NATIONAL BIOSAFETY AUTHORITY

SUMMARY OF AN APPLICATION FOR ENVIRONMENTAL RELEASE, CULTIVATION AND PLACING ON THE MARKET OF CASSAVA EVENT 4046 VARIETIES

1.0 General Information	
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2.0 Name and identity of the genetically modified organism

Cassava (*Manihot esculanta* Crantz) cultivar TME 204 was genetically modified to produce event 4046 cassava that is highly resistant to Cassava brown streak disease (CBSD). The resistance is mediated by ribonucleic acid interference (RNAi) via the expression of small interfering RNAs (siRNA) derived from the coat protein encoding sequences of Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), the causal agents of CBSD. Except for resistance to CBSD, cassava varieties containing event 4046 are not different from conventional cassava varieties.

2.1 Transformation Event

The unique identifier designation code for CBSD resistant cassava event 4046 is DPS-Ø4Ø46-8 (OECD unique identifier). Event 4046 contains sequences derived from the respective coat protein coding regions of two viruses known to cause Cassava Brown Streak Disease (CBSD), Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), which do not result in the expression of any new proteins.

2.2 Intellectual Property ownership of the novel trait

TheCBSD resistance trait of cassava event 4046 is not licensed and is royalty free. The Virus Resistant Cassava for Africa Plus (VIRCA Plus) project has developed the virus resistant cassava event 4046 for increased cassava production for the benefit of resource poor farmers in East Africa.

2.3 Technique used for modification

The CBSD resistant cassava event 4046 was produced via *Agrobacterium*-mediated genetic transformation of cassava cultivar TME 204 with plasmid p5001 resulting in the introduction of an inverted repeat of near full-length coat protein (CP) genes cloned from CBSV and UCBSV fused in tandem and separated by the *Flaveria trinervia* pyruvate orthophosphate dikinase (PDK) intron-3. The neomycin phosphotransferase II (NPTII) encoding gene from *Escherichia coli* was also introduced as a means of selecting transformed plants.

3.0 Summary of contained use and confined field trial data

3.1 Trials conducted in Kenya and Uganda

Cassava event 4046 was tested at the Kenya Agricultural and Livestock Research Organization (KALRO) confined field trials located at Kandara and Mtwapa. Similar trials were also conducted at the National Crop Resources Research Institute in Kasese and Namulonge in Uganda. The Mtwapa and Namulonge sites were chosen as they exhibits very high CBSD pressure to evaluate field level resistance of cassava event 4046. Evaluations were carried out over two cropping seasons at Namulonge and a single season at Mtwapa. For agronomic evaluation, trials were established in Kandara and Kasese over two cropping seasons (2017-2018) to generate regulatory data.

3.1.1 Results from greenhouse containment trials in Kenya

There were no greenhouse containment trials carried out in Kenya.

3.1.2 Results from confined field trials of event 4046 in Kenya

Event 4046 demonstrated a high level of resistance to CBSD, and recorded upto 20 times increase in marketable storage root yields compared to the conventional cassava which was the control.

One hundred (100) compositional components were assessed in samples of cassava Event 4046. There were no consistent patterns that emerged to suggest that biologically meaningful adverse change in the compostion nutritive value of the storage roots or leaves occurred as a consequence of the genetic modification resulting in cassava event 4046.

Multi-season analysis of the regulatory field trials showed that the genetic modification resulting in cassava Event 4046 did not have unintended effects on plant growth habit, general morphology, reproductive biology, diseases and pest susceptibility.

3.2 Global trials and approvals of event 4046

The stability of the inserted gene and gene expression of CBSD resistant cassava event 4046 was evaluated at the Donald Danforth Plant Science Center, Missouri USA, across four clonal generations. Event 4046 had consistently high siRNA expression across the multiple cycles of vegetative propagation. The T-DNA was stably incorporated and is likely to remain higly effective over multiple cycles of vegetative propergation.

Confined field trials were conducted for two cropping seasons (2015-2016) at the National Crops Resources Research Institute (NaCRRI) in Namulonge Uganda using micropropaged plants and stem cuttings.The trials were conducted in a location known to have high CBSD pressure.

Confined regulatory trials were conducted in Kenya at KALRO Kandara, while other trials were conducted at Kasese, Uganda over two consecutive growing seasons (2017-2018) for agronomic and phenotypic measurements as well as for compositional analysis.

3.3 Results from confined field trials of event 4046 in Uganda

The results from Uganda CFT studies consistently demonstrated high levels of resistance to CBSD. The agronomic and phenotypic measurements collected from the regulatory trials showed that the genetic modification resulting in cassava event 4046 did not have an unintended effect on plant growth habit, general morphology, disease and susceptibility to disease and pest. Among the 98 compositional components assessed, there were no adverse changes in composition or nutritive value of cassava leaves or storage roots.

4.0 Description of the Habitat where the Genetically Modified Organism may persisit or proliferate

Agronomic and phenotypic characteristics of cassava event 4046 were shown to be within the range of values displayed by conventional cassava, meaning that the growth habit of cassava was not altered. The introduced trait of resistance to CBSD does not confer a competitive advantage that would render cassava to be more weedy or invasive in natural habitats.

5.0 Donor organism (s)

Sequences were derived from respective coding regions of coat protein of CBSV and UCBSV, casual agents of CBSD. Both CBSV and UCBSV are (+) sense single strand RNA (+ssRNA) belonging to the genus Ipomovirus, family Potyviridae. Many plant viruses are common constituents in food and feed. No plant viruses are known to be pathogenic to animals or humans.

Escherichia coli a non-pathogenic bacteria strain K12, a normal inhabitant of intestinal flora of humans and animals where it does not cause disease.

6.0 Description of the proposed deliberate release, including the purpose(s) and foreseen products

This application seeks approval for commercial release in Kenya of cassava event 4046 as a solution to the threat posed by CBSD. Currently there are no cassava varieties with natural resistance to CBSD. If approved, event 4046 will be introgressed into farmer preferred cassava varieties adapted to different agro-ecological regions. Cassava event 4046 will benefit Kenyan cassava farmers as they will have access to CBSD resistant cassava germplasm, thus contributing to food security, and, providing economic opportunities for the future generations in Kenya.

The scope of this application covers environmental release of cassava event 4046 and encompasses commercial seed production for cultivation in all cassava growing areas in Kenya.

6.1 Forseen dates and quantities of genetically modified organisms to be released

This application seeks approval for the environmental release, so that event 4046 can be used to develop cassava varieties subject to the Seeds and Plant Variety Act of Kenya, that requires a new variety to be released according to the Governments' varietal release requirements. Event 4046 will be crossed with local farmer prefered varieties, and the resulting progenies will be entered into variety release evaluation that requires tests for Distinctness, Uniformity and Stability (DUS) along with the National Performance Trials (NPTs) in addition to on-station and on-farm demonstrations. The NPT's will be performed for two propagation cycles in at least three locations .The commercial release and cultivation of cassava varieties is planned for 2022.

6.2 History and results of previous Environmental Releases, as well as uses of the Genetically Modified Organism

There has been no history of environmental release of cassava event 4046 or use of its derivatives anywhere in the world.

7.0 Summary of the risk assessment of genetically modified organism

The potential adverse effects that could occur during environmental release and cultivation of cassava event 4046 in Kenya are described around the weight of evidence approach:

i) Molecular characterization
ii) The safety of the expressed protien
iii) Food and feed safety assesment of products derived from cassava event 4046
iv) Environmental safety of event 4046

7.1 Molecular characterization of cassava event 4046

The CBSD resistant cassava event 4046 contains a single, intact copy of p5001-TDNA integrated at a single site within the cassava genome, The introduced DNA does not contain any sequences derived from the plasmid back bone

There were no new novel open reading frames tht have the potential to encode protiens with amino acid sequence similarity to known or suspected toxins/allergens

7.2 Safety assessment of the expressed protiens

The NPTII protein from the NPTII encoding gene, a widely used selectable maker in plant transformation, has the longest history of safe use (Bevan *et al*., 1983; Fraley *et al*., 1983: Herrea-Estella *et al*., 1983).

Bioinformatic analyses has shown that the NPTII protien does not display significant amino acid sequence similarity to known putative protien toxins or allergens.

Food consumed by humans, whether of plant or animal origin, naturally contain dietary nucleic acids deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). There is a long history of exposure and safe consumption of food and fed containing plant and virus-derived dsRNAs.

7.3 Food and feed safety assessment of products derived from cassava event 4046

Key nutrients, including proximates, starch, fibre, fatty acids, amino acids, minerals, vitamines and ant-nutrients were analysed on samples of storage roots and leaves of cassava event 4046 and non-transgenic casssava. Analysis was compared to the range of values in the OECD consecus document for new cassava varieties (OECD 2009) This analysis was carried out over two cropping seasons in two locations (Kandara-Kenya and Kasese-Uganda). The results showed that in most cases there was relatively small differences observed, where larger differences were observed they were beneficial eg higer vitamin C in roots, reduced insoluble tannins in leaves) or related to a minor component (nervonic acid). The OECD has designated cassava event 4046 a unique identifier DP-Ø4Ø46-8.

Analysis of cyanogenic glycosides (HCN) concentrations in roots and leaves, of cassava event 4046 over two seasons in two locations, showed significant reductions in HCN concentrations. The range of measured HCN concentrations were within the the ranges of normal variations reported in literature

7.4 Environmental safety assesment of cassava event 4046

Cassava event 4046 was developed for resistance to CBSD, and not for pest resistance. There are no 'target species' and no non-target species. There are no plausible risk hypothesis that would indicate higher trophic interactions with non-target organisms would be negatively affected.
The introduced trait of CBSD resistance in cassava event 4046 does not confer a competitive advantage that would render it more weedy or invasive of natural habitats sine none of the reproductive or growth characteristics were modified

Cassava is propagetd exclusively by stem cuttings (stakes). The only naturalized relative of cassava is *Manihot carthaginesis* subsp *glaziovii* (Ceara rubber tree), it is not widely distributed, not invasive and dos not have weedy characteristics. There is no adverse environmental consequences arising from the potential intogressionof CBSD resistance trait from cassava event 4046 into *M. glaziovii*.

From the foregoing environmental release, cultivation and placing on the market of cassava event 4046 in Kenya is unlikely to have any adverse cosequences to human and animal health as well as the receiving environment

7.1 Risk management plans for deployment of cassava event 4046 in Kenya

The assessment of CBSD resistant cassava event 4046 has not identified potential environmental hazards or health and safety concerns relative to conventional cassava varieties. The environmental release of cassava event 4046 varieties will not result in any additional risks than those associated with the release of conventional cassava varieties. Therefore, there are no requirements for risk management plans to be implemented following environmental release.

7.2 Stewardship programme for deployment of cassava event 4046 in Kenya

VIRCA Plus project has developed a robust stewardship programme, to be implemented during the release of new cassava varieties containing event 4046, KALRO will ensure that the stewardship practices are adhered to by various aspects:

- Identify farmers that can serve as cassava basic seed multipliers in selected release areas. Contact will be maintained with these farmers to facilitate follow up of product performance assessments, training, and multiplication of certified planting material. These farmers will include those that participated in varietal selection and advancement decisions in the breeding programme.
- Establish agreements with producers (private companies or organizations) of certified cassava planting material to ensure they abide by published quality guidelines for bulking and distribution of the new varieties in Kenya.
- Publish and distribute a product user guide for farmers and extension agents throughout the cassava growing areas of Kenya including versions translated into the native dialects. The guide will contain information on how to distinguish CBSD from other cassava diseases, levels of damage necessary to report as well as recommendations on the frequency on replenishing planting material with certified planting stakes (e.g., every fourth growing season).

- Educate farmers and extension agents, seed multipliers, and KALRO scientists on the production of event 4046 cassava-derived varieties, and on the identification and estimation of CBSD symptoms and damage.
- Periodically inspect and confirm by molecular tests that producers and distributors of cassava varieties containing event 4046 are meeting quality and identity standards.
- Respond with remediation procedures in the case of confirmed breakdown of resistance to CBSD.

In addition, the applicant shall institute a monitoring programme in line with the requirements of Kenya's Biosafety Act (Environmental Release) Regulations, 2011.