



NATIONAL BIOSAFETY AUTHORITY

Summary Risk Assessment Report for the Application for Confined Field Trial (CFT) on the Evaluation of Transgenic Cassava Expressing African Cassava Mosaic Virus (ACMV) and Cassava Brown Streak Virus (CBSV) Resistance

Background information

The National Biosafety Authority received an application on 16th September 2013, from Masinde Muliro University of Science and Technology (MMUST) to conduct a confined field trial on cassava (*Manihot esculenta crantz*) using rDNA.

Cassava, *Manihot esculenta* is susceptible to two main viral diseases namely; African Cassava Mosaic Disease (ACMD) caused by cassava mosaic virus and the Cassava Brown Streak Disease (CBSD) that is caused by two different single strand RNA viruses namely; Cassava Brown Streak Virus (CBSV) and Uganda Cassava Brown Streak Virus (UCBSV) which are both monopartite ssRNA viruses transmitted by whitefly vectors. There are no known natural sources of resistance to CBSD that can be incorporated into cassava through conventional breeding; hence the introduction of the resistance using RNAi technology by use of *Agrobacterium tumefaciens* based vectors.

The objective of the proposed CFT is to evaluate the robustness and stability of engineered resistance against cassava viruses; ACMV and CBSV under natural field conditions. This RNAi technology has been successfully used to confer virus resistance in other transgenic crops such as papaya, squash and plums. The genetic modification is intended to introduce CBSD resistance to cassava variety TME7 (which is known to have natural resistance to ACMD) and ACMD resistance to cassava variety 60444 through RNAi technology by expressing viral sequences derived from truncated full length coat protein.

Summary details of the application

Title of application: Application for Confined Field Trial (CFT) on the Evaluation of Transgenic Cassava Expressing African Cassava Mosaic Virus (ACMV) and Cassava Brown Streak Virus (CBSV) Resistance in Kenya

Applicant: Masinde Muliro University of Science and Technology (MMUST)

Collaborating Institutions: KALRO (Kenya), ETH-Zurich (Switzerland)

Type of Application: Confined Field Trial (CFT)

Location of Research: KARI Alupe Research Sub- Centre (Busia County) GPS coordinates: 0°29'52.2"N 34°07'30.3"E

Parental Organism: Cassava (*Manihot esculenta crantz*)

Trait being modified: Disease resistance (cassava brown streak and cassava mosaic viruses)

Genetic modification method used: Agrobacterium mediated transformation (RNAi technology)

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)	Risk management measures	Conclusion (Acceptable or Manageable)
1	Gene flow	Possibility of out-crossing with the African wild type sorghum relative (<i>Manihot glaziovii</i>) or other cultivated sorghum varieties in the neighborhood	Unlikely	Severity of crossing with neighboring plants is minor	Low risk	<ul style="list-style-type: none"> • <i>M. glaziovii</i> plants are not known to be within the vicinity of the trial sites. • Continuous monitoring of isolation zone for 1 year will ensure continued absence of any cassava or <i>M. glaziovii</i> • All flower buds to be removed before maturity • Recommended isolation distance of 100 metres between the trial sites and other cassava plants to be maintained. • Rows of non-transgenic cassava surrounding entire experimental plots will act as guard rows 	Acceptable
2	GMO handling	Inadvertent loss of propagative material	Unlikely	Severity of unintended dispersal or movement of vegetative material is intermediate	Moderate risk	<ul style="list-style-type: none"> • Material is under confinement and chances of escape are low. • Staff involved in the trial including security personnel will be trained on biosafety containment measures before project commencement 	Acceptable
3	Dispersal mechanisms	Persistence and invasiveness	Unlikely	Severity of wild uncontrolled growth is minor	Low risk	<ul style="list-style-type: none"> • Cassava is not considered to be a weed nor invasive in an agricultural setting; however, one characteristic of weed i.e. “discontinuous germination and long-lived seeds” appears to apply unreservedly for cassava. • In unmanaged habitats, the viability of cassava is limited and the plant does not have a tendency for weediness and is unlikely to persist in the environment unless it is maintained • No flowers or seeds will be 	Acceptable

						<p>allowed to form in the CFT site.</p> <ul style="list-style-type: none"> Cassava plants do not propagate from storage roots, and the role of these organs in persistence is not of concern 	
4	Gene safety	Adverse effects on human and animal health (Allergenicity, Toxicity)	Unlikely	Minor	Low risk	<ul style="list-style-type: none"> The protein derived from CP gene of CBSV and UCBSV is not an allergenic to humans or animals. The vector is not naturally pathogenic 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 	Acceptable
5	Stability of inserted gene	Gene disintegration leading to cassava lines being exposed to CBSD disease	Likely	Severity of gene disintegration in subsequent generations is minor	Low risk	<ul style="list-style-type: none"> The coat protein is constitutively expressed and stably integrated. Agrobacterium provides clean insert with low copy numbers. Only lines with low copy numbers (1 or 2 copies) will be selected thus reducing the possibility of losing resistance expression. 	Acceptable
6	Non target organisms	Effect on other organisms	Unlikely	Mortality and/or effect growth characteristics is minor	Low risk	<ul style="list-style-type: none"> No new proteins are produced by use of RNAi technology. The products arising from this technology are not known to be toxic when ingested by native fauna populations including mammals, birds, reptiles and insects 	Acceptable
7	Environmental issues	Loss of biodiversity	Unlikely	Intermediate	Moderate risk	<ul style="list-style-type: none"> The products from gene silencing are not toxic when ingested by native fauna populations including mammals, birds, reptiles and insects. CFT sites are sufficiently isolated and confined. All materials in site to be destroyed at the end of the trial. Cassava is not indigenous in East Africa (Linked to Brazil and Mexico). Therefore the risk of loss of biodiversity is low. 	Acceptable
8	Resistance	Development of more virulent strains of cassava	Likely	Minor	Low risk	<ul style="list-style-type: none"> RNAi has been used in other transgenic crops and more virulent strains have not been reported so far. Development of resistance with time is a natural phenomenon with all disease control strategies and if it occurs in the long term, scientists will devise suitable counter-strategies. 	Acceptable

Overall conclusion on risk and risk management

RNAi technology used in developing the transgenic lines for the proposed CFT has been applied successfully to protect other crops from RNA viruses and has led to the development of virus resistant crops. The possibilities that the protein from the coat protein gene as an allergen/toxin are remote considering that the RNAi technology has been commercially adopted for crops like papaya, squash and plums that are consumed by humans in China and USA without any known human/animal health implications. The proposed CFT work is an experiment and no materials will be released to farmers at this stage. The facilities involved in the conduct of the CFT activities and the proposed contingency measures are adequate to prevent exposure of humans, animals and the environment to unnecessary risks. Additionally, experiments of this nature at CFT (cassava mosaic virus and cassava brown streak virus) have previously been approved by the NBA Board.

Decision

The application is approved with the following conditions.

Approval conditions

1. Applicant to obtain a plant import permit (PIP) from KEPHIS. On importation, the transgenic material must be escorted by officers from NBA/KEPHIS from the port of entry to the experimental site who shall ensure proper packaging during transport.
2. Strict adherence to the set operation and regulatory guidelines for cassava
3. Staff to be involved in the trial to be trained on handling transgenic plants and overall biosafety matters.
4. Train IBC members on overall biosafety matters and specifically on their roles in the project.
5. To aid in the planning of monitoring activities, a schedule of activities need to be provided to NBA and KEPHIS before commencement of the trial.
6. Put and implement measures to ensure that no plant material from the confined field trials may leave the site and enter the human food or animal feed chain. In instances where sample tissues will be taken from the CFT site for further analysis, Authority must be sought from the Authority and escorted as directed.
7. Provide quarterly and annual progress reports to NBA in the prescribed format

8. Notify the NBA and other relevant regulatory agencies of any changes to the experiment that might affect the risk status of the introduced material or those generated.
9. 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.

Approval details

Approval number: NBA/GMO/CO9/18/10

Approval Date: 6th March 2014

Duration of approval: 5 Years (Renewable)

Approved by,



Prof. Dorington O. Ogoi
Chief Executive Officer
National Biosafety Authority - Kenya

Date: 18th April 2020