



# **A handbook for the conduct of confined field trials of transgenic cassava in Uganda**



**Uganda National Council for Science and Technology**





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transgenic cassava in Uganda

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May 2009

**Published by:**

The National Biosafety Committee (NBC) Secretariat  
Uganda National Council for Science & Technology  
P.O Box 6884, Kampala, Uganda

**Preferred way to cite this publication**

UNCST, 2009. A Handbook for the Conduct of Confined Field Trials of Transgenic Cassava in Uganda. In Compliance with the Standard Operating Procedures for Conducting Confined Field Trials. UNCST CFT Publication Series

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*Photo credits:* Cassava Program, NaCRRRI

*Cover photo:* Host resistance to CMD, tolerant cultivar (L) and susceptible cultivar (R) at Nacri.

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## **Acknowledgement and disclaimer**

The Authors would like to express their thanks and appreciation to all officials and individuals who kindly provided valuable input and information during the preparation of this handbook.

Special thanks go to Dr. Theresa Sengooba and Dr. Karen Hokanson of Program for Biosafety Systems and Ms. Ruth Mbabazi Tugume of Uganda National Council for Science and Technology for their input, guidance and reviews on the handbook.

This activity was conducted as a consultancy for the Program for Biosafety Systems (PBS), under the Uganda Associate Award Project United States Agency for International Development Funded. The Project National Executing Agency is the Uganda National Council for Science and Technology (UNCST).

This handbook is not in any way meant to substitute the already existing Guidelines and Regulations for Genetically Modified crops in Uganda but is rather a supportive document that gives specific guidance on conduct of CFT of genetically modified cassava. This handbook is not meant to be used alone but rather in reference to the Trial Manager's Handbook that contains Standard Operating Procedures for Confined Field Trials in Uganda.

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## SECTION 1: INTRODUCTION TO CONFINED FIELD TRIALS

### 1.1 Introduction

Utilization of genetic engineering for development of agricultural products, while holding tremendous promise, has also been received with some concerns perceived risks human health and the environment. For this reason, international obligations have been put in place that require every country with an intention of introducing genetically modified (GM) crops in the environment to put in place systems to test these products before open release to the environment.

Before any GM crop is released to the public, it progresses through a product development pathway that includes contained investigations, confined testing, unconfined evaluation and then commercial release to farmers and the markets.

#### Contained Use

Contained use involves work performed on the GM crop within contained facilities, such as a laboratory, a greenhouse or a screen-house. During contained use there is physical isolation from the environment and strict measures have to be put in place to avoid introduction into the environment of any viable GM plant or plant part. National Guidelines for Containment have been published and all activities carried out within contained facilities should comply with the requirement depending on the level of containment (Biosafety Level of the facility). These Guidelines are available on request from the Uganda National Council for Science and Technology (UNCST) Biosafety Office ([uncst@starcom.co.ug](mailto:uncst@starcom.co.ug)) and UNCST website [www.uncst.go.ug](http://www.uncst.go.ug). The institutional biosafety committee (IBC) and biological safety officer (BSO) are responsible for enforcement of these Guidelines.

#### Confined Use

Confined use refers to work performed on the GM crop under a confined field trial (CFT). A confined field trial (CFT) can be defined as “a small-scale experimental field testing of a GM plant species performed under terms and conditions that mitigate impacts on the surrounding environment.” Confined field trials represent the initial controlled introduction into the environment of a GM crop. The trial is conducted in confinement to prevent the dissemination of the introduced genes into the environment, to prevent the persistence of the GM variety in the environment, and to prevent the introduction of the GM plant or its products into the food/feed chain. The size of the trial is usually about one hectare (ha) or less, and mitigation measures include reproductive and physical isolation. During the confined testing, data is collected on the agronomic performance and potential environmental impacts. This environmental data is part of the environmental risk assessment of the GM crop.

Every confined field trial must be approved by the National Biosafety Committee (NBC). The IBC must work with the Principal Investigator (PI) to develop a comprehensive application for biosafety review by the NBC. The requirements for applying for and conducting confined field trials are stipulated in the National Guidelines for Conducting CFTs. These Guidelines are available on request from the Uganda National Council for Science and Technology (UNCST) Biosafety Office ([uncst@starcom.co.ug](mailto:uncst@starcom.co.ug)) and are also posted on the UNCST website [www.uncst.go.ug](http://www.uncst.go.ug). The institutional biosafety committee (IBC), biological safety officer (BSO) and authorized inspectorate are responsible for verifying compliance with these Guidelines, and the terms and conditions of authorization.

### ***Unconfined Use***

Unconfined use refers to the general release or introduction into the environment of the GM crop without the requirements for reproductive and/or physical isolation. Before a GM crop is approved for unconfined release, a comprehensive environmental risk assessment, food safety assessment and an assessment of non-safety issues like socio-economic impact will be conducted. Approval of unconfined release of a GM crop requires public participation in the decision making process. Once for open release the GM crop will undergo the variety registration systems used for conventionally bred plants. Presently, Uganda does not have guidelines for unconfined use and commercial release of GM crops. Commercial release requires a Biosafety Law, which is yet to be enacted. Uganda's stand is that a Biosafety law has to be enacted before commercial release of GM crops. Some Countries do not distinguish between unconfined use and commercial release.

### ***Purpose of Confined Field Trials***

Confined field trials (CFTs) are essential to the scientific, success of any biosafety system. They can serve a number of purposes:

- » Plant developers can use CFTs to evaluate the agronomic performance of the variety with the introduced gene(s) in the natural environment. Plant developers can use the CFT to test the efficacy of the introduced trait.
- » Authorized parties can use CFTs to measure the trait expression levels of the introduced genes in a range of plant tissues.
- » CFTs can provide data on the environmental impacts of the GM crop, which is part of the environmental safety assessment.
- » CFTs can provide sufficient quantities of plant material for use in food safety assessment.
- » Regulators can use CFTs to build public confidence in the biosafety regulatory system by demonstrating its enforcement during the review process and conduct of CFTs.
- » CFTs can provide farmers and other stakeholders with an opportunity to observe first-hand the potential risks as well as benefits of the GM crop.

## ***Purpose and scope***

This Handbook provides the general Standard Operating Procedures (SOPs) which provide guidance for the safe conduct of CFTs for GM cassava species in Uganda. Compliance with these SOPs is a requirement for abiding by the terms and conditions of the trial authorization. The manual also recommends a communication strategy to accompany the implementation of the CFT. This manual should be used in conjunction with the Confined Field Trial Guidelines and the Trial Manager's Handbook that details the Standard Operating Procedures for CFTs in Uganda.

## **1.2 Terminology**

***Applicant:*** A party submitting an application for a confined field trial. Typically, the Applicant is the same as the Authorized Party, or is acting in collaboration with the Authorized Party.

***Authorized Party:*** The addressee of the Letter of Authorization is called the Authorized Party. The Authorized Party shall be a permanent resident of Uganda, or shall designate an agent who is a permanent resident of Uganda. 'Authorized Party' is construed herein to include any designated agents thereof. The Authorized Party accepts full responsibility for compliance with the Terms and Conditions of authorization, including all associated legal and financial obligations.

***Biological Safety Officer (BSO):*** An officer appointed to act as a secretary to the IBC and ensure compliance to regulations by Authorised Parties.

***Compliance:*** Fulfilling the requirements of the Terms and Conditions of Authorization, especially with regard to confinement measures.

***Compliance Infraction:*** Violation of the Terms and Conditions of Authorization.

***Confined Field Trial (CFT):*** A small-scale field trial of GM plants not approved for general release, in which measures for reproductive isolation and material confinement are enforced, in order to restrict the experimental plant material and genes to the trial site.

***Confinement:*** Restriction of an organism and its genetic traits to a specific and defined area of the environment, herein called the 'confined field trial site' or the 'trial site'.

***Construct (n):*** A segment of DNA to be transferred into a cell or tissue in the process of 'genetic modification'.

**Event:** A single instance of genetic modification of a specific plant species and type using a specific genetic construct.

**Following Crop:** A crop planted on a trial site after harvest or termination of a confined field trial.

**Free-living:** A plant living outside cultivation, or surviving without human intervention.

**Genetic Confinement:** Measures put in place to ensure that introduced genes do not escape from a field trial into the surrounding environment through pollen flow or through propagative material that can potentially grow into mature plants and reproduce.

**Genetic Engineering/Genetically Engineered (GE):** The genetic modification of organisms by recombinant-DNA techniques. For the purposes of this document, the terms 'genetically engineered (GE)', 'transgenic', 'genetically modified (GM)', 'genetically modified organism (GMO)', 'living modified organism (LMO)' and 'regulated' are equivalent.

**Genetic Modification/Genetically Modified (GM):** See 'Genetic Engineering'.

**Incident:** Any occurrence that causes, or threatens to cause, a breach of confinement of a confined field trial..

**Material Confinement:** Measures taken to ensure that all GM plant parts or tissue is materially maintained within the confines of the approved field trial site or storage facility and does not enter the food or feed supply

**NBC:** The National Biosafety Committee, hosted by UNCST, which is responsible for regulation of biotech products especially GMOs.

**PI:** Principal Investigator, the lead scientist in a confined field research study. He/she may himself/herself be the Authorised Party if he/she is the applicant or he/she may be a designated agent or lead scientific collaborator of the Authorised Party.

**Pollen-mediated Gene Flow:** The transfer of genes from one plant to another in pollen by successful fertilization.

**Prohibited Plants:** Plants that are sexually compatible with the GM plants being grown under confinement, and are thus prohibited from the established spatial isolation distance of a confined field trial.

**Propagative Plant Material:** Plant material such as seeds or cuttings capable of establishing and surviving in the natural environment without human intervention.

**rDNA:** Recombinant deoxyribonucleic acid

**Regulatory Authority:** The government body having the statutory authority to regulate an activity. For the testing and introduction of GMOs in Uganda, the Regulatory Authority is vested in UNCST (see), and exercised by the NBC.

**Reproductive Isolation:** Measures taken to prevent, principally, pollen-mediated gene flow from plants in the trial site to nearby sexually compatible species.

**Sexually Compatible:** Capable of cross-pollinating and forming viable hybrids without human intervention.

**SOP:** Standard Operating Procedure

**TMH:** Trial Manager's Handbook

**Trial Manager:** The individual(s) at a particular trial site, designated by the Authorized Party as responsible for management and compliance of an authorized confined field trial. Trial Managers are authorized to complete and sign documentation, forms and notes for the Trial file.

**Trial Site:** The area of a field trial that is confined by one or more continuous methods of reproductive and/or material isolation

**UNCST:** The Uganda National Council for Science and Technology, which is the body responsible for regulating the testing and release of GMOs in Uganda.

**Volunteers:** Progeny arising from the GM crop in a confined field trial site after the trial has been terminated.

### 1.3 Importance of cassava

Cassava (*Manihot esculenta* Crantz) is a staple food for over 600 million people in large parts of sub-Saharan Africa, South America and Asia. More than half of the world's cassava is produced in Africa, where it is a cheap and major source of calories for about 40% of the population. The crop is efficient in production of carbohydrates and is adapted to a wide range of environments. The crop is preferred by most resource-constrained farmers because of its low input requirement, tolerance to low rainfall and poor soils and ease of propagation by use of vegetative stem cuttings compared to most other crops. Cassava can be planted any time of the year, harvested year round, and harvesting for some varieties can be 'piece-meal' and take place for up to four years. These attributes make cassava one of the most reliable famine reserve and food security crops in Africa. The wide flexibility in planting and harvesting time enables farmers to allocate their spare time to cassava after attending to more season-bound crops.

Cassava is grown in Africa mostly for use as food. By contrast, about half of total cassava production in Asia and less than half of cassava produced in South America are used as food. Total consumption of cassava in Africa more than doubled from 24 million tons per year in 1961 to 1965 to 58 million tons per year in 1994 to 1998, after accounting for waste. It is a widely grown staple crop in Uganda, covering about 0.5 million hectares, with estimated annual production of 5.4 million tons of fresh roots in 2005. About 74% of farm households (each 5-6 people) in Uganda grow cassava, mostly for food. The roots of sweet cassava varieties are eaten raw, roasted, fried or boiled. Fresh cassava roots may be sliced, grated, fermented or pounded, then dried and further processed into dried chips and balls. The bitter type of cassava (cyanogenic) can only be used after fermentation. The dried chips are milled into flour which can be used alone or as a composite with millet, sorghum, maize flour to make a pasty product commonly called *ugali*, *atap* or *kalo* in Uganda. Other food products in West Africa and Latin America made from cassava include *gari*, *attieke*, and *tapioca*. Besides the cassava storage roots which are rich in energy, cassava leaves are edible and provide nutritive values similar to other green leaf vegetable that are good sources of vitamins A and C, iron, calcium, and protein.

Increasing populations and need for food in most parts of Africa have stimulated greater interest and demand for cassava production and consumption. To meet this demand, cassava has increased in importance as a food crop and also as a cash crop. The contribution of cassava to farm incomes is growing in Uganda and other cassava growing regions of Africa. Cassava is a source of cash income from sale of fresh storage roots, dried chips, flour, stems and processed products in rural and urban markets. In Latin America and the Caribbean, about half of the total cassava production is used as livestock feed. In Africa, cassava has played a minor role as an ingredient in livestock feed. Other uses of cassava are in the soft drink, malt beer, spirits and ethanol industries. High quality cassava flour can substitute wheat by up to 30% in the making of bread, cakes, biscuits and other confectionaries and pastries.

The most important cassava product is the starch contained in its storage roots. Cassava starch is a raw material in numerous industries, but it faces stiff competition from starch that is derived from numerous other plant sources. However, the resilience of cassava to environmental conditions and the fact that it is vegetatively propagated offers competitive advantage for cassava starch over other starches. Cassava starch can be used in confectionary industries, automotive and dry cell batteries, petroleum drilling, paint, textiles, pharmaceuticals, adhesives, iron ore mining, foundry, paper, soap and detergent, packaging, and cosmetic industries. Starch when subjected to an acid or enzyme can be used to derive sweet substances called starch hydrolysates which include glucose, sucrose, maltose, fructose, and syrup. It can also be heated in a dry form with an acid or alkali to produce dextrans which are sold as powders, granules, and pastes. Adhesives are made by cooking a dextrin in water. Adhesives are used in making corrugated boxes, sealing cartons, grocery, and multi-wall bags in the packaging industry; for lamination in plywood, paperboard, foot wear, and cables industries; for the production of paper tubes, cans and cones.

#### 1.4 Biology of cassava

##### ***Origin and wild relatives of cassava***

The modern cultivated cassava, *M. esculenta* subsp. *esculenta*, is thought to have been directly derived from the wild subspecies *M. esculenta* subsp. *flabellifolia*. It was first domesticated in the southern Amazon basin areas stretching from Brazil to Bolivia. The primary center of diversity in the genus *Manihot* is in northern South America (~80 species), and a secondary center of diversity occurs in Mexico and Central America (17 species). Central Brazil has the highest diversity of *Manihot* species, and is home to about 40 wild species. Most *Manihot* species occur in dry or seasonally dry conditions. Although a few species are found in rainforests, they tend to be sporadic in their distributions. The morphology of *Manihot* species ranges from low herbaceous vines to trees exceeding 12 meters in height. In Africa, the only naturalized relative of cassava is *Manihot glaziovii* (Ceara rubber tree). Although hybrids between cassava and *M. glaziovii* are fertile, these are not common and are normally obtained after deliberate hybridization in cassava breeding programmes. *M. glaziovii* is a tree species which is not widely distributed and does not have weedy characteristics.

##### ***Flowering***

Cassava plants are monoecious, producing separate male and female flowers on the same plant. Male and female flowers are borne on the same branched panicle, with female flowers at the base, and male flowers toward the tip. The flowers are small, with the male flower being about 0.5 cm in diameter, and the female flower slightly larger. In a given inflorescence, female flowers open first and the male flowers follow from one to a few weeks later. In general, flowering stages in cassava are as follows:

- » Branching begins from 2-6 months after vegetative planting.
- » A flower bud is produced at the branching point within 1 week of branching.

- » Female flowers are receptive to pollinations about 15 days after floral initiation.
- » Male flowers on the same branch open about 20-30 days later.
- » Fruits mature and are ready to dehisce (open) within 2-3 months of fertilization.

Time to flowering varies by genotype and environment and can range from 1 month to more than 2 years. Flowering is also dependent on plant habit, with more highly branched genotypes flowering more prolifically than those with a sparsely-branched habit. Therefore, apical branching is a good visual indication of plants that are about to start flowering. Environment can have a major impact and some cassava clones will flower profusely in one environment and not flower at all in another environment.

#### **Pollen production and viability**

Cassava pollen grains are large and sticky, and rapidly lose their viability after pollen shed. Due to the large size of cassava pollen, wind pollination is unlikely to occur. Several species of wasp (mainly *Polistes* spp.) and honeybees (*Apis mellifera*) are considered the main pollinators of cassava in Africa. In practice, cassava breeders perform pollinations within 1 hour after collecting pollen, since cassava pollen viability declines rapidly after this time.

#### **Seed production, dispersal and dormancy**

Developing seeds are viable about 2 months after pollination, and the fruit becomes mature 1 month after that. Dehiscence is explosive and the seeds fall close to the mother plant. Cassava seeds are adapted to ant dispersal and ants may transport the seed up to several meters from its place of origin. Newly harvested seed exhibits physical dormancy and requires 3 to 6 months of storage before they will germinate. Seeds remain viable for up to 1 year when stored at ambient temperature. However, germination percentage declines after 6 months. Seed germination is favoured by dry heat and complete darkness.

#### **Vegetative reproduction**

Botanical seed is not usually used for commercial propagation because cassava is highly heterozygous, and propagation from sexual seed results in wide and unpredictable diversity of phenotypes. Cassava cultivars are propagated almost exclusively by stem cuttings, referred to as stakes. Vegetative propagation is preferred by cassava farmers because it is the only way to maintain the desirable trait combinations present in the farmer-preferred cultivars. When cassava plants are harvested, nodal cuttings derived from the stem are cut for production of the next crop. These stakes are typically about 25 cm in length and have 4 to 5 nodes each with a viable bud. Most cassava varieties grown in Uganda attain physiological maturity 12 months after planting.

The viability of cassava in unmanaged ecosystems is limited by the habitat available. It does not survive well in abandoned fields or as an escape from cultivation, and its propagation by stem cuttings minimizes unintentional spread of the crop. The low fecundity and physiological dormancy of seeds also limits the spread and establishment of the crop into unmanaged habitats. Cassava is not considered to be a weed in agricultural settings, and is not invasive.

## 1.5 Priority transgenic cassava technologies for Uganda

### ***Gene transfer technologies***

The application of transgenic technologies to integrate desired traits into farmer preferred cultivars and elite varieties is an attractive option for genetic improvement of cassava in Uganda. This will allow the crop to fully benefit from major advances occurring in the genomic and post-genomic era. The transgenic approach can be used to transfer beneficial traits from one cassava cultivar to another and from wild relatives to cassava. Transgenic cassava technologies provide options to overcome bottlenecks that impede conventional breeding such as the crop's long breeding cycle, high heterozygosity, poor and non-uniform flowering and inbreeding depression. In addition, integration of genetic material from non-traditional sources such as viruses for pathogen-derived resistance strategies, and genes from other plant species for nutritional enhancement, can be used to benefit cassava farmers and consumers.

Transgenic systems in cassava are reliant on the development of tissue culture systems capable of generating totipotent cells and tissues. These act as the target for transgene insertion, after which selection for the successful integration events takes place and fully transformed plants are regenerated. The systems are based on the production of embryogenic tissues from *in vitro* leaf-lobe explants. In the most preferred system, friable embryogenic callus (FEC) is generated from the somatic embryogenic structures and it is the target tissue for transgene insertion. Subsequent culture of FEC on selection medium ensures proliferation of only transgenic tissues. Successful transformation events mature into somatic embryos which are recovered and germinated to produce whole plants. The *nptII* gene, which imparts resistance to the amyloglycoside antibiotics, is used routinely as a selectable marker. FEC, and the embryogenic suspensions established from them, have been successfully employed as target tissues for integration of transgenes in cassava using *Agrobacterium tumefaciens*.

### ***Virus resistance***

The threat of viral diseases, namely, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) make virus resistance a primary trait of interest for transgenic cassava research in Uganda. Since 1990, a pandemic of CMD has resulted in losses estimated to be equivalent to US\$ 60 million per annum at its peak in Uganda. Although deployment of CMD-resistant varieties contributed to reduced prevalence of the disease, most of them have poor storage root cooking qualities and many farmers have reverted to susceptible varieties resulting in resurgence of 'CMD hotspots'. Until recently, CBSD was restricted only along a small belt on the East African coast, but losses exceeding US \$100 million were

reported by CGIARNEWS in 2003. Since 2004, CBSD has emerged at high incidences in Uganda and other hitherto unaffected areas in East Africa. By 2008, the CBSD epidemic had spread to at least 25 districts of Uganda. Up to 100% incidence and total crop losses have been reported on many farmers' fields, especially in central Uganda. The severe necrosis caused by CBSD on roots only becomes evident at harvest and affected roots can not be consumed. The threat to food security by CBSD and CMD demands for new development of resistant varieties.

To address CMD, scientists from the Cassava Research team at NaCRRI, Namulonge, working alongside a team of scientists at the Donald Danforth Plant Science Center (DDPSC), Missouri, USA, have developed transgenic cassava with genes conferring resistance to CMD. The transgenic plants have been initially produced and challenged in the greenhouse at DDPSC and plans are underway to test the ability of the genes to protect cassava from CMD under natural conditions in confined field trials in Uganda. In addition, the potential to control CBSV using transgenically imparted CBSV coat protein and post transcriptional gene silencing are being investigated. Transgenic plants of the model host species *Nicotiana benthamiana* and cassava have been generated at DDPSC in which the full length coat protein (CP), C- and N-terminal portions of the CP gene from a Ugandan strain of CBSV were integrated into the plant genome. Analysis of siRNA production in these plants has indicated that the transgenic lines expressing high levels of the relevant siRNAs, therefore, are capable of controlling CBSV. Challenge of homozygous *N. benthamiana* plants lines by sap inoculation with the pathogen has shown highly effective control of CBSV in plants transgenic for all three of these genetic constructs. However, the cassava lines need to be tested in a CFT to determine the effectiveness of the genes.

### **Root quality enhancement**

Cassava storage roots are rich in starch but deficient in proteins and micronutrients. The nutritional profile of cassava considerably varies with the plant part and among genotypes. Future programmes will aim at enhancing these nutrients in cassava roots. Deployment of cassava varieties with enhanced nutritional value will especially target elevated protein and  $\beta$ -carotene content, starch modification, reduced cyanogenic content and post-harvest physiological deterioration.

### **Capacity building for transgenic cassava research**

The National Agricultural Research Organization (NARO) together with a number of development partners has embarked on building capacity for transgenic cassava research in Uganda. A number of scientists and technicians have attended short term and graduate training in cassava tissue culture, genetic engineering, molecular biology and biosafety. This

has enabled the efficiency of Ugandan farmer preferred cultivars for genetic transformation and regeneration to be evaluated. Tissue culture conditions have been optimized for a number of Ugandan farmer preferred cultivars which are now capable of producing high quality FEC, and some have been transformed and regenerated. A cassava *mock* CFT was conducted at Namulonge in 2008 to provide key scientists and technicians with skills and hands-on experience of conducting a CFT. In addition to re-furbishing a molecular biology laboratory, new facilities have been constructed at Namulonge including a tissue culture laboratory, BSL II screenhouse and confined field trial site. Efforts at developing infrastructure and human resource capacity for transgenic cassava research in Uganda will continue and are aimed at creating a solid base for routine development of new technologies and adaptation of technologies that have been developed elsewhere to elite Ugandan landraces. All such technologies will progress through CFTs.

These SOPs have been developed to minimize the potential risk of conducting cassava CFTs. A risk is a product of the likelihood of exposure to a hazard and the likelihood of the hazard causing serious harm (Risk = Exposure × Hazard). During the conduct of CFTs, when the plants are still being developed and tested for efficacy and performance, the level of hazard may not be assessed, but the risk is minimized by focusing on minimizing exposure through risk mitigation.



Plate 1. Host resistance to CMD. Tolerant cultivar (L) and highly susceptible cultivar(R).

## SECTION 2: STANDARD OPERATING PROCEDURES (SOPs)

### 2.1 3Ps for Safe Conduct of CFTs

Risk mitigation measures governing the safe conduct of CFTs are comprised of three major pillars: 1) prevent the pollen- or seed-mediated dissemination of the introduced genes into the environment; 2) prevent the persistence of the GM variety in the environment; and 3) prevent the introduction of the GM crop products into the food/feed chain.

#### ***Preventing the Dissemination of Introduced Genes***

This can be accomplished through reproductive isolation of all plants within the confined trial site. Reproductive isolation refers to the means used to control the movement of pollen from the confined trial site – thus ensuring that new genes are not introgressed into neighboring plants of the same or a sexually compatible species. Under natural conditions, pollen-mediated gene flow and introgression can only occur when the two plants are sexually compatible, male and female flowers mature at the same time, a pollen vector is available, and the progeny produced are viable, fertile, and able to persist in the environment.

Reproductive isolation in cassava can be achieved through one or a combination of measures including: (1) removal of flowers; (2) bagging of flowers to prevent open pollination; (3) termination of the trial prior to flowering; (4) spatial isolation from other sexually compatible plants; (5) use of border rows of conventional plants of the same variety to act as pollen traps for insect-pollinated species; and/or (6) temporal isolation of pollination by planting earlier or later than any nearby sexually compatible plants.



*Plate 2: Removing cassava flower buds.*

### ***Preventing Persistence into the Environment***

CFTs must be conducted in such a manner to ensure that the GM plant or its progeny will not persist in the environment. At the termination of the field trial, any viable plant material should be destroyed to prevent volunteer plants in subsequent growing seasons. Post-harvest monitoring for a specified duration and interval must be carried out to manage any volunteers that may be found.

### ***Preventing Introduction into the Food Chain***

CFTs must be conducted in such a manner that maintains the integrity of the food and feed chains. Measures must be put in place to control the movement of plant material to and from the trial site through proper transportation, cleaning of any machinery used, proper storage of seed and other plant material, appropriate disposal of plant material, appropriate disposition of retained material, and controlling unlawful harvest from the trial site.

Safe conduct of CFT requires having in place good record keeping and reporting, and regular inspections. Also, a contingency plan must be developed to minimize risk through risk management in the event of an accidental release of the experimental GM plant material.

## **2.2 SOPs for Transport and Storage**

These SOPs apply to the packaging, labelling, transportation or shipping, receipt, shipping documentation, as well as storage of genetically modified cassava in Uganda.

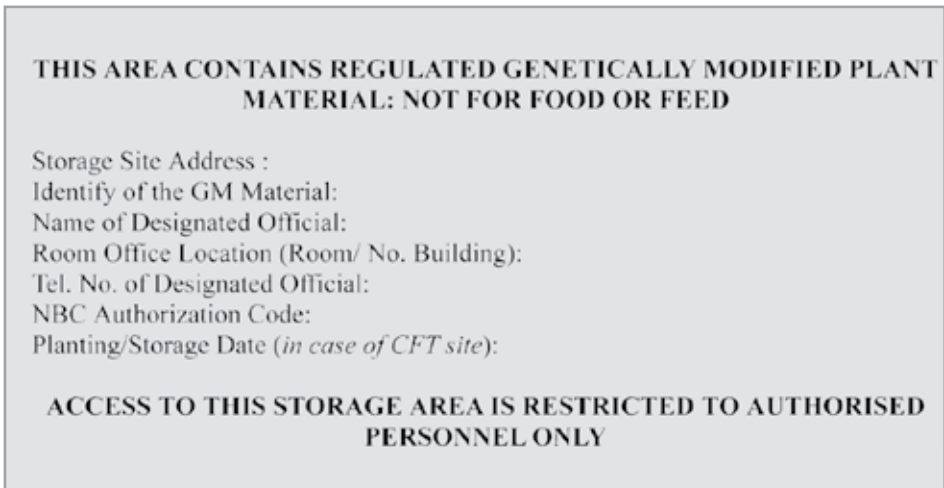
### **2.2.1 Packaging**

- i. Tissue cultured GM cassava plantlets are generally recommended for transboundary shipment or transport and must be packaged in such a fashion to prevent any accidental release.
- ii. The packaging must contain at least three layers of packaging: the primary, secondary and tertiary layers whereby the one in contact with the GM material is the primary container.
- iii. Each layer of packaging must be of such construction and sturdiness to independently prevent the release of the material under normal conditions and must be independently closable or sealable.
- iv. The name of the genotype of the plantlets, their source (institution), date of packaging shall be indicated on each container and a copy of the import permit enclosed in each layer of packaging.

### 2.2.2 Labeling

- i. Each layer of required packaging must be clearly labelled with sufficient information to establish the identity of the contents, and the contact details of an official contact person.
- ii. The label must also contain the following statement or equivalent verbiage: 'Genetically Modified Plant Material for Research Purposes Only. Do Not Use for Food or Feed'.
- iii. The Authorization Code Number issued by NBC shall be included on the label pasted on all packages.
- iv. The labelling on the stores, greenhouses and CFT entrance and other facilities shall also indicate the authorisation code, and disclaimer 'Genetically Modified Plant Material for Research Purposes Only. Do Not Use for Food or Feed' (box 1).
- v. Name and telephone contact of the Trial Manager shall be indicated on these labels at the entrance of the store or CFT site

#### Box 1. Recommended Signage for Storage/experimental facilities



### 2.2.3 Disposal or Recycling/re-use of Packaging Materials

- i. All primary containers and their associated labelling should be thoroughly checked to be devoid of GM material and shall be destroyed by incineration after removal of the materials, or thoroughly sterilized before reuse, according to the terms and conditions specified for the CFT.
- ii. Secondary and tertiary containers may be retained for re-use but after confirmation that they do not contain any GM material and after thorough cleaning. Otherwise, they should be destroyed at the same time with the primary containers.

## 2.2.4 Receipt and Shipping Documentations

- i. A Shipping Form that establishes the identity of the GM cassava planting material and the identity of the originating and receiving parties must accompany all shipments. Any additional inventory lists may be attached to the Shipping Form, if necessary, in order to list all items in a particular shipment.
- ii. The recipient of the shipment shall retain a copy of the completed Shipping Form, and all other documentation including the Import Permit.
- iii. Copies of all documentation associated with shipment of GM plant material shall be copied to UNCST and the Inspectorate.
- iv. The Authorized Party or his agent shall retain original copies of the documentation, copies of which should be kept in the compliance document binder at the trial site at all times
- v. The Authorised Party or his agents, the NBC or their representative, and the inspectorate shall be present at the time of receipt of planting materials at the port of entry and should observe the following:
  - » Complete the recipient information on the Shipping Form
  - » Verify that packaging is intact, and that no release of GM material has occurred.
  - » Note any damage to containers in the space provided on the Shipping Form and if any release of GM material is suspected or occurred, notify UNCST immediately and complete the Incident Report Form
  - » Verify that all items listed on the Shipping Form and any inventory lists have been received.
  - » Notify the shipper and/ or carrier immediately to locate any missing packages or items. If a package or item cannot be located, notify UNCST immediately, following the procedures and complete Incident Report Form.
- vi. The Authorised Party shall arrange appropriate transportation of the GM planting materials to a store or any other designated facility that will ensure continued containment or for direct planting in the field trial site as the case may be.
- vii. This transportation should be escorted by an Inspector and/or a designated agent of the NBC.
- viii. The entire shipping and transportation process shall be recorded in the Shipment and Transportation Form, copies of which shall be kept in the compliance document binder at the trial site.

## 2.2.5 Secure Storage

- i. All GM cassava planting material must be stored and maintained in such a fashion as to preserve its identity, security and integrity, and to prevent it from being consumed by humans, livestock or other animals.
- ii. Access to the storage facility should be restricted to authorised personnel only.
- iii. The storage facility which may be a store in the laboratory or a section in the screen house must be appropriately labelled as described in Section 2.13 to avoid misidentification.

## 2.3 SOP for Trial site management

This SOP applies to site establishment, access and hygiene of cassava CFTs in Uganda

### 2.3.1 Site establishment

The following SOPs must be observed during establishment of cassava CFTs in Uganda. These SOPs apply to all personnel involved in cassava CFTs and it is the responsibility of the Authorised Party to ensure compliance by all personnel to these SOPs.

- i. The CFT area must be fenced with wire mesh and adequately locked. The keys to the CFT site will be kept by the Trial Manager or another officer designated by him/her.
- ii. A store that is large enough to secure garden tools, seed, harvested produce and CFT clothing must be erected inside the confined area.
- iii. The Authorized Party shall ensure that there is 24 hour security at the site through the entire period of the cassava CFT. The guard house must be located outside the CFT site.
- iv. A map of the trial site must be prepared by the Trial Manager or PI and appended to the Record of Planting. Instructions for the preparation of maps are provided in Box 2. See example Map Below.
- v. Planting of the transgenic cassava shall be done only after authorized dates given by UNCST
- vi. The Authorized Party must notify the NBC Secretariat of the planting date at least three (3) working days prior to planting
- vii. A Record of Planting must be completed for each field trial site. A copy of the Record of Planting, with the appended map, must be submitted to the NBC within five (5) days following the completion of planting. The original Record of Planting must be retained by the Trial Manager, and copies made available to regulatory officials upon request.
- viii. Excess planting material shall be rendered non-viable by burning. Any retentions of planting materials shall be recorded, transported and stored in accordance with the respective SOPs but the NBC Secretariat must be notified of any retentions desired.
- ix. The Trial Manager must mount a sign at the trial site indicating the purpose and duration of the CFT conducted at the trial site and the Authorization Code under which the CFT was approved.
- x. An isolation distance of 100m must be observed for all cassava CFTs in Uganda. The isolation area should be monitored at least once every eight (8) weeks and all observations entered into a record book. Any prohibited plants within the isolation area must be removed before they flower.
- xi. Any flower buds developing on the test lines must be removed and destroyed.
- xii. The Trial Manager must ensure that only personnel authorized by the Authorized Party are permitted on the trial site. A bound book including the name, address and affiliation of all personnel and visitors who enter the trial site must be maintained.

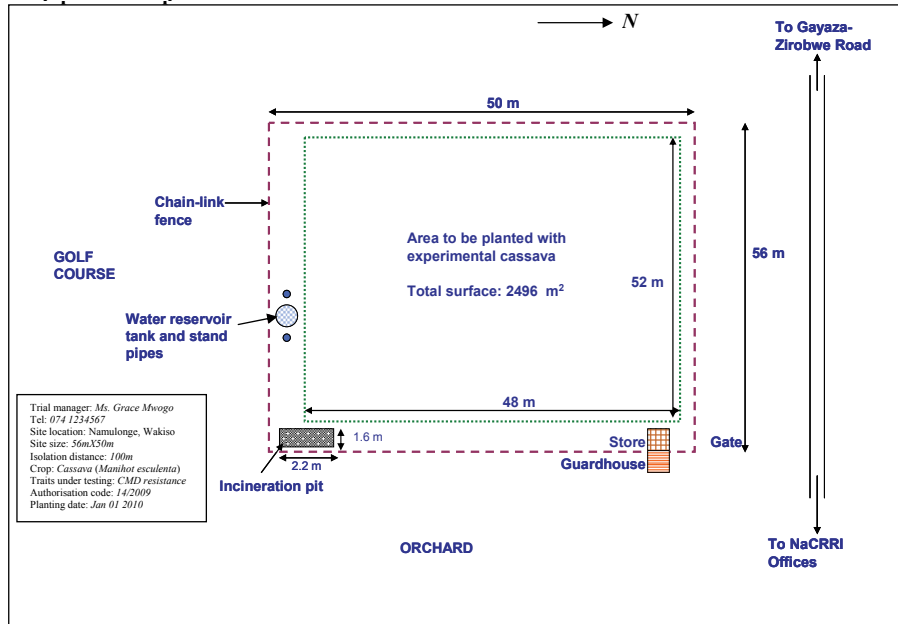
### 2.3.2 Access

- i. Access/entry to the CFT is restricted and shall be at the discretion of the Trial Manager.
- ii. Access to the trial site for the purpose of inspection shall be authorized by the IBC Secretariat upon request, for official use only and preferably during regular working hours.
- iii. A logbook providing details of the names, dates and time of entry of each individual shall be available.

### 2.3.3 Site and machinery hygiene

- i. All tools and machinery used to plant the GM cassava or to maintain the field trial site shall be free of any planting material prior to use at the trial site.
- ii. All equipment and tools used to plant GM cassava or used in the maintenance of the trial site must be cleaned on the trial site prior to their removal to eliminate unintended transport of regulated cassava from the trial site. Acceptable methods of cleaning include hand cleaning and use of high-pressure water. Any plant material recovered must be rendered non-viable by burning at the trial site.

### Example Site Map



## 2.4 SOP for Termination or harvesting and disposal

This SOP applies to termination of cassava CFTs in Uganda

- i. When the experimentation purpose of the trial has ended, it must be terminated using methods recommended by the NBC such as burning in the incineration pit located at the trial site
- ii. The NBC shall be notified at least 5 days before termination day and the notification shall indicate the exact time of the planned exercise
- iii. Unless otherwise permitted by the NBC, no materials should be taken out of the field trial site at the time of harvesting or during the trial termination exercise

### Box 2: Instructions for preparation of site maps

1. A map of the trial site will be prepared by the Trial Manager and appended to the Record of Planting.
2. Maps must provide sufficient detail to allow regulatory officials to locate each field trial site during the planting season and any required period of post-harvest land use restriction.
3. Maps must provide details on the layout of the site and distances between the field trial site and surrounding features.
4. The dimensions of the trial site and distances to physical landmarks must be accurately reported.
5. The following items shall be included on each map of a field trial site:
  - a. Trial Manager's name and contact details.
  - b. Permit number from the regulatory authority.
  - c. Legal or descriptive land location.
  - d. Accurate distances to physical landmarks such as telephone poles, fences, alleys or roads.
  - e. Total area planted with the regulated material, including border rows when used (hectares or square meters).
  - f. Label the map with all fields within the isolation area by the common name of the crop.
  - g. Label the map with any fields of cassava that fall within, or border on, the isolation area.
  - h. Include any natural ecosystems adjacent to the trial site (natural habitats, waterways, forests, and woodlots, hedgerows), where reasonable.
  - i. Planting date.
  - j. Compass directions, with North at the top of the page.

- iv. An Inspector or the agent of the NBC shall be present at the time of termination of the trial
- v. All the staff to be engaged in the termination of the field trial must first be briefed on the appropriate procedures of the termination exercise
- vi. If there are any materials that need to be taken out of the trial for experimental purposes, the NBC should be notified and such materials shall be subjected to the provisions of the Shipment and Storage SOP
- vii. The equipments and tools used during termination of the trial must be thoroughly cleaned before they are taken out of the trial site
- viii. This entire activity shall be recorded in the Crop Destruction Form

## 2.5 SOP for Inspection

This SOP applies to inspections by regulatory authorities on cassava CFTs in Uganda

- i. Inspection shall commence with the assessment of proposed site for conducting CFT, followed by regular visits during and after the experimental testing of the GM crop.
- ii. Inspections shall be carried out by the NBC members and authorized inspectorate to ensure adequacy and compliance with the terms and conditions of the trial authorization.
- iii. Inspection during the CFT shall be conducted at least once every month starting at the time of planting until the time of termination of the trial. The NBC reserves the right to organize impromptu inspection of the trial during its progress.
- iv. After CFT, inspection shall be conducted at least once every three months for the subsequent year. Inspection of the CFT shall be implemented in accordance with the Biosafety Inspection Manual available on request from the Uganda National Council for Science and Technology (UNCST) Biosafety Office ([uncst@starcom.co.ug](mailto:uncst@starcom.co.ug)) and UNCST website [www.uncst.go.ug](http://www.uncst.go.ug).

## 2.6 SOP for Post harvest management

This SOP applies to the post harvest monitoring and management of all cassava CFTs in Uganda

- i. The post-harvest period for cassava CFTs is one year and begins immediately upon harvest or termination of the CFT.
- ii. The Authorized Party must ensure that the CFT site remains restricted during the post-harvest period.
- iii. During the post-harvest period, the Trial Manager must ensure that any cassava volunteers or prohibited plants are removed from the trial site before flowering and are rendered non-viable by burning at the trial site. Volunteers must not flower at the trial site.
- iv. The cassava CFT site must be monitored at least once every three (3) months and records of presence and growth stages of volunteers and their method of destruction shall be made.

- v. Post-harvest monitoring and related activities must be recorded in the Volunteer Monitoring Form.

## **2.7 Contingency planning**

In the unlikely event of any accidental release of transgenic material from the CFT site, or at any other time during the transport of the materials to the CFT site, the following course of action will be taken:

### ***i. Notification of authorities***

The NBC Secretariat will be notified immediately by phone of any accidental release of genetically modified plant material and in writing within 24 hours. Biosafety inspectors will accompany the material during each stage of transport of the material. An incident and corrective action form will be completed for each case of accidental release. The completed incident and corrective action forms will be incorporated into the compliance binder maintained at the CFT site. Other authorities to be notified in case of emergency include Director of Research NaCRRI, Team Leader Cassava Programme, Administrative Officer NaCRRI, trial Principal Investigator, Trial Manager and local government authorities.

### ***ii. Recovery of materials***

If any transgenic plantlets should accidentally be released from the sealed plastic tubes, these plantlets will be immediately recovered, returned to the appropriate storage tubes and marked for incineration. If any plants are accidentally removed from the CFT site after planting, UPQIS officials will be immediately notified of the event and NaCRRI staff will attempt to recover the plant material under the guidance of UPQIS. All actions related to the recovery of the materials will be recorded on the appropriate incident and corrective action form.

### ***iii. Confinement of materials***

Uganda Plant Quarantine Inspection Service (UPQIS) inspectors will be notified immediately of any unintended violation of reproductive isolation. If the breach in the isolation area is due to a cassava plant flowering inside the CFT site, the 100 m isolation distance will ensure that genetic confinement is maintained. In the unlikely event that a sexually-compatible flowering plant within the 100 m isolation area appears to have been fertilized, an additional 100m isolation distance will be re-established around the plants.

### ***iv. Other***

If civil unrest, theft or natural disaster affects the integrity of the CFT, UPQIS will be notified. If necessary, all of the experimental plants will be destroyed. In case of theft, local council authorities and police will be notified as soon as possible.

## 2.8 SOP for Record keeping and Reporting

This SOP applies to keeping records and making reports on all cassava CFTs conducted in Uganda

### 2.8.1 Record keeping

Adequate records are critical to establish the compliance to the terms and conditions of the trial authorization. Clear, authentic and readily accessible records shall be maintained, documenting critical activities including:

- i. Transportation:* Records shall contain a description of the material transported, method and dates of transport and authorized custody. All movement made by the GM material shall properly be recorded. Communications between the shipper and the recipient shall also be recorded.
- ii. Storage:* An inventory of all GM plant material in storage and of portions removed from the storage area, dates and the purpose for removal shall be recorded. A daily record for responsible person and security of the storage facility shall be maintained.
- iii. Material confinement:* A record of all activities carried out to ensure material confinement shall be kept, including the site map, site security, log book for entry and exit, personnel training, planting, cleaning of equipment and disposal of excess to avoid removal of any planting material from the trial site.
- iv. Reproductive isolation:* A record including a description of the measures put in place such as flower bud removal, the temporal/spatial isolation used shall be kept.
- v. Harvesting:* A record shall document the amounts and fate of all harvested material, the disposal of any unwanted plant material, how much was retained and its disposition.
- vi. Post-harvest monitoring:* All activities related to post-harvest monitoring of a trial site shall be recorded.
- vii. Accidental release of GM plant material:* A record of all activities related to accidental release including corrective actions taken and communications shall be kept.

All records must be retained by the Trial Manager and they must be available for inspection by the BSO, IBC, authorized inspectorate and NBC.

### 2.8.2 Reporting

Reporting allows the Authorized Party to inform NBC on progress and results of the CFT, including unanticipated effects or occurrences. Preparing reports is a responsibility of the Principal Investigator and Trial Manager and all reports shall reference the authorization code assigned to the trial. Reports shall be submitted to NBC secretariat and copied to the IBC secretariat. Terms and conditions for Trial authorization require the following reports:

- » *Trial Establishment Report:* The Authorized Party shall submit details of site establishment within five (5) working days after the completion of planting at the site. The report will include the planting date, the amount of material planted, disposal and storage of any surplus GM plant material remaining after planting, and the size of the trial site. A final field site map shall also be submitted at this time.
- » *Trial Progress Report:* The Authorized Party shall submit a progress report within five (5) working days after completion of the flowering period of the crop. The report will include flowering information and results of activities enforcing reproductive isolation.
- » *Harvest Report:* The Authorized Party shall submit details of site harvest within five (5) working days after the completion of harvest at the site. The report will include the date and method of harvest, the storage or disposal of any harvested materials, and the method of destruction of any residual plant material on the site.
- » *Incident and Corrective Action Report:* In case of an accidental or unauthorized release of GM material, the Authorized Party shall orally notify NBC secretariat immediately, and in writing within 24 hours after the occurrence of the incident. The report will include any corrective actions taken or planned to contain GM material and assuage the impact of the incident.
- » *Unanticipated Effects Report:* In case of unanticipated effects, the Authorized Party shall notify NBC secretariat in writing within five (5) working days if the GM material exhibit any substantial unanticipated characteristics or effects, or if any unusual event occurs that may jeopardize the confinement of the material.
- » *Final Reporting:* An **Interim Report** shall be submitted by the Authorized Party within six (6) months after the harvest or termination of the trial, summarizing all activities, methodologies, data and analysis of experimental results concerning the trial, and any unanticipated effects observed. Within six (6) months after the completion of the post-harvest period, the Authorized Party shall submit a Final Report summarizing all observations including occurrence of volunteers and their disposal.

## SECTION 3 CAPACITY BUILDING AND COMMUNICATION

### 3.1 Training of staff

Training of staff and personnel that shall be engaged in conduct of the CFT and its management is mandatory to ensure compliance with the national guidelines and regulations for CFTs in Uganda. It is the responsibility of the Authorised Party to ensure that all personnel are adequately trained.

- i. All staff including scientists, technicians and other staff that will be involved in the implementation of the trial shall be trained on procedures, guidelines and standard operating procedures to ensure compliance with Government regulations
- ii. The trainings shall be conducted before the start of the trial and as appropriate during trial implementation
- iii. From time to time, specific briefings to certain categories of staff shall be given by the Authorised Party, Trial Manager or their authorised competent agents in preparation for key activities on or off the trial site

### 3.2 Strategic communication about the trial

A communication strategy for public engagement during the implementation of the trial is recommended. This strategy should be put in place prior to implementation of the CFTs and should be based on the National Strategy for Biotechnology and Biosafety Communication. Basing on the strategy developed, the following activities should be implemented:

- i. Sensitize community leaders and farmers in the vicinity of the trial;
- ii. Involve the relevant journalists at before, during and after the trial and communicating clear messages that they should relay to the public. This should also include development of a media package;
- iii. Develop a Frequently Asked Questions (FAQ) document that clearly answer issues about the background to the project, its objectives, targeted outcomes as well as key stakeholders, funders and supporters;
- iv. Design a standard presentation (s) on the trial that can be used in workshops and meetings of stakeholders to give clear messages on the objectives of setting up the trial, its progress and the targeted benefits and beneficiaries;
- v. Develop a 'seeing-is-believing' programme through which key stakeholders and policy makers can be brought to visit the trial as a means of laying strategy for acceptance of the technology under tri

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