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**Pre-basic cassava tissue culture-derived
plantlets — Requirements for certification**

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Requests for permission to reproduce this document should be addressed to:

Rwanda Standards Board

P.O Box 7099 Kigali-Rwanda

KK 15 Rd, 49

Tel. +250 788303492

Toll Free: 3250

E-mail: info@rsb.gov.rw

Website: www.rsb.gov.rw

ePortal: www.portal.rsb.gov.rw

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Foreword

Rwanda Standards are prepared by Technical Committees and approved by Rwanda Standards Board (RSB) Board of Directors in accordance with the procedures of RSB, in compliance with Annex 3 of the WTO/TBT agreement on the preparation, adoption and application of standards.

The main task of technical committees is to prepare national standards. Final Draft Rwanda Standards adopted by Technical committees are ratified by members of RSB Board of Directors for publication and gazettment as Rwanda Standards.

DRS 643 was prepared by Technical Committee RSB/TC 32, *Seeds and Planting Materials*.

Committee membership

The following organizations were represented on the Technical Committee on *Seeds and Planting materials* (RSB/TC 32) in the preparation of this standard.

Africa Food Supply Ltd

Cooperative KOPUAIM

International Institute of Tropical Agriculture (IITA Rwanda)

INGABO Farmers Association

Ministry of Agriculture and Animal Resources (MINAGRI)

One Acre Fund TUBURA

Rwanda Agriculture and Animal Resources Development Board (RAB)

Rwanda Inspectorate, Competition and Consumer Protection Authority (RICA)

Rwanda Institute for Conservation Agriculture (RICA University)

University of Rwanda- College of Agriculture, Forestry and Food Sciences (UR-CAFF)

Rwanda Standards Board (RSB) – Secretariat

Introduction

Rwanda's cassava sector has been severely affected by viral diseases, particularly Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD), threatening food security and farmer livelihoods. A major crisis occurred in 2014/2015 when the Kinazi cassava plant nearly closed due to insufficient raw materials, prompting the government to import clean planting materials as a temporary fix. Cassava production is constrained by the same viral diseases and low multiplication rates. Farmers often reuse infected planting materials, worsening disease spread and impact.

To address these challenges, the International Institute of Tropical Agriculture (IITA), in partnership with the Rwanda Agriculture and Animal Resources Development Board (RAB) and other stakeholders, launched several initiatives from 2017 onwards. These focused on developing disease-resistant cassava varieties and establishing sustainable clean seed systems. Rapid multiplication technologies such as tissue culture, Semi-autotrophic Hydroponics (SAH), and screenhouse propagation offer opportunities to improve ways of controlling diseases. These techniques emphasize on virus testing to ensure the distribution of clean, high-quality planting materials, thereby strengthening the cassava seed system and improving overall productivity.

A key breakthrough was the introduction of Semi-autotrophic Hydroponics SAH technology, which significantly improved plantlet survival rates and increased multiplicative rates of clean planting materials. This innovation has strengthened Rwanda's cassava pre-basic seed production capabilities, and ensured a steady supply of high-quality cassava seeds for farmers.

As a result, cassava seed production has expanded considerably, supporting higher productivity and improved disease control. Complementary efforts, such as improved propagation techniques and local substrate development, have further enhanced efficiency and reduced costs.

This guideline lays down requirements for inspection and certification for Tissue culture (TC), Semi-autotrophic Hydroponics (SAH), and Screenhouse (SH) which in return facilitate regulatory agencies to control seeds breeders, avoiding unfair practices and sub-standards seeds that may introduce cassava seeds diseases.

Pre-basic cassava tissue culture derived plantlets — Requirements for certification

1 Scope

This Draft Rwanda standard specifies the certification requirements for pre-basic cassava (*Manihot esculenta* Crantz) tissue culture-derived plantlets grown under controlled environments (facilities) such as Semi-Autotrophic Hydroponics (SAH) laboratory, SAH greenhouse, and greenhouse facilities.

It covers requirements for eligible varieties, their multiplication in controlled environments, application for certification, inspection, labelling and packaging for transportation.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

RS EAS 12, *Potable water - Specification*

3 Terms and definitions

For the purposes of this standard, the terms and definitions given in UPOV and the following apply.

3.1

batch

group of cassava plantlets produced at the same time, using the same starter materials, methods, and growing conditions during one production cycle.

3.2

breeder seed

initial high-quality seed produced and maintained by the plant breeder, serving as the source for all other categories of seeds

3.3

explant

small piece of plant tissue taken from a parent plant and used to start tissue culture, they are typically taken from parts such as shoot tips (meristems), nodes (stem sections with buds) and young leaves. These tissues are placed on a sterile nutrient medium where they grow and develop into plantlets.

3.4

plantlets/ in-vitro plants or clean planting materials

small, complete young cassava plants from explants, grown in bottles/tubes which later transferred to semi-autotrophic hydroponics (SAH) or screenhouses and then to the field.

3.5

pre-basic seed

seed that is derived from breeder seed or nuclear material and is used to produce basic seed through one cycle of multiplication.

Note to entry: It is categorized as pre-basic seed if it is directly derived from a generation of multiplied breeder seed

3.6

screenhouse

protective structure covered with insect-proof fine mesh (netting) which allows air and light in, which is used to house growing and maintaining clean cassava plantlets.

3.7

seed

plant or plant part which is used or is intended to be used for propagation and includes stem cuttings/plantlets of cassava

3.8

seed lot

group of cassava plantlets identified with a unique number for quality testing, storage, and distribution.

3.9

seed lot size

maximum quantity of seed of the same crop, variety, and category treated as one unit for inspection, sampling, testing, and certification.

3.10

seed producer

individual person approved by the relevant authority, licensed to produce quality seeds in accordance with relevant requirements and regulations.

3.11**semi-Autotrophic Hydroponics (SAH)**

plant propagation system where plants are grown in a soil like medium (peat moth) fortified with nutrient solutions, under conditions that allow them to photosynthesize (semi-autotrophic), used to harden and multiply plantlets efficiently, producing strong, healthy seedlings with well-developed roots.

Note to entry This is an intermediate hardening/propagation stage that uses modified substrates, little water, and photosynthesis to make roots robust and well-hardened before field transfer.

3.12**substrates**

clean and disease-free growing medium that supports plant growth, such as soil, sand, peat, cocopeat, or agar medium.

3.13**tissue culture-derived plantlets**

disease-free in-vitro plantlets produced in a laboratory from small pieces of plant tissue (such as cells, leaves, stems, or roots) grown on a nutrient medium under sterile conditions.

4 Requirements**4.1 General requirements****4.1.1 General**

4.1.1.1 Pre-basic seed (plantlets) shall be produced from breeder seed of eligible varieties, under the responsibility of the seed producer.

4.1.1.2 Seed producer shall be registered by the relevant national competent authority.

4.1.2 Eligible varieties

4.1.2.1 Varieties eligible for seed certification shall be those that have been registered in the national list of varieties or national variety catalogue.

4.1.2.2 National seed certification authority shall keep the official electronic or hard copies of descriptors of all registered varieties which shall be made available on request.

4.1.3 Facilities: controlled environments

4.1.3.1 Semi-autotrophic Hydroponics (SAH) Facilities

4.1.3.1.1 SAH may consist of SAH laboratory and SAH screenhouses

4.1.3.1.2 SAH facilities used for the multiplication of cassava seed shall be registered and approved by the relevant competent Authority.

4.1.3.1.3 SAH facilities used for multiplication of cassava plantlets shall be maintained free of cassava pests or vectors of cassava pathogens.

4.1.3.1.4 All containers or boxes and substrate shall be sterile and free from pathogens and pests.

4.2.2.1.5 Distilled water shall be used in SAH laboratory while in SAH screenhouse potable water complying with RS EAS 12 shall be used.

4.1.3.1.6 Hygienic conditions of equipment, working area, and other SAH practices shall be strictly maintained during receiving, cutting, and planting to guard against the spread of pests and diseases. Records shall be kept.

4.1.3.1.7 SAH laboratory growth room shall be kept hygienic, painted in white, with proper lighting, and temperature maintained at 26°C to 28 °C.

4.1.3.1.8 Metallic racks fitted with mesh at the tops (to allow light penetration, aeration and water drainage) shall be installed in the SAH screen house to hold boxes with growing plantlets.

4.1.3.1.9 The persons entering in the SAH laboratory shall wear laboratory shoes and coats in the changing area to reduce risk of contamination.

4.1.3.1.10 To avoid risk of mixing varieties, each of the boxes containing a given variety shall be properly labelled and kept well arranged in a well labelled shelf or shelves.

4.1.3.1.11 All plantlets in the SAH facilities ready for distribution shall be certified and a certificate copy shall be kept.

4.1.3.2 Conventional Screenhouse

4.1.3.2.1 All screenhouse facilities used for multiplication of cassava seed shall be registered and approved by relevant competent authority.

4.1.3.2.2 Screenhouse facilities used for production of cassava plantlets shall be maintained free of cassava diseases, pests or vectors of cassava pathogens and weeds.

4.1.3.2.3 All containers/boxes and substrates shall be hygienic and free from pathogens, pests and weeds.

4.1.3.2.4 Water used in conventional screenhouse shall be potable water complying with RS EAS 12.

4.1.3.2.5 The screenhouse shall be located in a protected environment. The facility shall be insect-proof, equipped with a double-door entrance, provision for footwear disinfection at the entrance, and insect-proof screens on all ventilation inlet and outlet openings to prevent insect entry.

4.1.3.2.6 Hygienic conditions shall be strictly maintained during potting, planting, irrigating, movement and use of equipment and other screenhouse practices to guard against the spread of diseases or pests in the conventional screenhouse.

4.1.3.2.7 Only tissue culture-derived plantlets from SAH that have been inspected and certified by competent authority shall be sourced for screenhouse macro-propagation. A copy of the valid certificate shall be provided prior to sourcing of plantlets to be transferred to the screenhouse.

4.1.3.2.8 No field-sourced cassava plants shall be grown in the screenhouse along with tissue culture-derived plantlets.

4.1.3.2.9 In screenhouse, varieties shall be physically separated and properly labelled to prevent varietal mixture.

4.1.3.2.10 Before dispatch to the seed multipliers, the tissue culture-derived plantlets shall be certified by the relevant competent authority. Certificate copy shall be kept.

4.2 Internal quality control

Seed producer shall conduct internal quality checks to assess health status of the plantlets. The assessment may be done in SAH facilities and Screenhouse. Records shall be kept.

4.3 Application for certification

4.3.1 The application shall be done for plantlets no later than fifteen days after planting by filling the application form provided by the relevant seed certification authority.

4.3.2 The minimum information for application for certification of cassava plantlets shall include the following:

- a) name, address and any other contact details of the seed company/seed producer;
- b) crop and variety to be certified;
- c) period of production;
- d) physical location;
- e) source of seed plantlets and their reference number where applicable;
- f) number of plantlets and batch/lot number;
- g) category of seed to be certified; and

h) registration number of the seed company/seed producer.

4.3.3 The maximum Lot size of a seed lot for certification purposes shall be 20,000 plantlets; lots larger than this shall be divided and given a separate lot number.

4.3.4 The relevant seed certification authority shall prepare the inspections schedule for the inspectors, based on all necessary information on the application form, to ensure that inspections are done in due time

4.4 Inspection and certification procedures for cassava plantlets under SAH facilities and conventional screenhouse

4.4.1 In order to safeguard the quality of cassava plantlets supplied to basic seed producers, the following steps shall be followed by the inspector during inspection:

- a) Inspection shall start from the major doorway. This is often the location where disease and pest problems begin;
- b) An Inspector shall be able to identify different varieties to inspect in the SAH facilities or conventional screenhouses. Varieties shall be clearly separated for easy inspection;
- c) Inspection shall be done by visual assessment, however, in case there is assumption of disease, or presence of white flies in SAH screenhouse or conventional screenhouse, an inspector shall conduct disease testing. White flies and their damage, tolerance shall be zero in the SAH laboratory;
- d) An inspector shall check the underside of the leaves or tip shoots for any pest or disease symptoms;
- e) In SAH facilities and screenhouse, inspection shall be conducted for all plantlets. However, for diseases testing shall be 20 plantlets for < 1000 and 2 % for > 1000 cassava plantlets;
- f) Proper relevant records shall be kept and be observed by the SAH and conventional screenhouse owners and shall be available to the inspectors during inspection;
- g) In SAH facilities and conventional screenhouses, one (1) inspection shall be done atleast in one month for SAH and atleast two months for conventional screenhouses.

4.4.2 An inspector shall inspect the plantlets in SAH facilities and conventional screenhouses in accordance with the procedure provided in Annex A to check the conformity of the requirements provided in Table 1. If the plantlets are found to be in conformity with those requirements, they shall be approved.

4.4.3 The inspection report shall indicate the plantlets status and comments for any corrective actions required such as re-inspection or other actions. All inspection reports shall be provided to the seed producer right after each inspection. The inspection report shall be signed by the inspector and, where applicable, the seed producer or the producer's representative.

4.4.4 The validity of the inspection report shall be 30 days from the date it was issued.

4.4.5 The requirements provided under subclause 4.2 and 4.4 on inspection and certification exclude breeder seed.

4.5 Requirements for tissue culture derived plantlets

The lot of cassava plantlets shall be approved for certification if it complies with the requirements given in Table 1.

Table 1 — Requirements for tissue culture derived plantlets in SAH Facilities and Screenhouse

S/N	Characteristic		Cassava plantlet		
			SAH laboratory	SAH Screenhouse	Conventional screenhouse
i.	Number of macropropagation cycles, max.		4	4	3
ii.	Isolation distance (meters), min.	Between SAH/Screenhouse and open cassava field	N/A	50	50
		Between SAH/Screenhouse and controlled cassava seed field			
iii.	Diseases				
	Cassava Mosaic Disease (CMD), %, max.		0	0	0
	Cassava Brown Streak Disease (CBSD), laboratory testing, %, max.		0	0	0
	Cassava Brown Streak Disease (CBSD), %, max.		0	0	0
	Cassava bacterial blight disease, %, max.		0	0	0
iv.	Off-types, %, max.		0	0	0
v.	Pests				
	Cassava mealybug, % of infested plants, max.		0	0	0
	Cassava green mite, % of infested plants, max.		0	1	1
	Scale insects, % of infested plants, max.		0	0	0
	White fly, max.		0	0	1
vi.	Plantlets:				
	Height, cm.		10 - 15	10 - 15	20 -30
	Number of leaves.		4 - 10	4 – 10	10 - 15
	Node's length, cm.		0.5 – 2	0.5 – 2	2 – 5

	Number of roots.	5 - 10	5 - 10	N/A
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5 Records and documentation

The following documents and records shall be available in SAH and conventional Screenhouse, and available on request by an inspector:

- a) records of crop variety;
- b) number of plantlets;
- c) planting date;
- d) cutting dates;
- e) dispatch date;
- f) substrate used;
- g) weeds and pest control and irrigation/manual watering schedules;
- h) date for transfer of the first breeder seed;
- i) lab testing reports;
- j) inspection report and certificate copy; and
- k) staff and facility sanitation records.

6 Lot size

The maximum size of a seed lot for certification purposes shall be 20,000 plantlets; lots larger than this shall be divided and given a separate lot number.

7 Certificates

If the plantlets are found to be in conformity with requirements provided in this standard, they shall be approved and certified. The seed test certificate for a seed lot shall be signed and issued by the seed certification authority and shall include all necessary information. This certificate shall be valid for a period not exceeding 30 days.

8 Labelling

8.1 Labelling in the SAH and Conventional screenhouses

Plantlets in the SAH and Screenhouses shall be arranged and labelled in blocks immediately after planting. Blocks shall be labelled to clearly distinguish the following:

- a) varieties planted;
- b) planting date;
- c) plantlets generation (G1, G2, G3, G4);
- d) source of the planting materials; and
- e) block serial numbers for easy reference.

8.2 Labelling by seed certification authority

8.2.1 Cassava plantlets that have been certified shall safeguard the quality of the seed and shall have the official label of the relevant seed certification authority.

8.2.2 The labels for the cassava plantlets shall be identified by a violet band on white.

8.2.3 The labels shall be issued by a seed certification authority and shall be prominent, indelible and legible.

8.2.4 The following information shall be included on the official labels:

- a) name and address of the seed producer;
- b) registration number of the seed producer;
- c) name and species of the crop;
- d) variety denomination;
- e) seed lot number;
- f) number of plantlets per package;
- g) name, address and logo of the relevant seed certification authority;
- h) certificate number;

- i) seed category; and
- j) production period (month and year).

9 Packaging for transportation

9.1 SAH Plantlets shall be packaged and transported in well-sealed transparent boxes.

9.2 Plantlets produced in conventional screenhouse shall be packaged and transported in a similar ways as other nursery plantlets.

Annex A (normative)

Inspection procedure

A.1 Purpose

This procedure is intended to provide guidelines that may be followed by seed inspector. It is intended to provide the methods used to determine the certification requirements of cassava plantlets.

A.2 Scope

This procedure covers inspection of the general status of the cassava plantlets, isolation, health status and number of macropropagation cycles requirements of the cassava plantlets.

A.3 Equipment and tools

An Inspector shall be equipped with the following:

- a) current national seed law, relevant regulations and standards;
- b) seed crop declaration form;
- c) variety descriptors;
- d) seed inspector service card;
- e) tally counter;
- f) support literature;
- g) measuring wheel and GPS;
- h) camera;
- i) calculator;
- j) protective clothing; and
- k) inspection report form.

A.4 Procedure details

A.4.1 Stage 1: Source of seeds

A.4.1.1 In order to authenticate the identity of the seed planted, the seed producer retains proof of origin (label/receipt) of each seed lot used to plant the crop.

A.4.1.2 The inspector checks the details provided on the label/receipt against those on the seed crop application form and checks the identity of the variety.

A.4.1.3 The inspector proceeds to next stage if the declared information corresponds to those on the label/receipt otherwise the Lot of plantlets shall be automatically rejected.

A.4.2 Stage 2: Confirmation of number of plantlets and previous macropropagation cycles

A.4.2.1 The seed inspector reviews number of plantlets and the details of previous macropropagating cycles.

A.4.2.2 In case the number of plantlets and the previous macropropagating requirements are met, the inspector proceeds to the next stage.

A.4.3 Stage 3: Checking isolation distance

The inspector checks isolation distance of the SAH or conventional screenhouses from other cassava fields whilst walking around its perimeter. If the required distance is not met, the inspector evaluates any risk of disease spreading by vectors and makes relevant decision/recommendation.

A.4.4 Stage 4: Checking the pest and disease infestation status of the plantlets

A.4.4.1 The inspector assesses the pest and disease infestation status of the plantlets and determines whether it is in satisfactory condition to permit the detailed examination of plantlets for varietal purity.

A.4.4.2 Stunted or poorly grown plantlets because of disease, pests or other causes and which cannot be assessed for other parameters shall be rejected.

A.4.5 Stage 5: Detailed examination of off-types, diseases and pests

The final stage in inspection is the assessment of varietal purity and health status of the seed crop. This shall be done when the source of seeds, varietal identity, isolation and plantlets conditions are all satisfactory. To do this, it is necessary to perform detailed examination of all plantlets.

A.4.5.1 Examination of off-types

By using the descriptor of the variety, the inspector evaluates the key characteristics of the variety, including but not limited to leaf shape, shoot; and leaf petiole colour; in each count and the number of off-types shall be recorded and the percentage calculated after examining all counts.

A.4.5.2 Examination of diseases

A.4.5.2.1 The inspector uses clear symptom description and chart (photo) or other available tools to recognize a diseased plantlet.

A.4.5.2.2 In each count, the inspector checks infected plantlets with the disease mentioned in Table 1, and takes record for each of them.

A.4.5.2.3 The inspector calculates the percentage of infected plantlets after examining all samples.

A.4.5.2.4 In case there is doubt in identifying the symptoms, the inspector shall conduct laboratory testing. Only laboratories authorized by the relevant seed certification authority to conduct seed testing for certification shall test seeds.

A.4.5.3 Examination of pests

The inspector examines the presence and damage of pests as listed in Table 1.

A.4.6 Stage 6: Decision taking

A.4.6.1 The inspector compares the calculated percentage for each parameter with acceptable limits provided in Table 1.

A.4.6.2 Based on the results of the comparison, the inspector may accept or reject plantlets.

Annex B

(informative)

Sample size and sampling strategy for the inspection and certification

B.1 Sample size

B.1.1 Sampling strategy of plantlets in screenhouse

A systematic sampling process should be applied for sample inspection and collection. Thus, for a batch of 40 plantlets, every second plantlet should be sampled (giving a total sample number of 20), for a batch of 1000 plantlets, one plantlet will be selected out of every 50 (giving a total sample number of 20), and for a batch of 10,000 plantlets, one plantlet will also be selected out of every 50 (giving a total sample number of 200). For all batch sizes from 1000 and above, the sampling intensity will remain 2%, which will always mean that one plantlet will be sampled for every group of 50.

B.2 Leaf sample quantity

For Screenhouse plantlets, one leaflet should be used for virus testing. This should be the central leaflet of the uppermost fully expanded leaf of the sampled plantlet. In SAH or Screenhouse, whole plantlets to be tested should be taken and placed either in a microfuge or falcon tube, or a small Ziplock plastic bag. These fresh samples should be held in a cool box for storage during transport back to the laboratory for testing.

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