NATIONAL BIOSAFETY AUTHORITY

Summary risk assessment report on the Application to introduce transgenic maize with stacked events MON 810 containing *cry*1Ab gene and MON 87460 containing *csp*B gene under confined field trials for efficacy against stem borer pests and drought tolerance in Kenya

Background information

The National Biosafety Authority received an application on 19th March 2015, from Kenya Agricultural and Livestock Research Organization (KALRO) to introduce transgenic maize with stacked events MON 810 containing *cry*1Ab gene and MON 87460 containing *csp*B gene under confined field trials for efficacy against stem borers pests and drought tolerance at KALRO Kiboko and Kitale centers.

Maize (*Zea mays*) is the primary grain food crop in Sub Saharan Africa (SSA) with more than 300 million people depending on it as their main food source. In Kenya it is a staple food crop. The average yield of maize in SSA is ~1.5ton/ha while that of farmers in developed world is ~5.0ton/ha. The low maize yield is attributed to a number of factors among them pest damage and drought. Strategies to address these challenges such as chemical control, cultural methods, biological control of pests, irrigation conventional breeding for drought tolerance are not sustainable due to costs and challenges of implementation. Therefore, genetic modification is a possible alternative strategy in addressing the constraints in maize production.

The objective of the proposed project was to assess the efficacy of transgenic maize with stacked event MON810xMON87460 obtained by crossing inbred lines of MON810 and MON87460 containing *cry*1Ab and *csp*B respectively against stem borer pests and drought stress under confined field conditions. The *cry*1Ab gene insert, from the soil bacterium, *Bacillus thurigensis,* encodes a delta-endotoxin protein that is toxic to a narrow range of stem borers notably *Chilo partellus* and *Buseola fusca*. The *csp*B gene from the soil bacterium *Bacillus subtilis* to confer drought tolerance characteristics in the maize. KALRO had tested these two events separately in KALRO Kiboko where the MON 810 event was evaluated for three seasons and MON 87460 for six seasons. The current application is to test the stacked events in lowlands (Kiboko) as well as highland ecological zone (Kitale).

Summary details of the application

Title of application: Application to introduce transgenic maize with stacked events MON 810 containing *cry*1Ab gene and MON 87460 containing *csp*B gene under confined field trials for efficacy against stem borer pests and drought tolerance in Kenya.

Applicant: Kenya Agricultural and Livestock Research Organization (KALRO)

Collaborating Institutions: CIMMYT; Monsanto (K) Ltd; AATF

Type of Application: Confined field trial

Locations of Research: (i) KALRO-Kiboko Research Centre, Makueni County, 2°12'42.7"S
37°43'01.6"E

(ii) KALRO-Kitale Research Centre, Trans-Nzoia County, 0°58'48.0"N
35°01'00.1"E)

Parental Organism: *Zea mays* (maize / corn)

Trait being modified: Insect resistance and drought tolerance (stacked event)

Genetic modification method used: MON 810 was generated through particle acceleration method while MON 87460 was generated through Agrobacterium mediated transformation. MON810xMON87460 hybrid was produced by crossing the two inbred lines.

Risk Assessment Summary Table

No	Issues of concern	Potential adverse effects (Hazard)	Estimation of likelihood	Consequences if the adverse effect were to happen	Estimation of risk (Likelihood x consequence)	Consideration of risk management	Acceptable/ Manageable
1	Gene flow	Vertical gene transfer: Possibility of out-crossing with conventional maize and wild relatives of maize leading to increased fitness thus causing increased competitive advantage	Unlikely	Marginal	Crossing with neighbouring sexually compatible plants is negligible	• There are no wild relatives of maize in Kenya • Teosinte and *Tripsacum dactyloides* (gamma grass) may cross with maize, however, none of these is native to Kenya or Africa. • The CFTs will be physically isolated with 400 metres hence possibility of out-crossing with conventional maize is remote	Acceptable
		Horizontal gene transfer with a possibility of causing antibiotic resistance	Unlikely	Marginal	Development of antibiotic resistance in humans is negligible	• The *npt*II which imparts kanamycin resistance is widely used in transgenic plants. The *nptII* genes have been thoroughly tested and found to be safe for use in transgenic crops. • Furthermore, experimental material will not be consumed either by human or animals as they will be destroyed at end of trial.	Acceptable
2	GMO handling	Escape during transport	Unlikely	Marginal	Possibility of escape during transport from airport to experimental site is negligible	• Three layer packaging to be enforced • Escort of GM material during transport from Airport and between containment and confinement facilities • Adherence to SOPs by the applicant and trial managers	Acceptable
		Possibility of inadvertent loss of propagative material	Unlikely	Minor	Possibility of escape of experimental is low	• Material is under confinement and chances of escape are low since the facilities have 24/7 security arrangement. • Staff involved in the trial including security personnel will be trained on biosafety matters. • Adherence to SOPS which must be retained at the two CFT facilities. • At end of trial, all GM materials will be destroyed and disposed at the respective CFTs. Any transfer of samples for analysis from any CFT site must be authorized by NBA who will also escort the GM materials.	Acceptable
3	Persistence and invasiveness	Possibility of increased fitness or competitive advantage	Unlikely	Minor	Risk of wild uncontrolled growth is low	• Maize is incapable of sustained reproduction outside domestic cultivation and non – invasive in natural habitats since it has lost its ability to survive in the wild as a result of intensive domestication. • Maize is not considered to be a weed nor invasive in an agricultural setting and is unlikely to persist in the environment unless it is maintained • No volunteer maize plants within the CFTs and isolation area will be allowed to flower or set seeds. Post harvest monitoring to be done for at least 12 months or 3 months for rain-fed and irrigated fields respectively. • Maize is not known to exhibit seed dormancy.	Acceptable

4	Gene safety	Adverse effects on human and animal health	Unlikely	Marginal	The risk of Allergenicity, Toxicity and Pathogenicity occurring is negligible	• No consumption of transgenic maize either by humans or animals is anticipated • Cry1Ab proteins are not known to be toxic or allergic to mammals. *Cry*1Ab protein is toxic only to targeted lepidopteran pests. • *csp*B protein has a history of safe use, has no structural similarity with known toxins or allergens, is present in many fermented foods • The source of genetic material including selectable marker gene *nptII* used for the constructs are safe and have been used successfully in other transformation work without any reported risks • The *A. tumefasciens* used in the transformationof MON87460 is naturally pathogenic to many plant species; however, the TI plasmid of the Agrobacterium has been disarmed thus eliminating any pathogenicity potential to plants. • The two plasmid vectors used in MON810 are not from viral or bacterial pathogenic sources. • *E.coli* bacteria, the source of *nptII* occurs naturally in the human gut and is not pathogenic • *csp*B is not pathogenic and is often used in culinary formulations associated with beneficial effects to humans and animals.	Acceptable
5	Stability of inserted gene	Gene disintegration	Unlikely	Minor	The risk of gene disintegration in subsequent generations is low	• Agrobacterium transformation method has been demonstrated to result to more stable gene integrations due to low copy numbers. • At the proof of concept stage, it is difficult to determine long term stability of the two genes but this will be assessed during the CFTs. • The interaction of the two genes is still unknown; it would of interest to collect data on this.	Acceptable
6	Non target organisms	Adverse effect on other non-targeted organisms leading to loss of bio-diversity	Unlikely	Marginal	The risk of the inserted genes causing adverse effects on non-targets is negligible	• The mode of action of Cry1Ab protein in MON810 indicates that Cry1Ab has selective toxicity against certain lepidopteran pests and not other insect orders. • No receptors for Cry proteins have been found in fish or birds; hence adverse effect on non-target organisms is not anticipated. • Cold shock proteins *csp*B are from naturally occurring bacteria	Acceptable
7	Resistance	Development of insect resistance	Highly Likely	Minor	The risk of developing resistance is moderate	• Development of resistance with time is a natural phenomenon with all pest management strategies and if it occurs in the long term, scientists will devise suitable counter-strategies such use of refugia and other insect pest management strategies (IPM). At this stage of CFT testing, this is not a major concern.	Manageable

Overall conclusion on risk and risk management

Both the cry1Ab (in MON810) and *csp*B (in MON87460) genes are from naturally occurring soil bacteria and have history of safe use. The regulatory elements used in both MON87460 and MON810 are from non-allergenic sources and have a long history of use without any documented adverse effects. The cp4-EPSPS and gox encoding for glyphosate tolerance, are segregated out and are absent in the final event plants. The cry1Ab gene encodes a nature identical *cry*1Ab insecticidal crystal protein, whose toxic effect is specific to certain Lepidopteran insects but not against other insect orders. The mode of action of *csp*B protein in plants is through a conserved stress adaptation mechanism common to plants and bacteria. The *csp*B protein has no structural similarity with known toxins or allergens. The stacked event will be tested in confined field trial sites in Kiboko and Kitale. The CFT in Kiboko has been used for testing MON810 and MON87460 events separately and is adequate to handle the new trial. Overall, the likelihood of risk arising from this research is low; the risk management measures as indicated in the dossier and proposed approval conditions are stringent enough to contain experimental materials within the proposed CFT facilities.

Decision

The application is approved with the following conditions;

1. Applicant to obtain a plant import permit (PIP) from KEPHIS. On importation, the transgenic seed maize must be escorted by officers from NBA and KEPHIS from the port of entry to the experimental or storage site.

2. Ensure that the trial site at KALRO Kitale is inspected and approved by NBA and KEPHIS before issuance of PIP. The trial site in KALRO Kiboko must also be re-inspected to assess its current status before use in the new project.

3. A detailed schedule of activities not exceeding 5 years from the date of approval should be provided both to NBA and KEPHIS before commencement of the trial to facilitate monitoring of the project.

4. Before commencement of the trial, staff on both Kiboko and Kitale CFT sites must be trained on biosafety matters and evidence availed to NBA.

5. Develop and avail operational manual and/or SOPS at both Confined field trial sites.

6. Put and implement measures to ensure that no plant material from the trial may enter the human food or animal feed chain.

7. All the transgenic plant material including leaves, stovers, grains, seeds and below ground debris must be rendered biologically inactive before disposal.

8. Notify NBA and KEPHIS of any changes to the experiment or change of protocol that might alter the risk status of the GM maize plants. Any unusual observation including but not limited to stacked genes interaction should be reported to the regulator immediately.

9. Post-harvest monitoring to be done for at least twelve months (if rain fed) or 3 months (for irrigated CFTs). All the volunteer maize plants in the CFT and surrounding 400 metres must be uprooted and destroyed before flowering.

10. Considering that there exists limited biosafety data on stacked events of this nature, the authorized party is encouraged to generate critical biosafety data that would support environmental release of the transgenic event in future should it be necessary.

11. If the project proceeds to environmental release, appropriate Environmental Impact Assessment (EIA) approval certificate or exemptions must be obtained from National Environmental Management Authority (NEMA) prior to such release.

12. Provide quarterly and annual progress reports to NBA in the prescribed format.

Approval details

Approval number: NBA/GMO/C09/18/21

Approval Date: 30th June 2015

Duration of approval: 5 years (Renewable)

Approved by,

Prof. Dorington O. Ogoyi **Date: 18th April 2020**
Chief Executive Officer
National Biosafety Authority - Kenya