Protocol to follow by enumerators to confirm genetic integrity of putative sweetpotato field plants

DECEMBER 2020

Contributors: Willy Pradel, Julius Okello, Marcel Gatto, Guy Hareau, Noelle Anglin, Clementina Banda, Rocio Alagón.

CIP CENTRO INTERNACIONAL DE LA PAPA

CGIAR

CGIAR RESEARCH PROGRAM ON Roots, Tubers and Bananas

Foreign, Commonwealth & Development Office

Protocol to follow by enumerators to confirm genetic integrity of putative sweetpotato field plants

1. Introduction

This activity has the objective to confirm that sweetpotato varieties identified by farmers' recall in household surveys match with the genotype of the varieties in the field. We will collect leaves samples of the varieties identified by farmers from the cultivated plots, followed by drying and crushing of the leave samples. DNA will be extracted from each sample and the genotype will be compared with the reference library for the respective varieties. We expect to collect one sample for each cultivated variety from a subsample of around 600 households.

2. Materials

The materials needed for this activity include. (1) for the field: gloves, tweezers, alcohol, water, squeeze bottle, masking tape, paper towel, pens, blocks, small container, measurement tape and measurement rope (2) for the sampling preparation place: gloves, alcohol, tweezers, silica gel, coffee filters, Ziploc bags, labels, permanent markers, template for sample identification and plot measurement, pencils and big containers for sample storage. (3) For silica gel drying: Microwaves, gloves for handling hot silica gel, bowls, stir. (4) For cleaning: Soap and Vaseline.

3. Protocol for collection and preparation of leaves samples

3.1 Coordination

- Before field work starts, enumerators will be trained on the sampling protocol, and plot measurement protocol.
- The selection of the households where to collect the samples from will be defined in advance in coordination with the household survey.
- Fill the template with the information of the household, plot and variety. Area will be calculated using measurement tapes or measurement ropes.
- The enumerators responsible to collect the leaves samples (two per household survey group) will assure that gloves, scissors, alcohol, silica gel, envelops or coffee filters, Ziploc bags, labels, permanent marker and containers (or buckets) are available and in good conditions.

3.2 Leaves samples collection

- Enumerators will review the data in the household survey to identify the sweetpotato varieties identified in the questionnaire and the specific plots. The varieties to be collected are: OFSP, Kenya Admarc, and the most important white sweetpotato in the district (The names and characteristics of the released OFSP, Kenya Admarc, and other white sweetpotato varieties is found in Appendix 1).

- Fill the template with the information describe above and the identifying notes on labels to ensure future identity of the leaves samples and matched to the survey questionnaire.
- Enumerators fill template (Appendix 2a) and labels (Appendix 3) with the information of the collector's group, district, household survey code, plot identification number and variety name.
- stick it into the paper coffee filters.
- Walk with the farmer with your container with the needed material to the selected plots and ask them to point out the sweetpotato varieties identified in the questionnaire.
- Collectors should untangle the vine of the selected plant to be sure you are taking the leave for the desired plant.
- It is preferable to pick the uppermost young leaves without necrotic areas or lesions (Figure 1.A), although older leaves which are not senescent may be used (B), diseased and dry leaves should not be collected (C).

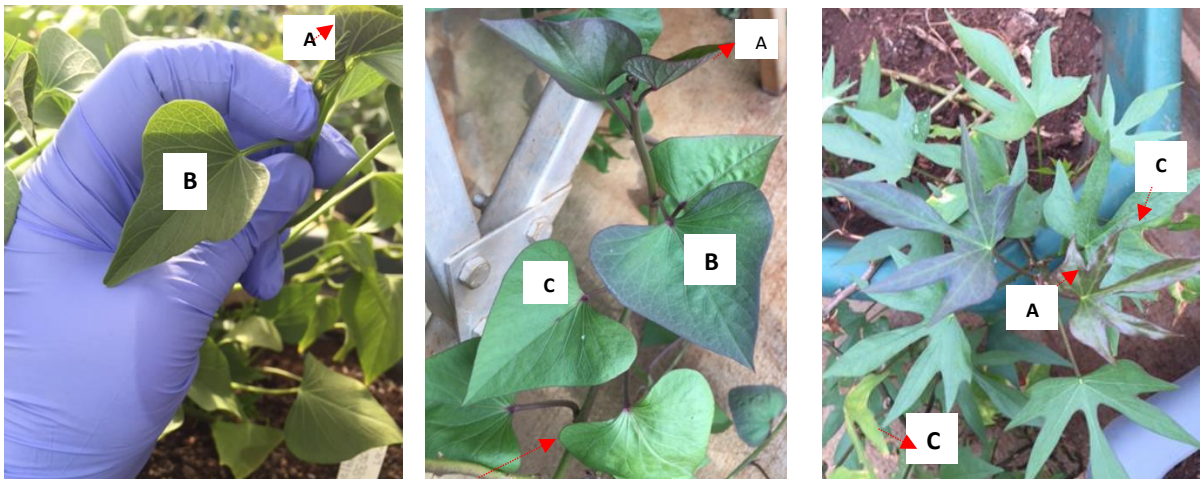


Figure 1. Different types of sweet potato leaves in the plant and preferences for leave collection

- Pick between 2-3 shoots or leaves, depending on what is available on the plot, using the tweezers to not touch the sample with hands (no need to change or clean gloves after each collection).
- Do not bend or fold collected leaves.
- When moving to the next sample, collectors should always disinfect the tweezers with ethanol. This is to avoid transferring DNA from one variety to another one, or an infected plant to a healthy one.
- If samples are dirty and there is no clean plants, clean the sample with water using the squeezer
- Put the leaves into paper towel and properly identified before moving to a place where to put shoots and/or leaves in the final coffee filter and Ziploc.

- If leaves are wet, use paper towel to dry them before transferring to the corresponding coffee filters with the identifier.
- Mark with pencil, the corresponding number from the template and the date of collection.
- Place the coffee filters/paper envelop in a Ziploc bag containing approx. 100 g orange silica gel.
- Before closing the Ziplock, take most of the air out of it.
- It is possible to keep from 6 to 9 coffee filters/paper envelopes in a Ziploc bag.
- Keep the Ziploc bags in an airtight plastic container in a dry place.
- After one or two days, replace the moisture saturated silica gel (change from orange to green when saturated) with another 100 g of orange silica gel. During the drying process, preserve the leaves as green as possible, and the best way to keep them green is to dry the leaves quickly.
- Leaves that are less dry should go to the bottom of the ziplock bag, to be closer to the silica gel.
- Moisture silica gel can be dried using a microwave (3 minutes). Then can be reused.
- Leaves can dry in silica gel between 2-7 days. Once leaves are dried, they can stay on the Ziplock with fewer amount of silica gel (10-20 grams).
- When the leaves are dry (crunchy), replace the moisture saturated silica gel with a small amount of the non-saturated with moisture ones (orange).
- Put 1 kg of silica gel in the big container for keeping low moisture in the large container.

The following pictures are an example of how to store the collected leaves in the Ziplock bags with silica gel and in the respective containers:

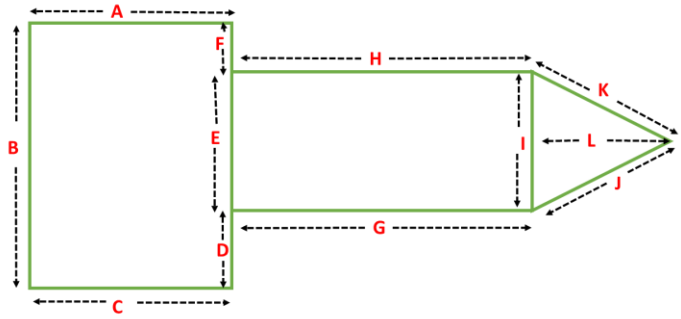
Figure 2. Orange silica gel with the leave sample inside a coffee filter into Ziplock bags

Figure 3. Plastic container to put 9 Ziplock bags filled with 9 leaves samples each.

- Once the large container is full of all leaves and/or shoot samples from the southern region, inform the supervisor that it is full, and it is needed to ship to the identified storage (either the CIP-Malawi office or the rented house in Lilongwe). Then a new large container will be used for central and northern region.
- Make prevision when any of the needed material is going to run out of stock (calculate when the material will last 7 days) to get more stock before is too late and there is no material to do the work properly.

4. Additional protocol for plot measurement.

- Plot measurement will be done to ALL plots where shoots and/or leaf collection was done. It means just plots that have OFSP, Kenya Admarc or the most important white sweetpotato in the District.
- After shoots and/or leaf samples are taken, the two collectors will prepare the material needed for the measurement: Blocks, pencils and measurement tape or measurement rope.
- *The selected farmer will show the limits of the plot where the variety was collected.*
- *The collector will draw the shape of the plot following the closest standard shape known (triangle, rectangle, square, circle).*
- *Put letters to each side that the drawn shape has (See example below)*



- *Start the measurement in one corner of the plot and make a mark (which is the point where the shape will start and finish).*

- *Measure each side of the shape, following the LETTERS included in the drawn shape.*, with the measurement tape or measurement rope.
- Write the measure in the template given by the trainer, together with the shape and the household and plot information (Appendix 2b).

Appendix 1. Main released varieties in Malawi

1. Anaakwanire

3. Kadyaubwerere

2. Chipika

4. Kaphulira

5. Mathuthu

7. Kenya

6. Zondeni

8. Lunyangwa

9. Mugamba

11. Sakananthaka

10. Nyamoyo

12. Semusa

13. Sungani

Appendix 2a. Template for registering leave and/or shoot samples

Format to fill leaf collection samples from the adoption and impact OFSP study in Malawi, 2019						Group : _____
N	DISTRICT	HH Nr.	RESPONDENT NAME	PLOT	VARIETY NAME	COMMENTS
1						
2						
3						
4						
5						

Appendix 2b. Template for registering plot measurement.

Format to fill plot measurement for area calculation of OFSP study in Malawi, 2019				Group : _____
N	HOUSEHOLD IDENTIFIER	PLOT #	PLOT SHAPE	SIDE MEASURMENTS
1	District: _____ Village: _____ Household Number: _____ Respondent name: _____			

Appendix 3. Template of information to be written in labels stick in coffee filters containing samples.