IITA MUSA IMPROVEMENT – MOLECULAR BREEDING

STANDARD OPERATING PROCEDURE (SOP)

FOR BANANA LEAF ARCHIVING

1. Authors & Contributors

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2. Introduction

Unlike seed crops, clonally propagated crops are difficult and expensive to conserve. With the rapidly evolving genotyping platforms, it is recommended to conserve leaf samples with the possibility of using them for genotyping, if the need arises. Banana leaf archiving involves the process of collecting, processing, and storing the leaves in a way that preserves the integrity of the DNA over time. Banana leaf archiving is important for maintaining a repository of genetic materials from different banana cultivars, which can be used for genotyping purposes in support of various breeding objectives.

The banana leaf samples collected are dried using the freeze-drying/lyophilizing method which preserves the integrity of DNA in leaf tissue samples for long-term storage and future use.

3. Purpose

The purpose of this SOP is to describe the procedure to be followed during leaf sampling, processing, and storing of banana leaf samples for archiving or storage.

4. Scope

This document describes clearly processes and procedures required for archiving banana leaf samples. It includes the items and equipment needed, preparation of sampling materials, sample collection, preparation of leaf samples for freeze-drying and storage of samples.

5. Definition of terms (in alphabetical order)

- **DNA:** Is the genetic makeup of an organism.
- **Freeze drying**: Is the process in which a completely frozen sample is placed under a vacuum to remove water or other solvents from the sample, allowing the ice to change directly from a solid to vapour without passing through a liquid phase.

6. Roles and responsibilities

Molecular banana breeder: Responsible for overseeing all molecular breeding activities, including setting up objectives, designing trials, data production, analysis, interpretation and reporting at the Trait Discovery stage specifically and across all the breeding stages that involves molecular breeding.

Research Associate: Supports the molecular banana breeder by coordinating molecular breeding activities including trial and experiment design and management, field and laboratory operation supervision, data collection, data analysis, synthesis, and writing of reports. Also responsible for coordinating and supervising the field, laboratory staff and trainees.

Laboratory Technician: Performs tasks such as preparation of buffers, DNA extraction and storage for genotyping, routine maintenance of laboratory machines and equipment. Conducts sample preparation processes, including field collection of leaf samples, plating of leaf samples in genotyping plates, freeze-drying of samples for shipping to genotyping platforms, and reporting the results.

7. Procedure

7.1 Banana leaf sampling procedure

Refer to **Banana SOP15_on-station leaf sampling** from **section 7.1-7.4**.

7.2 Processing of samples for leaf archiving

7.2.1 Required tools and equipment

- Leaf samples
- Stainless steel scissors or razor blades
- 2-ml vials such as Eppendorf tubes
- Sterile pierced microfuge caps
- Laboratory gloves
- Green mesh bags
- 70% Ethanol
- Permanent marker

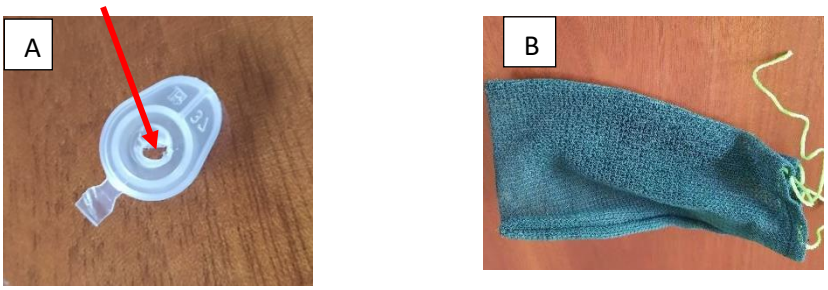

Figure 1: Figure 1A shows a vial cap with a hole created in it; B shows a picture of a mesh bag used for freeze-drying samples.

7.2.2 Leaf archiving procedure

i. Get a fresh leaf sample (cigar leaf), cut off the browning ends of the leaf sample, unroll it halfway (Figure 2, step 1).

ii. Cut a sizeable portion of rolled leaf using a pair of sterilized scissors.
N.B: Always sterilize the pair of scissors before and after cutting each leaf sample (Figure 2, step 2).

iii. Place the cut rolled leaf into a 2 ml vial and label it with the genotype name using a permanent marker(Figure 2, step 3). It is advised to sample each genotype in duplicate.

iv. Leave the lid of the 2 ml vial open (Figure 2, step 4).

v. Cap the leaf sample using a sterilized lid with a hole created in the middle to allow for air exchange (Figure 2, step 5).

vi. Place leaf samples in 2 ml vials in a mesh bag (Figure 2, step 6).

N.B: Always place the mesh bag with worked on samples on ice blocks to maintain the integrity of the samples.

vii. Place the samples in mesh bags in a -80°C freezer for at least 24 hours or longer in preparation for freeze drying.

Precaution

a) The lid with a hole is left covering the sample until the freeze-drying process is complete.

b) Once a leaf sample has been placed in a 2 ml vial, place it in a green mesh bag and keep it in ice cubes or ice blocks as you continue working on the other samples. And in between breaks of working on samples, please place samples already processed in a green mesh bag in a + 4 °C fridge. This is to minimize the deterioration of samples.

c) When all the samples have been prepared (placed in 2 ml vials), place them in a -80°C freezer for at least one hour before freeze-drying.

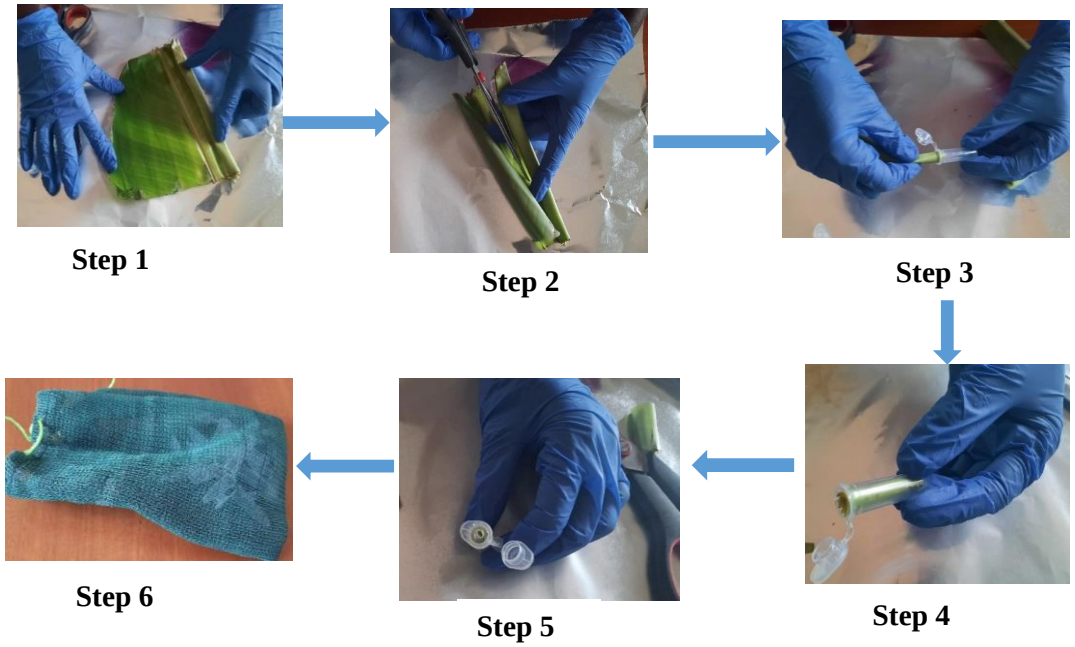

Figure 2: A stepwise process of sample preparation for leaf archiving.

7.2 Freeze drying of leaf samples

i. Switch on the freeze-dryer by first turning on the power source from the socket, then the UPS (uninterruptible power supply).

ii. Turn on the freeze-dryer by tapping on the switch button found on the interface (Figure 3, C).

iii. Ensure that the valves on the drying chamber of the freeze-drier are closed.

iv. On the interface, press the "collector" icon (Figure 3, A) to allow the freeze-dryer to run until the right pressure and temperature (-50 °C). Program the machine to run at a pressure of 0.214 Pa.

v. Open the valves at the drying chamber, place the samples in a total of 4 mesh bags (**150 samples per mesh bag**) in the drying chamber and dry them for at least 24 hours (this applies to a drying chamber of 18 litres).

vi. Once the cycle is done, tap the vacuum icon first, then the collector icon. Then open the valves at the chamber to release the pressure to open the lid, pick out the samples. Place the chamber lid back and turn off the freeze dryer from the switch button on the interface first, then from the UPS and then socket.

vii. Check that the samples are fully dried: they should be brittle when touched with forceps. If they are not fully dry, run them for another 6 hours.

viii. Remove them from the drying chamber promptly, and remove the perforated caps, and seal the sample with sealing caps immediately to limit air exchange.

ix. Remove the caps (lids) with holes from the samples that have been freeze dried and seal them with the cap of the 2 ml vial lid and store in a cool dry place with silica gel in the storage containers (Figure 5).

N.B: In case of moisture build up inside the freeze-dryer, insert firmly the tube attached at the lower end of the freeze-dryer and let out the water into a container (Figure 4, part E and F).

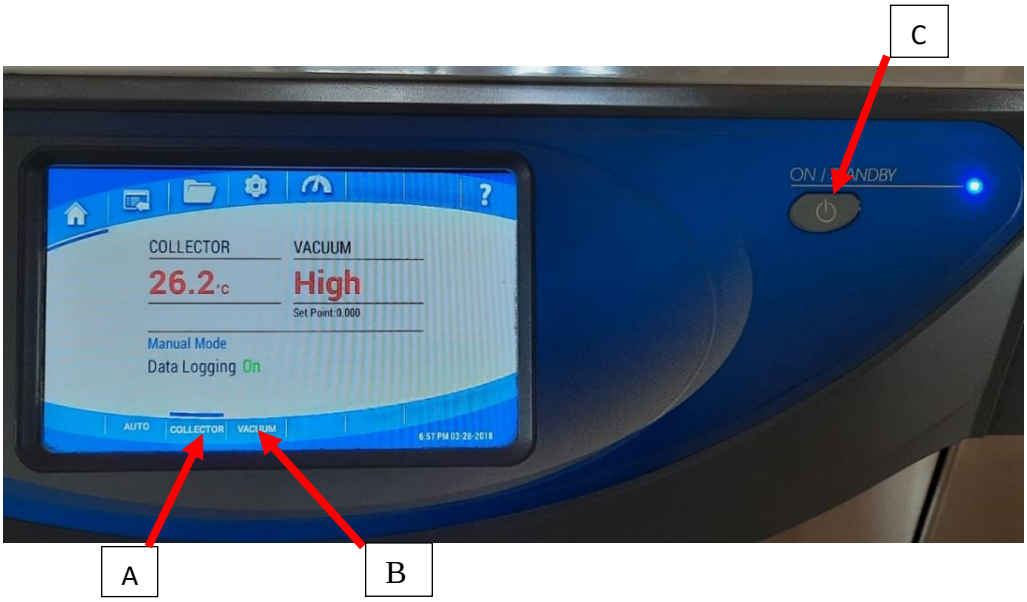

Figure 3: The interface surface for the freeze-dryer machine (LABCONCO-800-522-7658 model, Catalogue No- 701811030, Serial No-171049234). "A" shows the collector icon;" B" shows the vacuum icon and "C" shows the "switch" button.

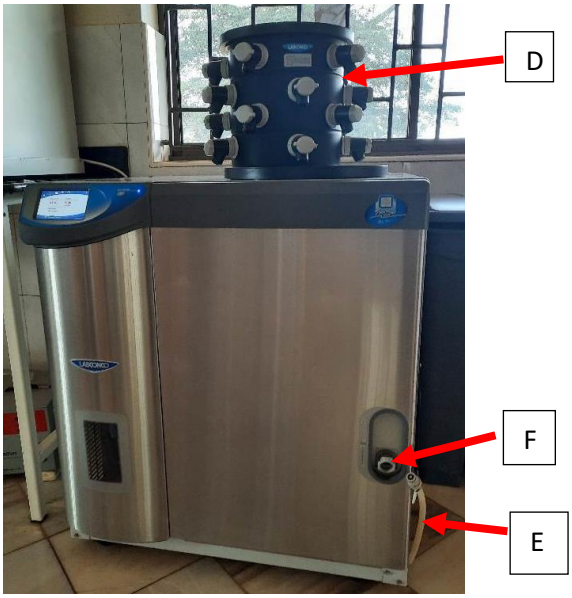

Figure 4: The freeze-dryer machine." D" shows the freeze-drying chamber where samples are placed." E" shows the tube used for letting out water from the freeze-dryer machine;" F" shows the valve where the tube (E) is placed.

7.3 Storage of freeze-dried samples

- Store the freeze-dried samples in sealed clear jars with well-dried silica gel (Figure 5).
- Label each jar with a number, the trial name and date of freeze-drying of the samples.
- Create an Excel file recording each freeze-dried sample and the corresponding data:
    - Breeding station
    - Breeding program
    - The trial where leaf samples were collected from
    - The field position of the sample
    - Date of freeze-drying
    - Jar number

- Check the jars every 3 months and replace the silica gel if it has turned pink (a sign of moisture absorption). The pink silica gel can be dried in a oven until they regain their blue colour and reused.

Figure 5: Freeze dried samples stored in a storage container with silica gel.

- The stored freeze-dried samples can be used for DNA extraction or treated as off-station samples for processing with the purpose of outsourced genotyping (See SOP 14, section 7.5).