NATIONAL BIOSAFETY AUTHORITY

Summary risk assessment report on the application to conduct confined field trials (CFT) to assess resistance of Banana to Banana Xanthomonas Wilt (BXW) Disease

Background information

The National Biosafety Authority (NBA) received an application from Kenya Agricultural and Livestock Research Organization (KALRO) on 22nd June 2016. The application was administratively screened and acknowledged within the stipulated 30 days. The main objective of this confined field trial is to evaluate transgenic banana (cultivars 'Gros Michel' and 'Cavendish Williams') expressing ES*Pflp* or stacked (*Hrap-Pflp*) genes for resistance against *Xanthomonas campestris* pv. *musacearum* under field conditions. The specific objectives of the trial are:

1. To carry out food safety assessments tests (allergenicity and toxicity) of the transgenic bananas.
2. To carry out comparative compositional analysis of banana fruits in order to compare the quality of transgenic banana with that of control non-transgenic banana.
3. To carry out environmental safety tests. Soil samples from the CFT site will be analyzed for non-target effect of transgenes on the soil microflora and microfauna.
4. To test the agronomic performance (growth and yield) of transgenic bananas.
5. To test the durability of the trait (bacterial resistance) for three cycles under field conditions.

Banana Xanthomonas Wilt (BXW) is a bacterial disease caused by *Xanthomonas campestris* pv. *musacearum* and was first reported in Ethiopian more than 45 years ago and has spread to Kenya, particularly to Nyanza and Western parts. Currently, there are no commercial chemicals, biocontrol agents or resistant cultivars available to control the pathogen. BXW can be managed by cultural methods; however the success of such approaches has been inconsistent due to the labour requirements. There are no known sources of genetic resistance to bacterial wilt. The lack of natural resistance, labour intensive cultural control methods and the challenges of conventional breeding makes genetic modification if successful a better strategy in addressing this economically important disease. This project proposes to use two genes {ferredoxin –like amphipathic protein (*Pflp*) and hypersensitive response-assisting protein (*Hrap*) genes all isolated from sweet pepper} either singularly or stacked to evaluate whether the Banana Xanthomonas Wilt (BXW) disease will be managed or completely controlled.

Summary details of the application

Title of application: Application to conduct Confined field trial of transgenic bananas for resistance to Banana *Xanthomonas wilt* (BXW) Disease

Applicant: Kenya Agricultural and Livestock Research Organization (KALRO)

Collaborating Institutions: International Institute of Tropical Agriculture (IITA)

Type of Application: Confined field trial

Location of Research: KALRO Alupe Research Centre, Busia County - 0°29'52.2"N 34°07'30.3"E

Parental Organism: *Musa spp*, Banana

Trait being modified: Disease Resistance.

Genetic modification method used: *Agrobacterium* mediated transformation

Risk Assessment Summary Table

	Issues of concern	Potential adverse effects (Hazard)	Likelihood of occurrence of adverse effect	Consequences should the adverse effect occur	Estimation of risk (Likelihood x Consequences)	Consideration of risk management	Conclusion on risk (Acceptable/ Manageable)
1	Gene flow	Vertical gene transfer: Possibility of out-crossing between transgenic banana and wild relatives of banana and other conventional banana cultivars leading to increased fitness	Unlikely	Marginal	Crossing with neighbouring sexually compatible plants is **negligible**	• The center of origin and diversity for banana is South East Asia. There are no wild and/or weedy relatives of banana in Kenya, except for the ones introduced and contained at the research stations. • The risk of gene transfer from genetically engineered bananas into the environment and or from one population to the other through cross-pollination is very low and this is attributed to very low pollen production of edible bananas due to the extremely low male and female fertility. • However, in order to exclude any possibility of this phenomenon, the inflorescence will be bagged and the male buds will be removed from the all plants in CFT and the isolation distance between the CFT and any relatives will be maintained at a minimum distance of 200m around the CFT	Acceptable
		Horizontal gene transfer with a possibility of causing antibiotic resistance	Highly unlikely	Intermediate	Low	• nptII which has been used as selection marker has a history of safe use and has not been reported to cause antibiotic resistance. • Gene transfer from plants to microorganisms under natural conditions is unlikely • No consumption of transgenic bananas by humans or animals is anticipated at this stage.	Acceptable
2	GMO handling	Possibility of inadvertent loss of propagative material during movement between containment facilities as well as escape from the CFT	Unlikely	Minor	Possibility of escape of experimental is low	• Material is under confinement and chances of escape are low. All materials except those for further analysis will be destroyed and disposed on site. • Staff involved in the trial including security personnel will be trained on biosafety matters. • There are operational manuals that will be placed at the CFT • There will be limited	Acceptable

						movement of transgenic material as the GM banana has been generated locally (BeCA-ILRI) and the only movement is from the glasshouse (Nairobi) to the CFT (Busia). • The experimental materials will be transported in a three tier, secure packaging system and transported in an enclosed vehicle to the CFT site at KALRO-Alupe, accompanied by NBA and KEPHIS officers and KALRO scientist. • Isolation distance of 200 metres provided • Chain link fence and security provided 24/7	
3	Weediness	Possibility of transgenic bananas becoming weedy or invasive	Unlikely	Minor	Risk of wild uncontrolled growth is low	• Banana is not considered a weed nor invasive in an agricultural setting as it doesn't possess the characteristics of weeds among which there is "discontinuous germination and long-lived seeds" which is exhibited only in wild bananas. • The introduced genes are from natural sources (sweet pepper) which is not known to have weedy or invasive characteristics. • Cultivated bananas have been domesticated for quite a long time and cannot survive for long in abandoned fields. In addition, bananas are not invasive since they survive under conditions of strict management • However, being vegetatively propagated, their underground corms enable them to withstand drought and weedy conditions for a limited period of time. If bananas persist in fields in an invasive manner, they can be controlled by chemical methods, manual or mechanical removal from undesired fields. • The CFT site will also be monitored for 6 months after harvest to collect any volunteers, which will be incinerated to prohibit any vegetative dispersal	Acceptable
4	Gene safety	Adverse effects on human and animal health ie potential to cause Allergenicity, Toxicity and Pathogenicity	Highly unlikely	Intermediate	Low	• PFLP and HRAP proteins are widely distributed throughout a broad range of plant species including rice and vegetables like sweet pepper, which is eaten raw.	Acceptable

						• The HRAP and PFLP proteins were evaluated using bioinformatics approach to identify any potential protein sequence matches with any toxin or allergens. Preliminary bioinformatics comparisons conducted on the proteins expressed showed no similarity to known allergens or toxins. • The *A. tumefasciens* used in the transformation is naturally pathogenic to many plant species; however, the TI plasmid of the Agrobacterium has been disarmed thus eliminating any potential of causing any plant crown disease	
5	Stability of inserted gene	Gene disintegration	Unlikely	Marginal	The risk of gene disintegration in subsequent generations is negligible	• There has been no reported reversal or loss of genetic modification for stably integrated genes in banana. This has been confirmed from the confined field trial in Uganda, where transgenic plants were evaluated for several generations over 5 years in two confined field trials. • Agrobacterium-transformation method has been demonstrated to result to more stable gene integrations due to low copy numbers	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	• *Pflp and Hrap* were isolated from the sweet pepper, *Capsicum annuum* which has a history of safe use *and the two genes* intensify hypersensitive response (HR), a plant defense mechanism against invading pathogens commonly found in disease resistant Plants. The genetic modification does not change the banana plants except for the introduced bacterial resistance trait. •	Acceptable
7	Target organisms	Development of resistance to the BXW disease and/or emergence of more virulent	Likely	Intermediate	Moderate	• Development of resistance with time is a natural phenomenon with all disease control strategies and if it occurs in the long term,	Manageable

		strains				scientists will be expected to devise suitable counter-strategies • Use of two stacked genes, *Pflp* and *Hrap* is expected to delay breakdown of bacterial pathogens resistance	

Overall conclusion on risk and risk management

The likelihood of risk arising from this project is low because of the following reasons. *A.tumefaciens* mediated transformation is a natural method that has been used for decades and has not posed a threat to animals, plants or the environment. *Pflp* and *Hrap* proteins, which were isolated from sweet pepper, can intensify hairpin HPSS-mediated hypersensitive response. They were analysed using bioinformatics and their sequences did not match with any known substances that can cause allergenic or toxic effects in their hosts. Genetic regulatory elements the project will utilize like CaMV35, Nos P, Ubiquitin P, Nos Poly A are isolated from natural sources and are not known to cause any allergic response and have been used for generation for transgenic studies and as such have a history of safe use. Risk management measures such as minimum isolation distances of 200m, 2m high chain linked fence, only authorized personel to access the site etc. are stringent enough to govern the confined field trial.

Decision

The application is approved with the following conditions;

1. At the commencement of the trial, transportation of the transgenic banana suckers must be escorted by Officers from NBA and KEPHIS from the BeCA BSL II green house to the experimental CFT site, to ensure proper packaging during transport.

2. A detailed schedule of activities for a period not exceeding five (5) years from the date of approval of the project must be provided both to NBA and KEPHIS before commencement of the trial to aid in monitoring purposes.

3. Avail evidence of staff to be involved in the trial having been trained by regulators (NBA and KEPHIS) on biosafety matters before commencement of experiment.

4. The proposed CFT at KALRO-Alupe must be jointly inspected and approved first for use by NBA and KEPHIS before start of experiment.

5. Avail operational manual and/or SOPS at all points of use in the CFT.

6. Put and implement measures to ensure that no transgenic banana material from the laboratory, glasshouse and the CFT enters the human food or animal feed chain.

7. Adequate containment measures to eliminate incidences of accidental escape of

transgenic banana into the environment must be put in place. No volunteer banana plants should be allowed to flower at the CFT.

8. All the transgenic plant material must be rendered biologically inactive through burning and deep burial within the CFT. Strictly adhere to the proposed waste management plan and records must be maintained and availed to biosafety inspectors on request. Any material retained for further analysis shall be counted and accounted for at all times.

9. Provide quarterly and annual progress reports to NBA in the prescribed format. The reports should be discussed by and forwarded through the IBC.

Approval details

Approval number: NBA/GMO/C09/18/25

Approval Date: 7th November 2016

Duration of approval: 5 years (Renewable)

Approved by,

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