 2019. *Regulatory Policy in Argentina: Tools and Practices for Regulatory Improvement*. OECD Reviews of Regulatory Reform. OECD Publishing, Paris. Available at: https://doi.org/10.4060/cb4474en [Accessed 6 August 2024].
8. Pilly, S. and Thaldar, D.W., 2018. *S Afr. J Bioethics Law*, 11(2), pp.89-92.
9. Policy Framework for Applications of Genome Editing in African Agriculture, African Union
10. UNDP Zambia, n.d. *Sustainable Development Goals 2030*. Available at: https://www.mw.undp.org [Accessed 6 August 2024].