

INSPECTION AND CERTIFICATION MANUAL FOR CASSAVA PLANTING MATERIAL



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About this Manual

This manual is to be used by cassava planting material (seed) inspectors appointed by the National Seed Certification Services (NSCS), Department of Crop Protection, Ministry of Agriculture, Animal Industry and Fisheries (MAAIF). The inspectors will ensure that cassava seed produced conforms to the required standards. It will also be used by cassava seed merchants (entrepreneurs), seed growers and training institutions for whom the quality of seed produced is attested by an official certificate. The manual contains the minimum standards that must be met in the tissue culture laboratory, screen house and fields for cassava seed to qualify for certification. The standards include; land requirement, isolation distances, pest and disease tolerance levels and seed standards.

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List of acronyms

CBB	Cassava Bacterial Blight
CBSD	Cassava Brown Streak Disease
CGM	Cassava Green Mite
CM	Cassava Mealy bug
CMD	Cassava Mosaic Disease
CSS	Cassava Seed System Project
DCP	Department of Crop Protection
FAO	Food and Agriculture Organization
GPS	Global positioning system
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
NaCRRI	National Crops Resources Research Institute
NARO	National Agricultural Research Organization
NSCS	National Seed Certification Services
OECD	Organization for Economic Cooperation and Development
PCR	Polymerase Chain Reaction
VCU	Value for Cultivation and Use
VRC	Variety Release Committee

Definition of Seed Certification Terms

A: Cassava crop and seed classes

Cassava *Manihot esculenta*, Crantz: Is a perennial shrub with an upright woody stem that grows up to 4m or more and belongs to the Euphorbiaceae family. It propagated vegetatively producing storage root for food and other uses.

Parental Material: The smallest unit used by the maintainer to maintain the variety from which all seed of the variety is derived through one or more generations.

Pre-basic seed: is seed of a particular variety that is produced under the supervision of the plant breeder or owner of the variety which is the source of the initial and recurrent increase in seed production of a plant variety. It is sometimes referred to as **breeder seed** especially when referring to crops propagated by botanical seeds.

Basic Seed: Seed which has been produced under the responsibility of the breeder according to the generally accepted practices for the maintenance of the variety and is intended for the production of Certified Seed. This seed class is sometimes referred to as **foundation seed**. It must conform to the appropriate conditions in the certification scheme and fulfilment of these conditions must be confirmed by an official examination. Its production is carefully supervised or approved by representatives of an agricultural experimental station under accreditation by a designated authority. It is the source of all other certified-seed classes, either directly or through basic seed.

Certified seed: is a first generation of multiplication of Basic Seed of a variety and is a class of seed produced under a certification program. It must conform to the appropriate conditions in the certification scheme and the fulfilment of these conditions must be confirmed by an official examination. It is the seed produced on large scale by certified seed growers/seed companies for general crop production. Certified first generation and second generation

Standard Seed: is seed not grown under a certification program entering the market in case of certified seed shortage but meets the same laboratory seed testing standards as certified seed

B: General terms

Cassava seed entrepreneurs: Farmers who are actively engaged in production of certified cassava planting material as a business.

Cassava seed: In the context of this manual, cassava seed refers to quality cassava stems intended for use as planting material.

Cassava stakes: Planting material taken from mature stem portions approximately 20-25 cm long for purposes of planting

Country of Registration of a Variety: The country of registration of a variety is the country where the variety is registered on the National Official Catalogue, following satisfactory tests of Distinctness, Uniformity and Stability

Crop age: refers to the time from planting to the date of assessment/inspection.

Designated Authority: Authority designated by, and responsible to, the Government for carrying out certification schemes.

Farmer: This can be an individual, a group of individuals or an institution taking part in production of certified cassava seed

Field Inspection: means inspection of the growing plants in the field by a qualified inspector following specific procedures for the purpose of determining the varietal purity of a seed crop, plants affected by pests and diseases, presence of undesirable plants and general condition of the seed crop.

Field: means the entire area occupied by one variety and one seed source and which is covered by one inspection report.

Inspector: means an officer designated/assigned and responsible for conducting the work of inspection, sampling, testing, supervising, guide and control in the implementation of seed law.

Maintainer: Person or organization responsible for the production or maintenance of a bred variety included in a national list of varieties eligible for certification under the certification scheme.

Off-Type: means any volunteer plant or any stock plant different from the cultivar as stated on the application for certification.

Seed certification: is a quality assurance system of official control and inspection of seed intended for marketing. The system certifies that a sack, packet or bundle of seed contains what it says on the label and that the seed was produced, inspected and graded, in accordance with the requirements of a Certification Scheme

Seed inspection: Any activity conducted by a crop inspector from seed acquisition through site selection, land preparation, planting, agronomic practices during the growth period, harvesting, packaging, labelling, storage and transportation requirements.

Seed lot: A specified quantity of seed that is physically and uniquely identifiable.

Seed Quality means physical quality as measured by physical purity and freedom from undesirable materials, physiological purity as measured by germination and vigour, genetic purity as measured by varietal purity and health quality as measured by freedom from insects, pests and diseases.

Seed: is a propagative material, plants and parts of plants intended for the propagation and multiplication of variety

Source of planting material: It is the supplier/ grower of the mother-plants from which the cuttings planted at the site being assessed came from.

Standard: refers to reference frame for comparison

Tissue culture: refers to the production of cassava plantlets resulting from the growth of cells from the apical meristem or shoot tip of the cassava plant and is done under controlled and hygienic conditions in test tubes (*in vitro*) in a laboratory.

Variety: is a population of plants which have common ancestors and which have certain characteristics such as morphological, physiological, cytological and chemical or others of significance for the purpose of agriculture, horticulture or forestry and which when reproduced sexually or asexually retain their distinguishing characters.

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1.1 BACKGROUND

Cassava is a major staple, commercial and industrial crop in Africa. It is relied on for food and income by more than 200 million people in eastern, central and southern Africa. Uganda produces over five million tons annually covering about 500,000 Ha and ranks 6th in Africa after Nigeria, DRC, Mozambique, Ghana and Angola. Cassava ranks 2nd in terms of production after banana in Uganda and over 74% of farmers produce it. Since 1994, 19 cassava varieties (NASE 1-NASE 19) have been officially released and are in cultivation. The process by which planting material of these varieties are obtained by farmers has been informal. In this case farmers obtain planting material of new varieties during multi-location and on farm field trials. Thereafter they multiply in their own fields from which they continuously obtain material for planting in subsequent seasons and also distributing to neighbors. Several development initiatives have multiplied and distributed cassava stems to farmers with limited quality assurance. Such informal system of seed production and distribution facilitates accumulation of cassava pathogens that causes diseases like, cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and cassava bacterial blight (CBB). It may also facilitate the spread of insects' pests like cassava mealy bug (CM), and cassava green spider mite (CGM) as many farmers do not know how to identify these pests. Consequently, there is degeneration of such planting material leading to low or total yield loss.

To promote commercial cassava seed production, an efficiently working seed system must be established. One of the components for production of quality seed is seed inspection and certification. This component has not been fully implemented in the past for vegetatively propagated crops, yet it plays a role of ensuring supply of high quality seed to farmers, which is true to type, high in purity, excellent sprouting capacity, free from pests and diseases. In order to achieve quality assurance in cassava, the National Agricultural Research Organization (NARO) through National Crops Resources Research Institute (NaCRRI) and under the leadership of MAAIF have initiatives to address sustainable availability of certified cassava

seed to farmers. Such initiatives include development of field and laboratory standards for cassava seed that will enable inspectors from MAAIF's, department of crop protection (DCP) and accredited institutions to perform cassava inspection and certification, thus regulating the quality of seed.

1.2 Purpose of seed certification

The purpose of seed certification is to maintain and make available to the public, high quality cassava seed of specific variety grown and distributed to ensure genetic and physical purity, germination ability, and freedom from pests and diseases. In addition seed certification is designed to achieve prescribed standards and is the mandate of NSCS at MAAIF.

1.3 Stages involved in seed certification

- i. Registration of field , tissue culture laboratory or screen house for inspection by the National Seed Certification Service
- ii. Provision of proof of origin of the parental material of the varieties registered for inspection. According to the Seeds and Plant Act (2006), only varieties/cultivar officially released by the Variety Release Committee (VRC) or advanced breeders' lines awaiting release shall be eligible for certification. The variety must have been evaluated for qualities such as Distinctness, Uniformity and Stability (DUS) and Value for Cultivation and Use (VCU). The origin of seed must be known and traceable to the breeders' seed. If it is a new variety, this must include the descriptors of the parental material and for the new variety.
- iii. Actual inspection to ensure that seed resulting from a crop meant for seed purpose is of the designated variety (trueness to type) and has not been contaminated genetically or physically beyond certain specific limits. The crop must be healthy and free from seed borne diseases and pests
- iv. Seed testing should be done to ensure that the materials are free from seed borne pathogens

- v. Harvesting and packaging must be done in a manner that will not damage the planting materials
- vi. Labeling and sealing: upon satisfactory fulfilment of the prescribed requirement, every seed lot should be provided with a label and seal
- vii. Post control: this is a requirement that samples of certified seed lots are grown out in small plots to verify that the seed lot meets the necessary standards. These plots are monitored a number of times during the growing season, and may be viewed by seed growers should a problem arise with a seed lot.

1.4 Classes of cassava seed eligible for certification

Cassava seed can be categorized into three classes according to OECD standards and these include:

- i. Pre-basic seed: in this case the variety is released and maintained by the breeder. Breeder seed is used to produce basic seeds.
- ii. Basic seed: this is the progeny of breeder seed. Its production is normally under the control of mandated institutions under the direct supervision of NSCS of MAAIF. Basic seed act as source of certified seed.
- iii. Certified seed 1 and 2: this is produced through the multiplication of basic seed

Other classes of cassava seed may also include

- iv. Standard Seed: is not grown under a certification program but enter the market in case of certified seed shortage but meets the same laboratory seed testing standards as certified seed. For this class of seed, the Seeds and Plant Act (2006) allows the seed board to advise the minister on the informal seed sector and vegetatively propagated material in case of shortages of certified seed.

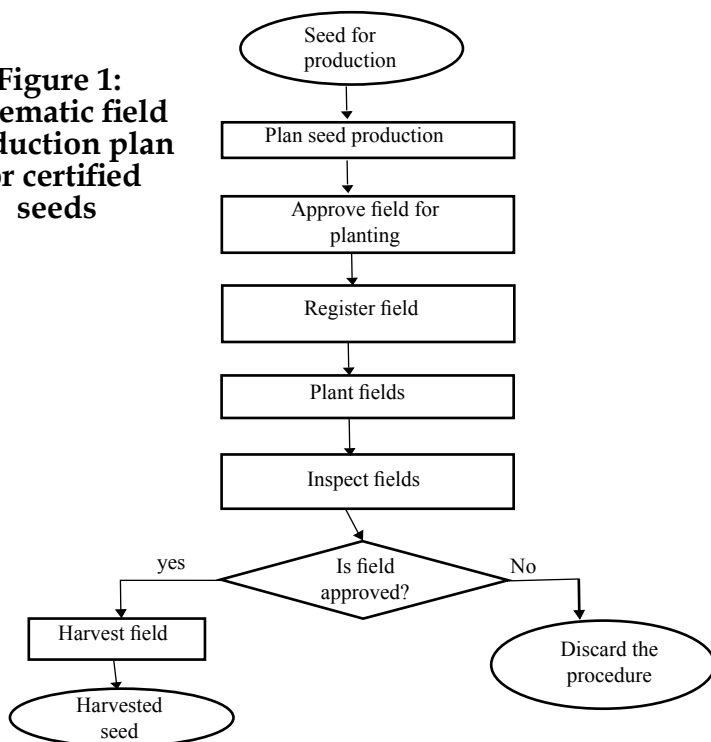
- v. Quality Declared Planting Materials (QDPM) also referred to as Quality Declared Seed (QDS), is an FAO designation of planting material intended for sale within a limited area under informal seed sector for vegetatively propagated material. A QDPM cannot be sold outside a designated area in which it is grown. This class of planting material is regulated at the local level through the district agricultural offices working under the national seed framework. However, this class of seed is not defined in Seeds and Plant Act 2006 of Uganda.

CERTIFICATION STANDARDS FOR BASIC AND CERTIFIED CASSAVA SEED PRODUCED UNDER FIELD CONDITIONS

2.1 Introduction

Basic and certified seed multiplication is normally done under field conditions and it must meet certain minimum requirements in order to be certified as a seed. Certification of cassava seed shall be accomplished by application for certification by the Seed Merchant or Seed grower (entrepreneur), verification of seed source by the inspection office, field identification and inspection, sampling and analysis for systemic pathogens and grant of certificate and certification tags as shown in the field production procedure in figure 1 below.

Figure 1:
Schematic field
production plan
for certified
seeds



2.2 Standards that must be met for certification of cassava seed under field conditions

2.2.1 Registration of cassava seed entrepreneurs

Any person who wants to take up certified cassava seed production must register with NSCS of MAAIF or through any agency accredited by MAAIF. Seed entrepreneurs must register their fields each year and request for inspections every season. For this reason, seed growers have to forward their application for field registration and inspection every season. Only approved seed fields by NSCS or accredited agency are eligible for issuance of a certificate of field inspection allowing the certification of the field for seed multiplication.

2.2.2 Verification of cassava seed source

The cassava seed entrepreneur should submit a planting return to the NSCS or through the accredited agency office to register the crop and season with a registration fee and prescribed certification charges as may be required and formalized by the NSCS of MAAIF. The seed entrepreneur shall also submit to the Certification Agency/accredited agency, one or more relevant evidence of the cassava seed source such as certification tags, seals, labels, purchase records, sales records or official letter from source etc., as may be demanded by the Certification Agency during submission of the application. These evidences shall be scrutinized in order to confirm if the seed used for raising the crop has been obtained from the approved source. The seed source used in the production of basic cassava seed must be obtained from pre-basic cassava seed while the one used in production of certified seed must be from basic seed material.

2.2.3 Field land requirements for basic and certified cassava seed production

Land for cassava seed production should meet the following requirements;

- i. Land should be at least 100 meters away from existing cassava fields.
- ii. Isolated from high pressure areas for cassava pests and diseases especially for basic seed production.
- iii. Free from volunteer cassava plants, preferably land not previously planted with cassava for at least 2 years.
- iv. Fertile, well drained soils (not swampy), not rocky, and not shaded with many trees.
- v. Accessible to beneficiaries
- vi. For Basic seed production, the land should be at least 2 acres while for certified seed production the land should be at least 5 acres

NB: For more details about land requirement, see Cassava Seed Quality Management Manual

Table 1: Isolation requirement for production of different classes/categories of cassava seed

Contaminants	Minimum isolation distance (Meters)			
	Pre- basic seed	Basic seed	Certified seed	Standard seed
Fields of the same or other variety not conforming varietal purity requirement for certification	Approved screenhouse or laboratory	100	100	100
Minimum distance between varieties in the seed field not infected by the disease	5	5	5	5
Rotation (years)	2	2	2	2

2.2.4 Tolerance levels major pests and diseases of cassava

Visual plant inspection can be done to identify major cassava pests and diseases. The common pests that attack cassava include cassava whitefly, cassava mealy bug, cassava green mite, and scale insects (Table 2). The major diseases of cassava in Uganda include cassava brown streak disease, cassava mosaic disease and cassava bacterial Blight (table 3). A maximum permissible limit for these pests and diseases must be observed and beyond which the seed must be rejected. This is summarized in the table 4

Table 2: Major pests of cassava and characteristic symptoms

 <p>Above: whiteflies under the surface of cassava leaf, and below; white flies as viewed under the microscope</p>	 <p>Sooty mould caused by heavy feeding activity of whiteflies</p>	 <p>Cassava mealybug</p>  <p>Bunchy-top symptoms caused by cassava mealybug</p>
 <p>Cassava green mite</p>  <p>Shoot Tip Die-Back caused by severe CGM infestation</p>	 <p>Characteristic foliar damage caused by cassava green mite</p>	 <p>Scale insects on cassava stem</p>

Table 3: Symptoms of major cassava diseases






 <p>Cassava mosaic disease foliar symptoms</p>	 <p>Chlorosis on cassava leaves due to CBSD infection</p>	 <p>Streaks on green parts of a cassava stem</p>
 <p>Constrictions and fissures on cassava roots due to cassava brown streak disease</p>	 <p>Dark brown necrotic spots in cassava root due to cassava brown streak disease</p>	 <p>Cassava bacterial blight</p>

Table 4: Pests and disease toleranc levels of tolerance for different categories of cassava seed

Factor	Maximum permissible limit			
	Pre-basic	Basic	Certified 1&2	Standard seed
CMD – Max incidence (%)	0.0	1.0	2.0	5.0
CBB – Max mean severity	0.0	2.5	2.5	3.5
CBSD – Max incidence (%)	0.0	1.0	2.5	5
CM – Max incidence (%)	0.0	0.0	0.0	0.0
CGM – Max mean severity	0.0	2.5	3.5	3.5
Scale insects – Max incidence (%)	0.0	0.0	0.0	0.0

Note: For CMD and CBSD the maximum disease severity must be 2 beyond which the seed should be rejected irrespective of the incidence level

Table 5: Cassava seed standards

Factor	Requirement			
	Prebasic	Basic	Certified 1&2	Standard seed
Genetic Purity (%)	100	100	99.5	99
Harvesting age of new crop (months)	10-18	10-18	10-18	10-18
Harvesting age of ratoon crop	6-12	6-12	6-12	6-12
Minimum diameter of cutting (cm)	1.5	1.5	1.5	1.5
Minimum length of stakes cutting (cm)	20-25	20-25	20-25	20-25
Minimum number of nodes per stake	4	4	4	4
Maximum damaged nodes (%)	5	5	5	5
Maximum number of stems per plant stand after ratooning	3	3	3	3

NOTE:

- I. Before seed harvesting inspectors need to ensure that off types are rogued to ensure 100% purity
- II. All infected plants are rogued to ensure disease free fields
- III. Fields with notorious weeds such as Spear grass, Couch grass

(*Lumbugu*), and star grass should be sprayed with recommended herbicides prior to planting. (For more details refer to seed quality management manual).

IV. Weed stressed cassava crop should not be considered as seed fields.

2.3 Application for cassava seed field inspection

2.3.1 Cassava seed inspection

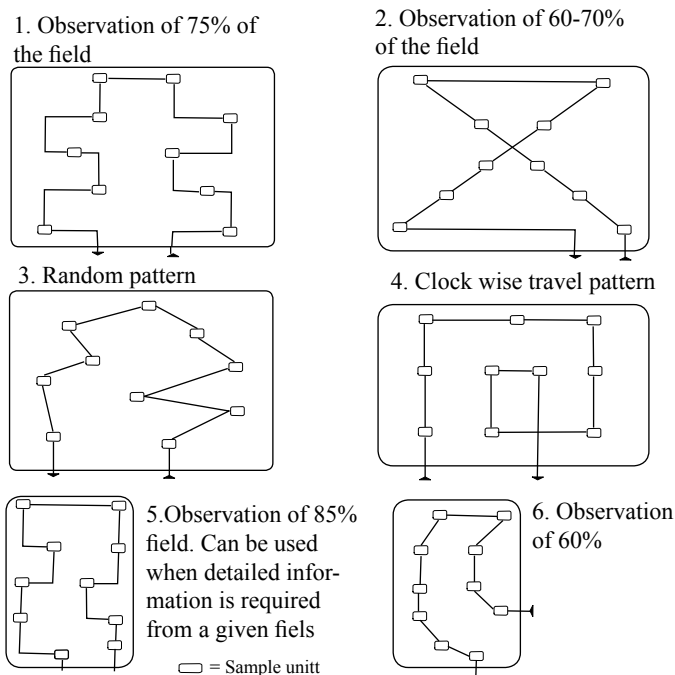
Field inspection is done by the crop inspector whose roles and equipment they need to execute their duty is defined in appendix 1 and 2 respectively. All cassava seed fields are inspected to confirm the identity of the variety, to ensure that they meet the minimum level of varietal purity and plant health standards. There are mandatory requirements with regard to the minimum isolation distance from cassava crops, and the crop rotation history of the land on which the seeds are grown. The seed entrepreneur is responsible for requesting for field inspection by applying using Form SR8 and a reply by NSCS on Form SR9. The specific date of inspection will be fixed by the NSCS/accredited agency upon receiving a request for inspection from the seed entrepreneur. Field inspection will be done four times for both basic and certified seed. The first inspection will be done at the site selection stage, second inspection is at 3 months after planting, third inspection at 6 months and fourth inspection at harvest, 9-16 months and is followed by issue of certificate. At harvesting time, the seed entrepreneur is issued with plant movement permit provided by NSCS to confirm that the seed being transported is from the certified field.

2.3.2 Steps followed during field inspection of basic and certified cassava planting materials

- i. Record the location of the sampling site preferably using a global positioning system (GPS).
- ii. Assess each cassava variety separately and record the data using the site data sheet and the Field Scoring Sheet (See appendix 4 and 5).
- iii. Assess pest and disease incidence / severity following standard protocol and record information in field scoring sheet.
- iv. In a one hectare field, one hundred plants (1%) are assessed following

any of the working pattern in (Figure 2) of each field for each variety for diseases, pests and varietal purity. The assessment is achieved by scoring every 10th plant and four other plants around it for pests and disease incidence and severity. The recommended pattern of inspection of cassava is diagonal method (pattern 2 in figure 2) but other patterns can be used depending on the objectives to be achieved.

**Figure 2:
suggested
patterns to
follow during
field inspection**



NB: Although pattern 2 is the recommended method, other patterns indicated above may also be used depending on the number of factors like type of cassava growing on the site, the denseness and growth stage of the crop, field shape, past history of the site and risk factors identified by the inspectors

- v. Out of the 100 plants sampled, 10 plants are scored for plant height

and number of stems produced during the fourth inspection.

- vi. Plant height is measured in centimeters using a measuring tape or marked stick from ground level to the apex of the tallest shoot of the sampled plant.
- vii. Data for pest/disease incidence and severity, varietal purity, and average plant height and stem number are calculated and entered into the datasheet (see the calculation in Appendix 3).
- viii. Also at the fourth inspection, each of the 10 plants assessed for plant height and number of stems is uprooted and the roots scored for CBSD. The uprooted roots are removed, cleaned and cut cross-sectional along the length of the root. Every slice is examined for the presence of CBSD symptoms, and where one or all are positive, that root is considered diseased. The number of diseased roots is recorded for each plant in the form (diseased roots/total roots) e.g. (3/7) for a case where a plant has seven roots of which three have symptoms of CBSD. This is converted to percentage to give disease incidence.

2.3.3 Site and field information considered during inspection

I Farmer and location information

- a) Farmer's name
- b) GPS reading
- c) Administrative levels-
 - Region
 - District
 - County
 - Sub county
 - Village
- d) Name of the inspector carrying out inspection
- e) Date of inspection - this should be recorded as Day/Month/Year.

II Field information recorded during inspection

- a) Size of the farm
- b) Crop age: this is given in weeks if the crop is less than a month old and in months if it's more than a month.
- c) Planting date: this is written as dd/mm/yy
- d) Soil type: this is determined by feel. There are three main types: sandy, clayey and loamy. Observe if the soil is well or poorly drained and record accordingly.
- e) Cassava variety: record the variety being inspected and off types
- f) Source of planting material: verify the source of the planting materials.
- g) Plant spacing
- h) Plant population
- i) Season prevailing during the field inspection must be noted i.e. 'Rainy' or 'Dry'.

III Management practices to note during field inspection

- a) Number of weeding: The seed entrepreneur will inform the inspector how many weeding he/she has done since planting to the date of assessment. This will be recorded in the datasheet
- b) Weed density
- c) Cropping type: observe how planting was done at the site i.e., on ridges, flat, using terraces or with soil mounding.
- d) Crop cycle: record the crop cycle i.e. first crop, first ratoon and second ratoon.

2.4 Procedure for plant sampling for laboratory analysis

Materials needed for taking plant samples

- i. 70% Ethanol
- ii. 2ml micro centrifuge tubes
- iii. Corrugated card board
- iv. News prints
- v. Masking tapes

Steps followed during field sampling for CBSD diagnostics

- i. Systematically collect samples from 500 plants per ha following the inspection pattern 2 described above
- ii. Pick a leaf from mature mid canopy of the plant and pluck off the central leaf lobe.
- iii. Fasten this onto news print using a masking tape.
- iv. Press the fastened leaf between corrugated card board sheets to allow the plant to dry without deterioration.
- v. Transport the sample to the laboratory for nucleic acid extraction and RT-PCR analysis. Other available laboratory techniques may also be used instead of RT-PCR
- vi. In the laboratory the samples can be kept in cool dry place as it awaits processing.
- vii. These samples will be pooled in tens during laboratory analysis.

Steps followed during field sampling for CMD diagnostics

- i. Collect samples from plants with suspected symptom of CMD
- ii. Pick the sample from fully open young leaf and put in Eppendorf tubes containing 70% ethanol.
- iii. Transport the samples to the laboratory for further processing.

2.5 Harvesting and packaging

Harvesting of cassava seed shall be done when the plants have attained complete physiological maturity. This will be done after supervision by a field inspector in order to ensure that all the required standards are achieved. Appropriate harvesting and packaging methods that minimizes the seed damage shall be used. Mature cassava stem cuttings 20-25cm length should be cut and 500 stakes packed in an aerated bag of dimension 110cm x 80cm and well labeled. Packing of long stems in bundles of 50 pieces may be about 1meter length and above depending on variety are tied with string or 100 pieces of 1 meter are packed in perforated bag of 110cm x 80cm bag and well labeled. The suitable packaging on advice of an inspector will depend on time when to plant. Short stems are planted before 2 weeks and long stems can store 1-4 months when kept under suitable environment depending on customer requirement. The inspector shall issue certification tags after verification of the harvested and packed material and issue a movement permit.



Stem bundles of 1m length having 50 pieces



100 pieces of 1m long stems parked in 110X80cm bag.



Cuttings of 20- 25cm ready for planting

2.6 Labeling and tagging of cassava seeds

- i. All cassava seed packages shall be officially tagged.
- ii. The label shall be properly written on water proof wax paper with the following descriptions for pre-basic, basic and certified seed.
 - a. Variety name.....
 - b. Name of seed entrepreneur.....
 - c. Purity.....
 - d. Seed class
 - e. Date of stem harvest.
 - f. Best before
 - g. Place of origin.....
 - h. Lot number.
- iii. The label shall have the following colors

Seed class	Color of the label
Pre-basic seed	White with violet diagonal stripe
Basic seed	White
Certified seed 1st generation	Blue
Certified seed 2nd generation	Red
Standard seed	Yellow
Partially certified seed (in transit)	Grey

TISSUE CULTURE LABORATORY CERTIFICATION STANDARD FOR PRODUCTION OF PRE-BASIC CASSAVA SEED

3.1 Introduction

Virus tested pre-basic cassava seed is produced in the laboratory using meristem tip or nodal tip culture through micro propagation technique which ensures rapid and true to type multiplication of plants on artificial nutrient media under controlled aseptic environment. To ensure quality control, certification program for the tissue culture plantlets is vital since unintended micro propagation of virus infected plants will not only result in its poor performance, but also in undesirable spread of viruses wherever such plants are grown. Also, failure to use prescribed standard protocols will result in variations in the plants produced. The most deleterious variants in tissue culture raised plants are those that affect yield, genetic fidelity and carry infection of viruses, and other fastidious pathogens, which are difficult to diagnose. This is an area of great concern, and requires a well-structured system to be put in place to provide support to the tissue culture laboratory for the commercialization of virus tested and high quality cassava seed.

3.2 Eligibility requirement for certification of tissue culture laboratory and *In-vitro* cassava seed

While in the tissue culture laboratory, the inspector will take note to ensure that the required standards are met and the plantlets are being handled in a manner that precludes, as much as possible, the re-introduction of pathogens. Also inspection of tissue culture laboratory is required to ensure that tissue culture plantlets are properly labeled and handled to assure genetic purity of the final certified product (a

single genotype and not a mixture of clones). During inspection of tissue culture laboratory, the inspector must be keen in four different sections of the laboratory; washing room, media (preparation and storage) room, inoculation room and culture rooms and the following eligibility requirement must be fulfilled.

- i. All micro propagation facilities must be registered and approved by the NSCS
- ii. Laboratory facilities used for production of cassava plantlets should be maintained sterile
- iii. All growth media should be sterile
- iv. Water sources used in the laboratory operation should be clean and free of cassava pests and pathogens.
- v. Hygienic conditions should be maintained strictly during micro-propagation. Use equipment and other laboratory practices which guard against the spread of diseases or insects in the facilities used for seed multiplication.
- vi. The persons entering the Tissue Culture laboratory should use personal protective equipment to reduce the chances of inadvertent introduction of vector or insects clinging to clothes.
- vii. The material being initiated for producing tissue culture plantlets must be of known variety and confirmed identity.
- viii. All details of the materials propagated must be maintained for inspection by the certification authority (NSCS). This may include inventory of all plant material in the laboratory, origin of initial material, individual record of each line showing step by step flow through various multiplication stages, test reports and any other information as may be required during inspection.
- ix. The plants of a cassava variety being initiated for tissue culture should be tested in an accredited laboratory for freedom from the following: CMGs, U/CBSV, CBB, endophytic or epiphytic bacteria and fungi. Test must be conducted by third party laboratory accredited and approved by NSCS.

3.3 Sources of planting material for production of pre-basic cassava tissue culture plantlets

- i. The seed source used for multiplication of pre basic in tissue culture must be from the breeder's mother garden and must be free from CMD, CBSD and CBB.
- ii. The breeder must be responsible for establishment and maintenance of the mother garden of all the released varieties that may be required for further seed multiplication.
- iii. Where no clean mother garden is available, the source material must pass through virus elimination methods and the generated tissue culture plantlets must be tested before further multiplication to ensure it is free from the above pathogens.

3.4 Testing of pre- basic cassava plantlets

3.4.1 Test requirement for mother plants

- i. All the mother plants should be tested for viral pathogens before selection as source of explants for tissue culture propagation.
- ii. All the plant materials to be further mass propagated should test negative for viruses.

3.4.2 Establishment of virus free plants *in vitro*

- i. Collect stem cuttings from desired variety and establish it in screen house.
- ii. Leave the stems to grow for 2-3 months and assess for virus symptoms.
- iii. Discard symptomatic plants and select symptomless plants and test them further using appropriate diagnostic method
- iv. Select those plants that tested negative for viruses and initiate in tissue culture laboratory.

- v. Before mass multiplication, wean part of the tissue culture plantlets above in screen house and leave it to grow for 3 months and observe any virus symptoms.
- vi. Test the symptomless plantlets using appropriate diagnostic tools.
- vii. Discard lines that show virus symptoms or those that tested positive for viruses.
- viii. Lines that tested virus free are then mass multiplied *in vitro* as clean material.

SCREEN HOUSE CERTIFICATION STANDARD FOR PRODUCTION OF PRE- BASIC CASSAVA SEED

4.1 Eligibility requirement for certification of cassava seed grown in screenhouse

- i. All screenhouse facilities used for multiplication of cassava seed must be registered and approved by the NSCS
- ii. Screenhouse facilities used for production of cassava plants should be maintained free of cassava pests or vectors of cassava pathogens and weeds
- iii. All potting and growth media should be sterile and free from pathogens, pests and volunteer plants
- iv. Water used in screenhouse must be from clean sources
- v. Hygienic conditions should be strictly maintained during potting, planting, irrigating, movement and use of equipment and other screenhouse practices to guard against the spread of diseases or insects in the facilities used for seed multiplication.
- vi. The screenhouse must be insect proof and be equipped with a double-door entrance, provision for footwear disinfection prior to entering the protected environment and insect proof ventilation screening on inlet and outlet openings. The persons entering the protected environment should use gum boots and change lab-coat in the changing area to reduce the chances of inadvertent introduction of vector or insects clinging to clothes.
- vii. The plantlets being grown in screenhouse must be of known variety or confirmed identity. It must be duly documented with respect to origin.
- viii. No field-sourced cassava plants should be grown in the screenhouse along with tissue cultured plants.
- ix. Varieties must be physically separated and properly tagged to prevent varietal mixture.

- x. Before dispatch to the seed multipliers, the tissue-cultured plantlets growing in the screenhouse should be tested for the absence of viruses and confirm clonal identity.
- xi. The proprietor of the screenhouse should obtain a certificate from the NSCS to prove that the cassava tissue cultured materials have been produced as per the certification guidelines before the plantlets are dispatched.
- xii. The laboratory producing cassava tissue culture material will follow the labeling procedures as described in 2.6 above.

4.2 Sources of cassava seed for screen house multiplication:

- i. The tissue culture plantlets that test free from the major pathogens (CMD, CBSD and CBB) is bulked and become the source of plant materials in screenhouse.
- ii. Valid pathogen testing results are required prior to the bulking up of the cultures to ensure that what is transferred to the screenhouse is free of known cassava pathogens.

4.3.1 Procedures followed during inspection of screenhouse for plant pests and diseases

Since most pests and diseases that attack screenhouse crops do not distribute themselves evenly throughout the crop, the inspector should never assume to know exactly where the pests/diseases are, or a serious misjudgment could occur and an entire infestation overlooked. During screen house inspection, the following steps will be followed.

- i. Inspection should start from the major doorway. This is often the location where disease and pest problems begin.
- ii. Random plant inspections should be conducted following zig-zag pattern to ensure that most areas of the screenhouse is covered.
- iii. Special attention should be paid to plants around any openings in the screenhouse, especially those plants on the outside rows of benches.

- iv. At least 20% of plants in screenhouse should be inspected, making sure that entire screenhouse is covered
- v. Individual plants should be chosen at random and inspection should include checking for insects pests (white flies and mites), or disease symptoms.
- vi. Inspection should be done on entire plant by visual observation
- vii. The inspector should check the underside of the leaves or tip shoots for any pest or disease symptom.
- viii. Root rot can be examined by inverting and/ or removing the pot.
- ix. In addition to random sampling of plants, the inspector should also observe the sticky traps hanged inside the screen house.
- x. Proper record keeping must be observed by the screenhouse owners and should be available to the inspectors during inspection process
- xi. The record should contain information on status of cassava seed being introduced in screen house, random and sticky trap inspection of the plants in screen house and any crop treatments must be recorded.
- xii. All plant protection inputs must be noted concisely and accurately. Detailed records of any pesticide application should be kept to compare with previous records to see if fewer applications have been made or if a less toxic chemical has been substituted.
- xiii. Maps of the screenhouse showing where each variety and sticky traps, are located should be maintained. Disease, mite, and insect infestations can be marked on these maps, and movement of the infestation can be monitored.
- xiv. Information on the pests detected, the counts, and any unusual circumstances found in the screenhouse should be summarized.

4.4 Laboratory test for screenhouse mass propagated cassava seed for pathogens

Any commercial screenhouse seed multiplier must obtain virus indexed tissue culture plantlets from recognized tissue culture laboratory and he/she must be issued with proof that the tissue culture plantlets have been tested for known viruses and is clean. But where the source of material is not clear or where, the screen house materials are doubtful probably because of pest infestation, the following must be done:

- i. All plant samples for pathology test must be taken and laboratory results completed prior to shipping of plantlets.
- ii. A minimum of 1% of the plants but not less than 20 samples (20%) of lots having 100 plants or less must be tested for major pathogens like CMD and CBSD.
- iii. The sampling will be taken under the supervision of seed certification agency. Tests are to be conducted by an independent diagnostic laboratory approved by NSCS of MAAIF.

Appendix 1: Roles of inspectors during cassava seed field inspection

- i. Verify the location of the farm and the seed field.
- ii. Verify cropping history of the field
- iii. Verify soil type
- iv. Confirm the seed source by observing the seed labels
- v. Affirm the acreage of the seed farm
- vi. Validate uniformity in planting and border rows
- vii. Authenticate the isolation distances as specified by the standards.
- viii. Guide the seed merchant/grower (entrepreneur) in identifying and removal of off-types and diseased /pest infested plants
- ix. Check for proper rouging
- x. Monitor for pests particularly CGM, cassava mealy bugs, white flies
- xi. Monitor for disease incidence and severity with specific reference to CMD, CBSD and CBB
- xii. Collect leaf samples for analysis of CMD, CBSD and CBB in the laboratory
- xiii. Prove the maturity of the seed
- xiv. Explain to the entrepreneur about when and how to harvest planting material
- xv. The inspector must conduct thorough field inspection without bias and produce detailed report indicating the remedial action that has to be implemented and the time limit. The report should also contain the sketch map of the farm with boundaries and directions. A copy of the report will be given to the seed merchant/grower(entrepreneur) and another copy to the NSCS

Appendix 2: Equipment and other materials needed for inspection of cassava seed

All cassava seed inspectors are required to have the following:

- i. A field inspection notebook into which are recorded all events noticed in the field.
- ii. A copy of a field inspection manual, pest and disease manual and variety descriptor manual.
- iii. Pen / pencil
- iv. Ruler
- v. Measuring tape;
- vi. Raincoat;
- vii. Gum boots;
- viii. Camera;
- ix. Map of the area;
- x. Sampling bags;
- xi. Overall
- xii. GPS
- xiii. Panga

Appendix3: Quantification of parameters assessed during inspection

i. *Percentage purity of the variety sampled*

- a. To determine this, count the number of off-types encountered during the assessment of the other parameters along the two diagonals within the 100 plants sampled. Write the total number of the off-types in the space provided in the data sheet.

$$\text{b. Then calculate the Percentage off types} = \frac{\text{Total number of off types}}{\text{Total number of plants assessed}} \times 100$$

- c. Therefore percentage purity = (100 – percentage off types)

ii. *Size of cassava farm (Ha)*

The size of the multiplication site is estimated by pacing with a 1m pace (or if possible by tape-measuring) its width and length, multiply these, then divide by 10,000 to give the area in hectares. = $\left(\frac{\text{length of the farm} \times \text{width of the farm}}{10,000} \right)$

iii. *Plant spacing*

- iv. Plant population per ha: is calculated following estimation of the plant spacing used at the site, using the following formula:

$$\frac{\text{total area of the site in metres squared}}{\text{spacing used in metres (a X b)}}$$

iv. *Average plant height from 10 plants*

The average plant height from 10 plants is obtained by measuring the heights of 10 out of the 100 sampled plants. The height is measured using a measuring tape (in cm) from the ground level to the apex of the tallest shoot of the sampled plant.

The average is then given by;

$$\frac{\text{Total number of stems for the ten plants sampled}}{10}$$

Appendix 4: Site data sheet

1	Farmer information	3	Quantification
1.1	Farmer's name.....	3.1	Percentage purity of the variety (sampled)
1.2	GPS reading:		
1.3	Long.....		
1.4	Lat.....	3.2	Size of cassava farm (Ha)
1.5	Alt.....		
1.6	Administrative levels (AL):	3.3	Plant spacing.....
1.7	Country.....	3.4	Plant population per ha.....
1.8	Region	3.5	Average plant height from 10 plants.....
1.9	District.....		
	County.....	3.6	Average number of stems from 10 plants.....
	Sub county.....		
	Name/institute of data recorder.....		
	Date of assessment.....		
2	Field information	4	Management practices
2.1	Crop age.....	4.1	Number of weedings.....
2.2	Planting date.....	4.2	Cropping pattern.....
2.3	Soil type.....	4.3	Stem harvest status: 1st crop (.....) 1st ratoon (.....)
2.4	Cassava variety.....		
2.5	Source of cassava planting material.....		
2.6	Season prevailing during the visit.....		

Appendix 5: Field Scoring Sheet

Location..... Block..... Variety
Date

Plant No.	CMD Severity	CBSD Severity	CM Incidence	CGM Severity	CBB Severity	WF No.		Plant No.	CMD Severity	CBSD Severity	CM Incidence	CGM Severity	CBB Severity	WF No.
1								26						
2								27						
3								28						
4								29						
5								30						
6								31						
7								32						
8								33						
9								34						
10								35						
11								36						
12								37						
13								38						
14								39						
15								40						
16								41						
17								42						
18								43						
19								44						
20								45						
21								46						
22								47						
23								48						
24								49						
25								50						

Plant No.	CMD Severity	CBSD Severity	CM Incidence	CGM Severity	CBB Severity	WF No.			Plant No.	CMD Severity	CBSD Severity	CM Incidence	CGM Severity	CBB Severity	WF No.
51									76						
52									77						
53									78						
54									79						
54									80						
55									81						
55									82						
56									83						
57									84						
58									85						
59									86						
60									87						
61									88						
62									89						
63									90						
64									91						
65									92						
66									93						
67									94						
68									95						
69									96						
70									97						
71									98						
72									99						
73									100						
74															
75															

Appendix 6: List of released cassava varieties

Variety name	Maturity period (Months)	Yield (Ton/Ha)	Year of release	Reaction to CMD	Reaction to CBSD
NASE 1	14	20-25	1994	Susceptible	Tolerant
NASE 2	14	30-40	1994	Susceptible	Susceptible
NASE 3	12	30-35	1994	Tolerant	Tolerant
NASE 4	12	35-50	1999	Resistant	Susceptible
NASE 5	12	30-40	1999	Resistant	Susceptible
NASE 6	12	25-35	1999	Resistant	Susceptible
NASE 7	12	30-45	1999	Resistant	Susceptible
NASE 8	12	30-40	1999	Resistant	Susceptible
NASE 9	12	30-45	1999	Tolerant	Susceptible
NASE 10	12	35-40	2000	Resistant	Susceptible
NASE 11	12	35-40	2000	Resistant	Susceptible
NASE 12	13	35-40	2000	Resistant	Susceptible
NASE 13	12	30-35	2011	Resistant	Susceptible
NASE 14	12	30-35	2011	Resistant	Tolerant
NASE 15	12	30 - 40	2011	Resistant	Tolerant
NASE 16	15	35-40	2011	Resistant	Susceptible
NASE 17	12	35-45	2011	Resistant	Susceptible
NASE 18	12	35-45	2011	Resistant	Tolerant
NASE 19	12	30-35	2011	Resistant	Tolerant
Advanced lines for release					
Nam 130	12	35-50	?	Resistant	Tolerant
MM06/0130	12	30-40	?	Resistant	Tolerant

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