

Malawi Government

Guidelines for Safety Assessment and Risk Management of Genetically Modified Organisms with Stacked Genes

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Malawi Government

MINISTRY OF NATURAL RESOURCES AND CLIMATE CHANGE

GUIDELINES FOR SAFETY ASSESSMENT AND RISK MANAGEMENT OF GENETICALLY MODIFIED ORGANISMS WITH STACKED GENES

SEPTEMBER 2022

FOREWORD

The Malawi Vision 2063 (MW2063) is a long-term vision which outlines the collective aspirations and goals of Malawians towards the year 2063. It is anchored on the three pillars, namely: Agricultural Productivity and Commercialization, Industrialization and Urbanization. MW2063 recognizes that there are several opportunities for enhancing agricultural production and productivity to catalyze the realization of Malawi's Vision 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. With the growing national population, shrinking agricultural land-base, and emerging threats of climate change, agricultural transformation is a high priority worldwide. Breeding stack is among few technologies that are being adopted globally to improve agricultural productivity to attain food security.

Considering the importance of biodiversity and its benefits to people Malawi ratified the Convention on Biological Diversity and the Cartagena Protocol on Biosafety in 1992 and 2009 respectively. The Cartagena Protocol on Biosafety seeks to protect biological diversity from the potential risks posed by living modified organisms resulting from modern biotechnology. Malawi government has domesticated the Protocol by putting in place the Biosafety Act (2002) and gazetted the Biosafety (Management of Genetically Modified Organisms) Regulations (2007) to ensure development, use and handling of genetically modified organism is well regulated.

Recently the world is registering rapid adoption of new technologies for the development of genetically modified organisms including stack events. However, the current Malawi biosafety legal framework is not precise enough on regulating stack events, despite being robust in regulating modern biotechnology. This prompted urgent need for guidelines to complement the Biosafety Act and Regulations in guiding safety assessments of genetically modified stacked gene events.

These guidelines were developed in a consultative manner, involving all relevant stakeholders in biosafety and biotechnology. It is my sincere hope that with the same participatory spirit, we shall join hands in ensuring effective and successful implementation of the guidelines to ensure safety of biodiversity and human health from any potential adverse effects of genetically modified organisms with stack traits.



Honourable Eisenhower Nduwa Mkaka MP

MINISTER OF NATURAL RESOURCES AND CLIMATE CHANGE

PREFACE

Gene stacking refers to the process of merging two or more genes of interest into a single plant, combined traits resulting from this process are called stacked traits. Biotech stacks are engineered to have better chances of overcoming the myriad of problems in the field such as insect pests, diseases, weeds, and environmental stresses so that farmers can increase their productivity. Experience has shown that the resistance conferred by a single Bt. gene has the potential to break down as the target insect pest mutates and adapts to defeat the Bt. Trait. The gene stacking approach has reduced the potential of resistance breakdown as it is more difficult for the pest to overcome multiple insecticidal proteins.

Considering the rapid adoption of genetically modified organisms (GMOs) with stack traits globally in addressing multiple challenges being faced in the agriculture sector, and also being mindful that Malawi is part of the global community, it is imperative that the country develop a guidance document to ensure precautionary measures are taken before their adoption or approval of imports from GMOs with stack events.

These guidelines were developed in a consultative manner involving various biosafety stakeholders. The guidelines highlight biosafety legal and institutional framework including processes that are followed in reviewing applications for use of genetically modified organisms in Malawi and a flow chart with a step-by-step procedure for determining how Genetically Modified Organisms with stack traits can be regulated.

These guidelines are by no means exhaustive; they will be updated from time to time as new scientific knowledge becomes available. It is my hope and belief that the National Biosafety Regulatory Committee, the academia, researchers and other stakeholders will find these guidelines helpful in determining the Regulatory Process for genetically modified organisms with stack traits.



James C. G. Ali

PRINCIPAL SECRETARY ADMINISTRATION

For: SECRETARY NATURAL RESOURCES AND CLIMATE CHANGE

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Memory Zikapanda
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ABBREVIATIONS AND ACRONYMS

CAMA	Consumer association of Malawi
CFT	Confine Field Trials
DARS	Department of Agricultural Research Station
DNA	Deoxyribonucleic Acid
EAD	Environmental Affairs Department
ERA	Environmental Risk Assessment
GM	Genetically Modified
GMO	Genetically Modified Organism
GS	Gene Stack
NBRC	National Biosafety Regulatory Committee
NCST	National Commission for Science and Technology
MBS	Malawi Bureau of Standards
PCB	Pesticide Control Board

DEFINITION OF TERMS

Breeding Stacks:	Are conventional plant breeding, where parents with single GM events are crossed to produce progeny that contain two or more GM events
Confined field trial:	a field experiment in which a regulated, GM crop is grown in the environment under specified terms and conditions that are intended to prevent the establishment and spread of the crop
Conventional counterpart:	the equivalent non-genetically modified crop variety or parental line or a near-isogenic line
Co-Transformation:	Techniques of modern biotechnology using two or more transformation vectors to produce a GMO. A plant is transformed with two or more independent transgenes. The transgenes of interest are in separate gene constructs and delivered to the plant simultaneously
Donor organism:	an organism from which genetic material is obtained for transfer to the recipient organism
Environmental release:	the introduction into the environment of a genetically modified crop for which an approval has been granted in accordance with these Environmental Release Regulations and for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment; and includes making genetically modified organisms available to the public for purposes other than sale
Environmental risk assessment:	the process of identifying significant risks to the environment, estimating the level of risk, and determining those risks that require measures to reduce the level of risk
Event:	a genotype produced from the transformation of a single crop species using a specific genetic construct
Exposure:	the contact or occurrence of a potential hazard with an environmental entity of value
Fitness	number of seeds produced per seed sown, and includes the whole life cycle of the crop

Genetically modified crop (GM crop):	a crop in which the genetic material has been changed through in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct introduction of nucleic acid into crop cells or organelles. Also referred to as a recombinant DNA (rDNA) or transgenic crop
Harm:	a negative outcome of effect of an action or event; in other words, an adverse effect.
Hazard:	anything, including a situation or state that may cause harm, without considering its probability or the consequences
Host organism:	the crop species that was transformed to produce the genetically Modified crop
Modern biotechnology:	the application of in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) 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 of crops/crops.
Molecular Stacks:	A plant transformed by using molecular methods, where two or more traits are simultaneously (Co-Transformation) or sequentially (Re-transformation) introduced into a host plant. This involves the introduction of gene constructs simultaneously or sequentially into the target plant by standard delivery systems such as Agrobacterium-mediated and biolistic methods
Re-transformation:	Use of modern biotechnology to produce a GMO where the recipient organism is already a GMO
Risk:	in relation to any crop, is the probability of an adverse effect on the environment and the severity of that effect, due to an environmental hazard
Risk analysis:	in relation to any crop, means a process consisting of three components, i.e., risk assessment, risk management, and risk communication
Risk assessment:	the evaluation of risks to human, animals and the environment, whether direct or indirect, immediate or delayed, which the environmental release or placing on the market of genetically modified organisms may pose

Stability:	Permanence of the transgene in a defined genomic context and without changes to its structure or phenotypic expression
Stacking:	A process that involves combining genes (at least two novel genes) of interest into a single plant line through conventional breeding or molecular techniques
Synergistic effect:	An interaction of elements that when combined produce a total effect that is greater than the sum of the effect of the individual elements
Transgenic crop:	a crop in which a transgene has been integrated into its genome
Transformation:	the unique DNA recombination event that took place though the integration of a transgene(s) in one crop cell for genetic modification, which was then used to generate entire transgenic crops
Vector:	an organism (e.g., virus) or a DNA molecule (e.g., plasmid, nucleic acid cassettes) used to assist the transfer of genetic material from a donor organism to a recipient organism
Vertical gene flow:	the transfer of genetic material between sexually compatible species

CHAPTER ONE

1.0. BIOSAFETY AND BIOTECHNOLOGY IN MALAWI

Malawi government recognizes the potential of biotechnology in contributing to national development. In order to ensure efficient and safe utilization of biotechnology the country has put in place biosafety legal and institutional framework

1.1 The Biosafety Regulatory System in Malawi

The Biosafety Regulatory System in Malawi is made up of the Biosafety Act of 2002, the Biosafety (Genetically Modified Organisms) Regulations of 2007 and the National Biotechnology and Biosafety Policy of 2008. Regulation 3 of the Biosafety Regulations provides for the establishment of a National Biosafety Regulatory Committee (NBRC) which is responsible for the safe management of biotechnological activities.

The specific responsibilities of the NBRC include;

a) evaluating all applications concerning or related to genetically modified organisms and products and making recommendations to the Minister;

b) advising the Minister on matters concerning genetic modification of organisms including; all aspects relating to the introduction of GMOs into the environment, proposals for specific activities or projects concerning GMOs, all aspects of Confined Field Trials, Import and Export of GMOs and amendments or withdrawals of licenses and permits issued;

c) liaising, through the relevant institutions, with international groups or organizations concerned with biosafety and biotechnology;

d) inviting knowledgeable persons to assist the Committee on any aspects related to genetically modified organisms; and

e) carrying out such other functions that are necessary for the effective implementation of these Regulations.

The NBRC, in accordance with the provisions of the Biosafety Act and Regulations, regulates all activities involving GMOs in environment, food, feed, research, industry and it fulfills its mandate by ensuring and assuring safe development, transfer, handling and use of GMOs in Malawi. In implementing biosafety legislation, NBRC collaborates with several agencies at national level including, but not limited to, National Commission for Science and Technology (NCST), Malawi Bureau of Standards (MBS); Consumer Association of Malawi (CAMA); Plant Protection Unit of Department of Agricultural Research Services (DARS); and Pesticide Control Board (PCB).

The NBRC, in collaboration with stakeholders, has facilitated the development of various Guidelines and Handbooks to encourage compliance with the Biosafety Act and Regulations and to enable it to fulfill its mandate effectively. These include: (i) the Trial Managers Handbook, (ii) Inspectors Handbook, (iii) Guidelines for Confined Field Trials and Multi Location Field Trials, and (iv) Guidelines for the Safety Assessment of Food and Feed Derived from Genetically Modified Crops.

The legal framework has enabled Malawi to utilize and regulate the utilization of modern biotechnology, including Genetically Modified Organisms in agriculture, nutrition, health, environment, industry and trade sectors. Malawi approved and regulated confined field trials for genetically modified cotton, cowpea and banana and has approved genetically modified cotton for general release with 4 commercial varieties registered in 2018. These developments demonstrate that Malawi has a robust legal framework and decision making pathway based on evidence to safely management of modern biotechnology.

Despite the robust biosafety legal framework and the rapid adoption of new technologies for the development of genetically modified organisms including stack events at international level, a review of the legal documents showed that Malawi does not have guidelines for the Safety Assessments for Genetically Modified Stacked Gene Events. The NBRC, in collaboration with stakeholders, has developed the *"Guidelines on Safety Assessments for Genetically Modified Stacked Gene Events"* to establish the safety assessment procedures for viable and processed products derived from GM crops with stacked traits.

1.2 Legal authority of these guidelines

Legal authority for these guidelines is derived from section 6 of the Biosafety Act which provides that the *Minister shall formulate and review guidelines on biosafety.* Regulation 25 of the Biosafety (Genetically Modified Organisms) Regulations provides that *in addition to the information required for all applications under the Regulations, the Minister shall issue guidelines to be followed by applicants in the conduct of biotechnology activities.* Regulation 6 (a) of the Biosafety (Genetically Modified Organisms) Regulations confirms the mandate of the NBRC to "[…] *evaluate all applications concerning or related to genetically modified organisms and products thereof and make recommendations to the Minister in that regard.* Furthermore, Regulations 15 (1) stipulates that: "*No licence shall be issued unless a suitable and sufficient assessment of the risks that may be created thereby to the environment or human health is undertaken.*"

1.3 Objective of the Guidelines

The objective of these guidelines is to provide guidance and information on the safety assessment and risk management requirements including procedures for plants with Genetically Modified (GM) events stacked through traditional breeding or molecular techniques. The safety assessment process is anticipated to identify and evaluate potential adverse effects whether direct, indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s).

1.4. Scope of the Guidelines

These Guidelines shall apply throughout Malawi to every person, institution or body (Annex 2) intending to carry out GM stacking or release of GM stacked events from containment, confined field trials, multi-locational trials and imports intended for direct use as food or feed, or for processing except for pharmaceutical processes. These guidelines are intended to apply to both imported and domestically developed stacked GM crops that are;

a) Intended for cultivation (intended for release into the environment);
b) Imported for direct use in food, feed, or processing and which, if unintentionally released into the environment, could become established and persist without human intervention
c) Unintended introduction into the environment
d) illegal transboundary movement

CHAPTER TWO

2.0 GUIDING PRINCIPLES FOR GM SAFETY ASSESSMENT

The risk associated with GM crops is weighed in comparison with non-GM counterparts. Any differences identified are then subjected to a risk analysis to determine if they pose any greater risks to humans, animals and the environment. The assessment of risk associated with GM crops focuses on the viable GM crop part and also the processed products on a case-by-case basis.

2.1 Concept of Safety Assessment

The safety of products from GM crops is established using data that establishes whether such products are substantially equivalent to non-GM counterparts and the newly introduced protein is safe at the level present in the GM plant. The GM products can be viable products (e.g. seed and grain) or non-viable material (e.g., flour, starch, crushed meal, or oil) derived from a GM crop. This comparative approach acknowledges that the goal of safety assessment is to establish whether the GM product is as safe as its traditional counterpart. The data on substantial equivalence gives reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption.

2.2 Substantial Equivalence

This comparative approach, embodied in the concept of substantial equivalence, is not a safety assessment. Substantial equivalence represents the starting point which is used to structure the safety assessment of a new GM product relative to its conventional counterpart. This concept has been described in international consensus documents, such as the Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Crops, on which these guidelines have been based; this process is used to identify similarities and differences between the modified GM product and its conventional counterpart. Any differences identified are then subjected to a risk analysis to determine if they pose any greater risks to human and animal health than the conventional counterpart.

Any type of genetic modification results in intended effects, but may also result in unintended effects. safety Assessment is concerned with the identification and characterization of both effects with respect to possible adverse impacts on human and animal health, and on the receiving environment. Effects can be direct or indirect, immediate or delayed, including cumulative long term effects. Intended effects are those that are designed to occur and which fulfil the original objectives of the genetic modification. Unintended effects of genetic modification are those which are consistent (non-transient) differences between the GM crop and its appropriate comparator, which go beyond the primary intended effect/s of introducing the transgene/s. As these unintended effects are event-specific, the applicant must supply data on the specific intended event. Data that may reveal such effects including those related to the properties of the plant before transformation, the method of transformation, the source of the donated gene, properties of the transformed plant and cultivation method.

2.2.1. Description of the non-modified conventional crop

The applicant must describe the biology of the plant before genetic modification. The applicant is expected to submit detailed information, including the sources of this information (e.g., literature citations). The submitted information includes common name, scientific name, taxonomy, biology, history of cultivation, crop importance in Malawi, crop production levels, variety, genotype, relevant phenotype, sensitivity to population as food/feed, hybridisation or gene flow possibility, and general interaction with other organisms.

2.2.2. Description of GM Crop

For the GM crops, the applicant should identify the GM event that is the subject of the application (including any commercial or trade names), viable products of GM crops for direct use in food, feed or processing (e.g., flour, starch, crushed meal, or oil derived from a GM crop), purpose of the genetic modification and intended uses of the GM crop in Malawi.

2.2.3. Description of the Donor Organisms

The applicant must describe the donor organism(s) including its common name, scientific name, taxonomic classification, history of safe use and including use as a food/feed in Malawi and elsewhere.

2.2.4. Description of the Genetic Modification(s)

The applicant must describe the method used for the modification, including citation of the source of the protocol. Donor organism, description, and characterization of all genetic materials used to modify the crop and their intended functions in the crop should also be provided. In the application to NBRC, the applicant must include details of any intentional modifications to the introduced genetic material, diagram of the plasmid used, restriction map of the introduced genetic material, including coding and non-coding sequences. For each sequence the applicant must provide information on the location, order, and orientation in the vector and the name and size of the sequences inserted.

2.2.5. Characterization of the Genetic Modification(s)

The applicant must provide a comprehensive molecular and biochemical characterization of the genetic material after transformation. The applicant must indicate the number of insertion sites, the organization of the inserted genetic material at each insertion site, sequence data of the inserted material, indicating whether the sequence of the genetic material was conserved or whether significant rearrangements have occurred upon integration and sequence data of the flanking regions bordering the site of insertion. The applicant must describe novel substances synthesized in the GM crop including the gene product, the gene product function, the level and site of expression of the expressed gene product(s), and the levels of metabolites, if any, in the major tissues of the crop. The applicant must also indicate whether all expected traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance.

2.2.6. Phenotypic Characteristics of the GM Crop

The GM crop should be compared with a suitable conventional counterpart (e.g., near-isogenic line or parental line) and commercial cultivars to demonstrate that, except for intentional phenotypic changes induced by the inserted genetic material, the field performance of the GM crop falls within the normal range for the crop. The applicant should indicate changes in the growth habit, life cycle of the crop, reproductive characteristics. Data should be collected from confined field trials conducted over multiple growing seasons in a range of environmental conditions representative of the intended area of commercial cultivation in Malawi.

CHAPTER THREE

3.0 SAFETY ASSESSMENT FOR GM PLANTS WITH STACKED TRAITS

3.1. Description of Stacked events

GM crops have been modified to have high tolerance to abiotic stress, improved disease resistance, herbicide tolerance, pest resistance, improved nutrition, improved shelf life, or the ability to produce useful by-products, among others. Recently, the combination or stacking of different traits or genes in plants has rapidly gained popularity in biotech crop production. The combination of two or more genes/traits of interest into a single plant is referred to as stacking GM events.

Trait stacking can be obtained two ways: 1) by conventional plant breeding, in which single GM event parents are crossed to produce progeny with two or more GM events referred to as stacked trait products (also known as "breeding stacks"), or 2) by molecular methods, in which two or more traits are introduced through transformation. The transformation process can introduce genes simultaneously or sequentially. Therefore, a single transformation process can introduce DNA with two or more traits or different transformation processes can introduce a different trait on top of the previously approved event in a crop.

Conventionally bred stacked trait products do not contain any new events that have not been evaluated and approved by regulatory bodies. Sometimes the process of breeding Stacked genetically modified crops (GMCs) with their improved traits, versatility and low cost, have been well received by many growers and researchers since their inception and are now leading transgenic crop developments. However, the requirements for the safety assessment of products obtained using conventional breeding stack strategies are not standardized and differ among countries. The main question is whether a breeding stack creates unintended effects and changes that require additional safety assessments. Two primary concerns are 1) whether a breeding stack can increase genomic instability (heritability) and, 2) whether potential interactions between the products of the transgenes in stacked GMCs impact on safety.

Generally, if the singles are safe, the stacks are also considered safe. However, there may be instances where questions of potential for interactions between the combined events may be raised. In such cases where the potential for interactions cannot be excluded based on the individual modes of action of the respective GM Events, and there exist a clear testable hypothesis of such interactions between combined events which may raise safety concerns, an assessment of such an interaction will be required. The applicant will therefore be required to provide relevant information for this assessment. - The request for information shall be made where it is not available from previous work and where there is evidence of possible interaction based on mode of action of the different gene products (Figure 1). However, for the case where high order stack has been shown to be safe, low order stack that develop naturally through open pollination in commercial fields and intermediates used for seed production will be considered safe in Malawi.

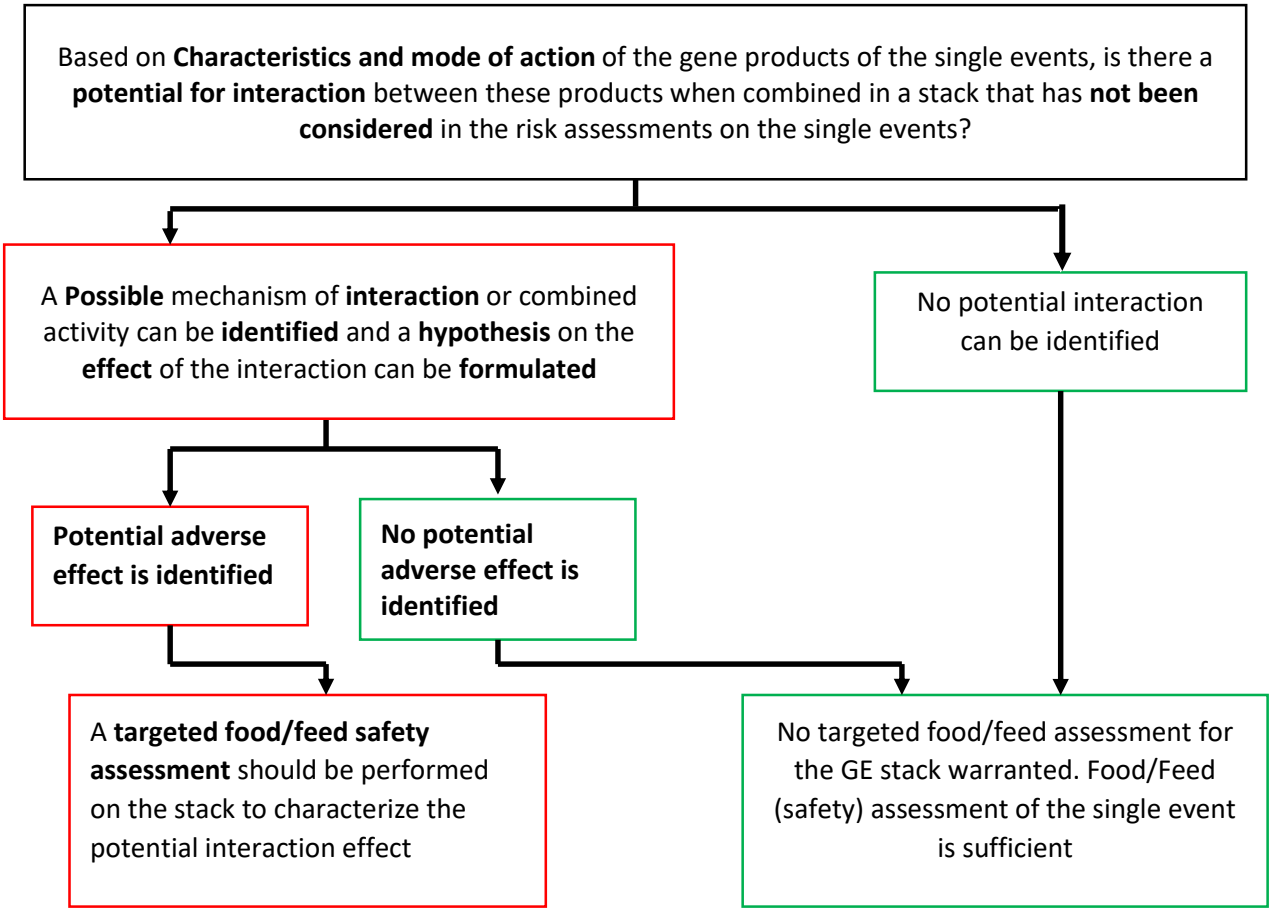


Figure 1: A possible decision-making process for food/feed crop with stacked events combined by conventional breeding (Adopted from Steiner *et al.*, Plant Physiology, 2013)

3.2 General Approach in regulation GM crop with Stacked traits

Regulating GM stacked events mainly focuses on two issues: previous information about the GM stack and / or the method of stacking used. For GM stacks produced through conventional breeding, safety assessment will focus on whether any of the events has undergone regulatory process in Malawi or a similar environment outside Malawi. Stacks produced in Malawi through molecular breeding will require similar steps, including the evaluation of intended and unintended effect of the transformation process and on the transgene at molecular, genotype and phenotype level of the stacked event. Regardless of method of producing the stack events, the NBRC framework identifies the following as possible Gene Stack (GS) events:

a) GM Stacks where the single events have already been approved,
b) GM stacks where at least one of the events is not approved,
c) GM Stacks where all the events have not been approved

3.3 Safety Assessment Approaches for Stacks produced through Conventional Breeding

The approach for safety assessment of stacks shall depend on the regulatory status of the single events used in producing the GM stacked product. In general, sub-combinations that develop through natural segregation in cultivated fields of approved higher order stacks are covered under the resulting safety assessment of the higher order stack. However, this approach can be reviewed at the point when the applicant decides to consider a specific trait within an already approved stack product to become commercially available to end users.

3.3.1 **Stacking or Importing of GM Events where the single events have already undergone regulatory approvals in Malawi.**

In cases where stacked traits are combined by breeding, and all parental events have biosafety regulatory approvals, a limited safety assessment will be conducted, and the following procedure shall apply;

i. An Applicant will be required to submit a Notification (Annex 1) to NBRC. The Notification will include information on data for/on possible gene interactions, gene stability for the singles, gene expression levels for the singles and on possibility of any synergistic or antagonist effects in the stacks.
ii. The Notification will be submitted by the applicant to the Biosafety Registrar (BR) who will vet general compliance and completeness of the application. The BR will be guided by the Biosafety Act in the assessment of the application.
iii. After being satisfied with the completeness of the Notification, the BR will send the application to the NBRC for comprehensive review.
iv. According to the Biosafety Act, the applicant expects to receive feedback from NBRC, through the BR, within 30 working days. When satisfied the NBRC, through the BR, will advise the Minister responsible.
v. If the decision of the Minister is to grant a permit or a license to the applicant, the permit comes with conditions as relevant. There are several types of conditions possible, such as those related to reporting, record keeping and training
vi. NB: Sub-combinations that develop through natural segregation in cultivated fields of approved higher order stacks are covered under the resulting safety assessment of the higher order stack and thus exempted from this notification.

3.3.2 Stacking or Importing of GM Events where one or more of the single events have not already undergone regulatory approvals in Malawi or elsewhere.

Where stacked traits are combined by breeding, and one or more single events do not have biosafety regulatory approval, a full safety assessment will be conducted on all new or unapproved single events (Table 1). The following procedure will apply;

i. An applicant will be required to prepare and submit a full data package for the NBRC A simultaneous review process will be adopted for the unapproved single event(s) and the higher order stack (concurrently) to ensure seamless decision making. Where the singles and the higher order stacks are found to be safe following risk assessment, the outcome of this risk assessment will also cover the lower order stacks, also called

intermediate or sub stacks including those that develop naturally through open pollination in commercial fields and those used as parental materials for seed production.

For the purpose of this document, full risk analysis process shall be as follows:

i. New application should be made in the prescribed format to the NBRC for an un-approved events.
ii. Provide information/review for possible gene interactions for the stacks
iii. Data on gene stability for the singles
iv. Data on Gene expression levels for the singles
v. Data on possibility of any synergistic or antagonist effects for the stacks
vi. Full Safety Assessment to be conducted

NB: CFT data will only be required for the combination product(s) that the applicant intends to make commercially available to end users and in accordance with existing guidelines. Where the applicant intends to commercialize any sub-combination other than the ones approved, supplemental efficacy data may be requested, as appropriate

3.3.3 **Stacking or Importing of GM Events where none of the single events have already undergone regulatory approvals in Malawi or elsewhere.**

Where stacked traits are combined by breeding, and all the single events **do not** have biosafety regulatory approval, this will be treated as a new application requiring a full Safety Assessment to be conducted on all the single events. In this case, the applicant will be required to submit full Data packages for the single events and the highest order stack in relevant application formats to the NBRC for full safety assessment (Table 1). A simultaneous review process will be adopted for the single event(s) and the higher order stack (concurrently) to ensure seamless decision making. Where the singles and the higher order stacks are found to be safe following risk assessment, the outcome of this risk assessment will also cover the lower order stacks, also called intermediate or sub stacks including those that develop naturally through open pollination in commercial fields and those used as parental materials for seed production.

For the purpose of this document, full risk analysis process shall consist of the following procedures:

i. An Applicant will be required to submit a prescribed application form to NBRC. The dossier should include: data for possible gene interactions; Data on gene stability for the singles; Data on Gene expression levels for the singles; Data on possibility of any synergistic or antagonist effects for the stacks; a Full Safety Assessment
ii. The dossier will be submitted to the Biosafety Registrar (BR) who will vet general compliance and completeness of the application. The BR will be guided by the Biosafety Act in the assessment of the application.
iii. After being satisfied with the completeness of the application, the BR will send the application to the NBRC for comprehensive review.

iv. According to the Biosafety Act, the applicant expects to receive feedback from NBRC, through the BR, within 90 working days. When satisfied the NBRC, through the BR, will advise the Minister responsible.

v. If the decision of the Minister is to grant a permit or a license to the applicant, the permit comes with conditions as relevant. There are several types of conditions possible, such as those related to reporting, record keeping and training

NB: CFT data will only be required for the combination product(s) that the applicant intends to make commercially available to end users and in accordance with existing guidelines. Where the applicant intends to commercialize any sub-combination other than the ones approved, supplemental efficacy data may be requested, as appropriate.

3.5 Safety assessment approaches for stacks produced through Molecular Techniques

The development of stacked traits using molecular techniques could be carried out through re-transformation, co-transformation or through the use of multi-gene cassettes. Whichever method is used; the following parameters need to be assessed prior to release:

A) Assessment of the Intactness of the Inserted Loci and Genotypic Stability

The following should be provided by the applicants:

I. Information on the genetic stability of the transgenic locus (loci); phenotypic stability and inheritance pattern(s) of the introduced trait(s).
II. Information showing that the integrity of the events is retained in the GM plant.
III. source of the material, the sampling design and the number of plants used for the analysis. When analysing the inheritance pattern(s), appropriate statistical methods should be applied.
IV. A comparison between the structure of the inserts in stacked traits and that of the insert present in the corresponding single events.

B) Assessment of Potential Interactions between Combined Events and the Resulting Phenotypic Effects

For GM plants containing stacked events for food, feed, import and processing purposes, the main objective of the analysis is to assess the potential for any interactions between the events which may raise safety concern. If newly expressed proteins are present in the GM plant, their levels should be compared, in the same field trials, with the levels in any set of GM plants that have been assessed for safety. Applicants will therefore be required to provide the information on the function of the nucleic acid region(s) intended for insertion. This information should comprise the following elements:

I) complete sequence of the nucleic acid intended to be inserted, including information on the donor organism and any deliberate alteration(s) to the corresponding sequence(s) in the donor organism(s);

II) history of safe use of the gene product(s) arising from the regions intended for insertion;

III) data on the possible relationship of the gene products with known toxins, anti-nutrients and allergens

C) Assessment of Cumulative/Combinatorial/ Synergistic or Antagonistic Adverse Effects of Stacked Transgenic Traits on the Conservation and Sustainable Use of Biological Diversity
Applicants are to provide information on the following:

i. Phenotypic characteristics, including the modified traits, compared to the parent GMOs and to relevant non-modified recipient organisms (plants);
ii. Compositional analysis, including the levels of toxins, allergens or anti-nutritional factors known to be present in the parent GMOs or non-modified recipients.
iii. Additional information depending on the nature of the combined traits. For example, further toxicological analysis of the stacked event may be required to address any synergistic effects arising from the stacking of two or more insecticidal traits that result in a broadened target range or increased toxicity.
iv. The levels of expression of any introduced proteins or modified traits, compared to the parent GMOs.

The general provisions of assessment shall comply with "*Guidelines for the Safety Assessment of Food and Feed Derived from Genetically Modified Crops*" of Malawi;

D) Development of specific methods for detecting individual transgenes combined in stacked events (To be researched further)

E) Compositional Assessment Using Substantial Equivalence
The provisions of section 5 of National Biosafety guidelines on GM food and feed shall apply.

CHAPTER FOUR

4.0 ENVIRONMENTAL RISK ASSESSMENT

Environmental Risk Assessment (ERA) shall be carried out in a scientific sound and transparent manner on case by case basis in line with the Fourth Schedule of the Biosafety (Management of Genetically Modified Organisms) Regulations of 2007. Environmental risk assessment includes three basic stages: problem formulation risk characterization and risk evaluation. Fig 2 provides detailed steps from problem formulation to overall risk evaluation.

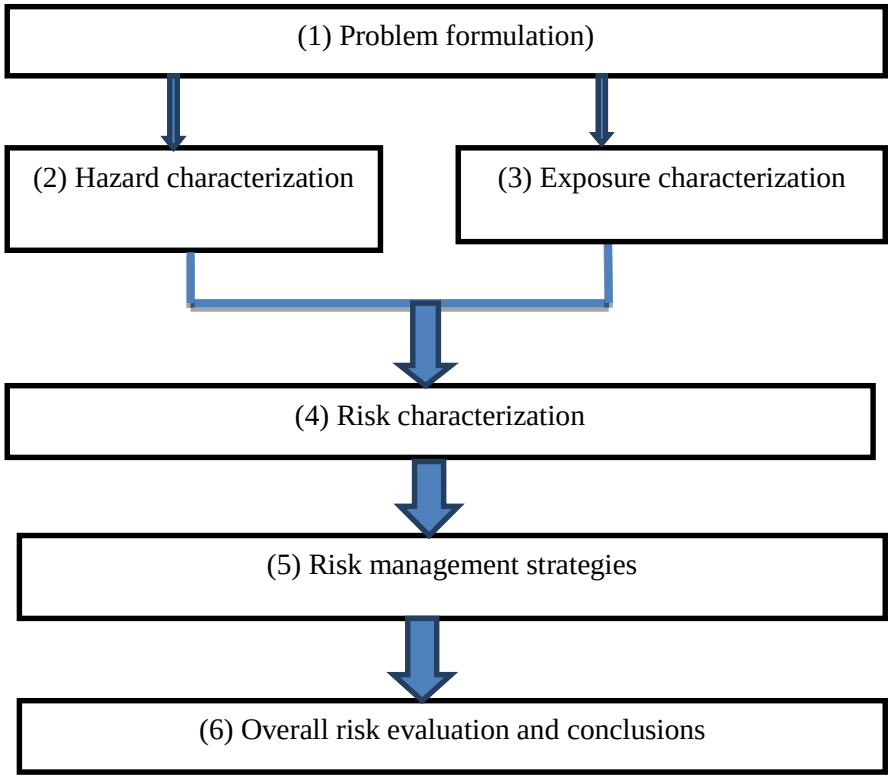


Figure 2: Environmental Risk Assessment Flowchart

4.1 Steps in Environmental risk assessment

Step 1: Problem Formulation

This is the first step in ERA. The applicant shall be expected to identify any possible harm that could arise from the GM crop. Problem formulation includes the identification of those characteristics of the GM crop capable of causing potential adverse effects to the environment, the nature of these effects, and the pathways of exposure through which the GM crop may adversely affect the environment. Problem formulation also defines the assessment endpoints and sets specific hypothesis to guide in the generation and evaluation of data in the next risk assessment steps. Problem formulation starts with identification of hazards arising from the GM crop and its use. A comparison of the characteristics of the GM crop with those of its appropriate comparator enables the identification of differences in the GM crop that may in a way lead to harm. The identified potential adverse effects need to be linked to the assessment endpoints (Assessment endpoints are sometimes called specific protection goals or operational protection goals) in order to derive testable hypothesis that allow for the quantitative evaluation of the harm posed to those assessment endpoints. An assessment endpoint must include an

entity (e.g. such as salmon, honeybees or soil quality) and a specific attribute of that entity (e.g. such as their abundance, distribution or mortality).

Step 2: Hazard characterization

This is the qualitative and/or quantitative evaluation of environmental harm associated with the hazard. Hazard characterization shall be categorized as having potential to cause major, intermediate, minor or marginal consequences.

(a) Major consequences: Significant changes in the numbers of one or more species of other organisms, including endangered and beneficial species in the short or long term. Such changes shall include total eradication or significant reduction of a valued species (protection goal).

(b) Intermediate consequences: Significant changes in population densities of other organisms, but not change which could result in total eradication of a valued species (protection goal). There could be long-term effects, provided there are no serious negative effects on the functioning of the ecosystem.

(c) Minor consequences: Non-significant changes in population densities of other organisms, which do not result in total eradication of any valued species, and have no negative effects on the normal functioning of an ecosystem. The only organisms that might be affected would be non- endangered, non-beneficial species either in the short or long term.

(d) Marginal consequences: No significant changes caused in any of the populations existing in the environment.

Step 3: Exposure characterization

This step evaluates the exposure i.e. likelihood of adverse effects occurring, and estimates the exposure quantitatively. For each hazard identified and characterized, it may not be possible to estimate the exposure (likelihood) precisely. However, applicants shall be expected to at least qualitatively give likelihood of occurrence expressed as highly likely, likely, unlikely or highly unlikely.

Step 4: Risk characterization

Risk is characterized by combining the magnitude of the consequences of a hazard and the likelihood that the consequences occur (Table 1)

Table 1: Risk Determination Matrix

		Risk Estimate			
Likelihood of hazard	**Highly Unlikely**	Negligible	Low	Moderate	High
	Unlikely	Negligible	Low	Moderate	High
	Likely	Negligible	Low	High	very high
	Highly Likely	Low	Moderate	High	very high

This model is a tool to enable identification of information and methodologies which might be useful for risk assessment. Then, in establishing that any of the steps is impossible or unlikely will lead to the conclusion of minimal risk.

Step 5: Risk management strategies

When risk characterization (step 4) identifies risks, then the applicant shall propose appropriate mitigation measures. These specific measures should endeavour to reduce the identified risks associated with the GM crop to a level of no concern, and should consider defined areas of uncertainties. Where the applicant has proposed mitigation measures, their adequacy shall be assessed by the NBRC before final determination of the application. The applicant should also give specific post-release measures in order to monitor and verify the efficacy of the risk management measures, and to allow changes in risk management strategies in case circumstances changes or when new scientific data emerges which require changes to the risk management.

Step 6: Overall risk evaluation and Conclusions

An evaluation of the overall risk of the GM stacked crop shall be made taking into account the results of ERA and associated levels of uncertainty, the weight of evidence, and the risk management strategies proposed in the receiving environment. The overall risk evaluation shall be categorized as either **acceptable** or **manageable**. In instances where the overall conclusion is that the risk is manageable, appropriate post release monitoring strategies shall be put in place and assessed progressively by the NBRC and any other relevant regulatory agency. High risk overall conclusion shall be considered unacceptable and the GM crop shall not be approved. On the other hand, negligible, low and moderate risks shall be mitigated by appropriate management strategies as appropriate.

4.2. Key Elements for Consideration under ERA

The following elements are key and need to be taken into consideration during environmental assessment for GM stacked events; invasiveness, Interactions between the stacked events and target organisms and potential impact the stacked events on non-target organisms

4.2.1. Invasiveness

Comparison between plants containing the stacked events and the most appropriate comparators during one growing season and multiple geographical locations representative of the various environmental conditions in which the GM plants will be cultivated are necessary. There is need for systematic and frequent observations for any changes. Once changes in behaviour, fitness, and reproduction are noted within a year, additional field data may be required.

4.2.3. Interactions between the stacked events and target organisms

The impact on target organisms should be examined in field trials in two growing seasons to identify and evaluate possibly altered efficacy of biocidal gene products in stacked events

compared to individual events. Additional research may be required if biologically relevant changes are discovered.

4.2.4. Potential impact the stacked events on non-target organisms

Stacked biocidal traits may have different effects on non-target organisms when compared with the single trait events. Therefore, there is a need to focus on changes in sensitivity of non-target organisms and specificity of biocidal gene products. To test the hypothesis that such combined events do not interact, a minimum of two season field trial is required. Where appropriate, further laboratory tests on a range of relevant non-target organisms representing ecological functions, using plant material containing the combined events may be required.

4.2.5. Environmental Monitoring Plan

Applicants intending to introduce stacked genetically modified organisms into the environment must put in place a plan for general monitoring that would be designed to identify any potential adverse effect that may have been overlooked during the risk assessment. Applicants may wish to adopt different approaches for monitoring plan such as:

- Monitoring of stacked events and their cultivation sites by using a questionnaire
- Utilization of data collected by existing monitoring networks

Applicants in collaboration with the biosafety competent authority should carry out monitoring and evaluation of risk for a specified period equivalent to the life cycle of the relevant species.

5. Decision Making

The outcome of the risk assessment of the higher order stacks and the singles will also apply to the sub-combinations. Where the singles and the higher order stack are found, following environmental and food safety assessment, to be as safe as their conventional counterparts, the sub-combinations or lower order stacks (also called intermediate or sub-stacks) will also be considered as safe as the higher order stack for purposes of decision making. This includes sub-combinations that develop naturally through open pollination in commercial fields and those used as parental materials for seed production. The decision to permit the environmental introduction of any of such events will however be on a case-by-case basis and will be at the sole discretion of NBRC taking into consideration other decision-making criteria.

REFERENCES

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6. Malawi Government (2017). The Malawi Growth and Development Strategy III
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8. Organization of Economic Cooperation and Development (2002). Report of the OECD Workshop on the Nutritional Assessment of Novel Foods and Feeds. Organization of Economic Cooperation and Development (OECD), Paris.

ANNEXES

ANNEX 1: STACK EVENT NOTIFICATION FORM

This form will guide in the determination of how stack events are regulated under Malawi Biosafety Act of 2002

SECTION I: APPLICANT INFORMATION	
Name of Applicant: Address: Email: Telephone:	
1.2. Affiliated Institution: Address: Email: Telephone: Website:	
SECTION II: SINGLE EVENTS (List all events/ traits in the Stack)	
2.1. Trait/ Event Name	Approval Status in Malawi (Approved/ Not Approved)
2.2. Intended use (Research, Import, Environmental release, Placing in the market, etc.)	
SECTION III: ORGANISM DESCRIPTION	
3.1. Description of the host organism (or parent organisms) before and after stacking: 3.2. Description of the gene products, their functions and the affected pathways before and after stacking (where applicable):	
SECTION IV: METHODOLOGY	
4.1. Give a summary of the techniques used to make events in the stack: 4.2. Indicate the method of stacking used (multigene cassette transformation, co-transformation, retransformation, conventional crossing):	

4.3. Provide the names of vectors used and their genetic map (where applicable)		
4.3.1 Is the vector naturally pathogenic?	4.3.2 Is the vector disarmed?	4.3.3 If yes, how was the vector disarmed?
Yes No	Yes No	
4.4. Describe delivery methods used for transformation, retransformation or co-transformation (where applicable)		
SECTION V: GENE AND/ OR GENE PRODUCTS		
5.1. Are there any interactions and/ or epistatic effects between the introduced genes or/ and their expression products?		
Yes No If Yes; Describe the possible interactions (synergistic, additive, antagonistic or silencing effects) taking into account their modes of action, metabolic pathways involved, etc.		
5.2. Has the stack event been approved anywhere in the World? If YES, where and for what purpose?		
SECTION VI: DETECTION METHODS		
6.1. Outline the detection methods applicable to this stack (if different from the singles)		
SECTION VII: REFERENCES		

SECTION VIII: DECLARATION OF CORRECTNESS OF INFORMATION

I certify that the above information is true to the best of my knowledge.
Principal Investigator/Applicant

Name _____

Signature _____ Date _____

Collaborator(s) (if applicable)

Name(s) _____

Signature _____ Date _____

Annex 2: Key players in safety assessment of stacked GM products

S/N	NAME OF KEY PLAYER / STAKEHOLDER	ROLE ON STACKED GM PRODUCTS
1	Applicant	• Fills out application to develop or introduce Stacked GM product • Submits required safety data package based on internationally accepted testing procedures • Ensures compliance with all conditions stipulated in the licence or permit
2	Environmental Affairs Department	• Through NBRC and Biosafety Registrar's office, reviews application in accordance with the Biosafety Act, the Biosafety Regulations and relevant guidelines • Consults the public following prescribed procedures • Incorporates public comments in its review and recommendations to the responsible Minister
3	Malawi Bureau of Standards	• Provides input in developing of standards for any type of stacked GM product • Acts as expert reviewer for stacked GM applications, as member of the NBRC
4	National Commission for Science and Technology	• Gives advice on science and technology (S&T) to Government and other stakeholders on all matters related to S&T including modern biotechnology • Promotes Science and Technology
5	Biosafety Registrar	• Ensures proper administration of the Biosafety Act (2002) and Regulations (2007) • Works in collaboration with applicants and NBRC, making follow-ups on progress of application • Works as secretariat for the NBRC and responsible minister before his final advice regarding the application
6	Minister responsible for environmental affairs	• formulate and review guidelines on biosafety; • establish contact and maintain liaison with appropriate bodies involved in biosafety; • promote awareness of biosafety issues; • maintain an inventory of all premises and persons involved in biotechnology; • approve safety aspects of import, export, manufacture, processing and selling of genetically modified organisms and products thereof; among others
7	Universities	• Supports scientists conducting research in biosafety and modern biotechnology

8	Department of Agriculture Research Services	• Supports scientists conducting research in biosafety and modern biotechnology • Hosts modern biotechnology testing programs, including CFTs and multi-location trials • Acts as expert reviewer for stacked GM products, as member of the NBRC
9	Consumers' Association of Malawi (CAMA)	• Provides advice to consumers on the safety of any products, including stacked GM products • As part of NBRC they participate in evaluation of applications providing expertise on the safety of any products to consumers

Annex 3: Summary of Breeding Stack Regulation in Malawi

ATTRIBUTES OF THE STACKED PRODUCT	TYPE OF APPROVAL	DATA REQUIRED FOR THE STACKED PRODUCT	DATA REQUIRED ON THE UNAPPROVED EVENTS (S)
Has each of its single events that have been approved previously	Notification: For tracking the stacked product, exempt from full assessment	(i) information on possible gene interactions, (ii) data on gene stability for the singles, (iii) data on Gene expression levels for the singles and (iv) data on possibility of any synergistic or antagonist effects in the stacks	Successful safety reviews of the higher order stack to also cover intermediates (also called sub-stacks or parental intermediates) that develop through natural recombination and/or those that are used for the commercial seed production purposes
Has at least one single event which has not been approved previously	Approval: Assessment is required	Interactions that have potential impact on safety.	Gene stability (heritability) Gene expression levels Full safety assessment. Successful safety reviews to also cover intermediates (also called sub-stacks or parental intermediates) that develop through natural recombination and/or those that are used for the commercial seed production purposes In addition, full CFT data will be required for the combination product(s) that the applicant intends to make commercially available to end users and in accordance with existing guidelines.
Has none of its single events approved previously	Approval: Assessment is required	Interactions that have potential impact on safety. Full safety assessment.	Gene stability (heritability) Gene expression levels Full safety assessment. Successful safety review to also cover intermediates (also called sub-stacks or parental intermediates) that develop through natural recombination and/or those that are used for the commercial seed production purposes

Note: Stack products for which the original intended use has changed will require full assessment.