**Manual on Standard Operating Procedures (SOP’s)** **and Critical Control Points**

**Harvest data Phenotyping**

This Standard operating procedures were updated with EMBRAPA activities and procedures using the IITA file available at this [link](ftp://ftp.cassavabase.org/documents/standard_operating_procedures/IITA/).

# Harvest data collection

Outline

* Objective of harvest data collection
* Traits of interest in harvest data collection
* Descriptors and scoring system
* Materials needed
* Standard Operating Procedures and Critical Control Points

Objective of harvest data collection

* To obtain data for agronomic traits for final decision on clone selection

Traits of interest

List of trait is present in appendix 1

Descriptors and scoring system

As indicated in the appendix 1

Materials needed

* 1x Tablet or mobile device with fieldbook app
* 1x Power bank
* 2x Portable scale with accuracy of 100 grams with maximum load of 45 kilos
* 1x Hook to grab the plants bunch
* 1x sisal rope roll
* 3x machete
* 3x picks
* 0.5 Liters of chlorine solution (Soup + liquid Chlorine + water)
* 0.5 liters of 0.5% iodine solution
* 4x Sprayers to apply the chlorine solution in machetes and iodine solution
* Onion bags per plot
* Plot self-tie labels of the trial
* 1x Notebook for annotation
* 3x Pencil
* 1x Erasor
* 1x Pencil sharpener
* 3x Batteries 9 volts
* 1x Battery charger
* 3x Pen marker
* 3x Pocket knifes
* 1x Tent cover

Table 1. Standard Operating Procedures (SOP) and Critical Control Point (CCP) for harvest data collection

|  |  |  |
| --- | --- | --- |
| **s/n** | **Standard Operating Procedures** | **Critical Control Points** |
| 1 | Write and understand the protocol for the harvesting activity and obtain approval | Validate written protocol |
| 2 | identify the harvesting team and assemble all necessary materials using the checklist | Ensure all equipment are functioning properly |
| 3 | Install the Tent cover to cover the harvesting team in rainy days | This reduces the missing teamworkers due sickness |
| 4 | Determine the net plot and avoid border plants | Consult the field records where necessary |
| 5 | harvest plot by plots to prevent mixing errors with picks to avoid breaking the roots | Validate each plot before harvest. Look carefully to avoid leaving roots in soil. |
| 6 | Specify where and when to stop the harvest in each day considering the analysis to be carried by the laboratory | Be guided by the quantity of samples the lab can analyze per day |
| 7 | Count the plant number per plot, while harvesting plants per plot | Keep looking for rooted roots in plants while harvesting |
| 8 | Take all necessary data from stem part of the plant (characterization – Appendix 1) | Trained personnel should be involved in weighing and recording |
| 9 | Use machetes to detach the roots after pulling | Clean the machetes with chlorine solution, to avoid spread diseases. |
| 10 | Pick, count, and weight an appropriate and sufficient root samples for dry matter by oven and specific gravity method, Cyanide content analysis (Picrato methodology), and starch content | Ensure samples are well labeled and avoid mixed up |
| 11 | Root samples must get to the laboratory in time (8:00am - first samples, 9:30am - last samples of the day | Avoid lost plot labels while carrying the vehicle with the onion bag fully with roots |
| 12 | Take root data (characterization – Appendix 1). In Waxy trials spray the iodine solution in a cut root to evaluate the presence of waxy starch in roots | Trained personnel should be involved in weighing and recording |
| 13 | Weight the plants bunch of each plot | Remember that some plots may have more than one bunch plants |
| 14 | Prompt submission of data to the analyst |  |
| 15 | Upload the data into Cassavabase |  |

# Dry matter content determination

Standard operation procedures for dry matter (DMC) using oven dry and specific gravity methodology

Outline

* Objective of DMC determination
* Sampling procedure
* Methodology for Oven dry and Specific gravity methods
* Standard Operating Procedures and Critical Control Points

Objectives of DMC determination

* To determine the percentage of dry weight in roots of clones

**Sampling procedures**

1. Oven dry method:

Materials needed

* 3x medium sized root samples per plot
* 1x Plastic bowl of 15 liters per plot
* 1x Aluminum container per plot
* 3x knifes
* 3x cutting tables
* 1x Tablet or mobile with fieldbook app
* Plots sticky labels
* 1x Power bank
* 2x electronic scale
* Plot labels of the trial
* 1x Notebook for annotation
* 3x Pencil
* 1x Erasor
* 1x Pencil sharpener
* 1x Air-forced oven

Procedures

* Receive the root samples from the field
* Change the roots from onion bags to plastic pots (15L) with the plot label
* Wash the roots
* Carefully peel the roots removing outer and inner skin (do not scrape flesh)
* Cut the root into pieces the middle part of the root
* Weigh ~200 grams into well labelled aluminum bowl and note the fresh weight (FW) information in fieldbook app
* Let the samples dry in oven at a temperature of 70°C for 72 hours
* Take the dry weight (DW) and compute DMC (%) using the following formula

1. Specific gravity method:

Materials needed

* 5kg of fresh root (without the extremities)
* 1x Tablet or mobile with fieldbook app
* 1x Power bank
* 2x Portable scale with accuracy of 10 grams with maximum load of 10 Kilos
* 1x basket for the roots
* 1x Iron structure to support the scales
* 1x sisal rope roll
* 1x Barrel fully with water
* 3x machete
* 3x picks
* Onion bags per plot
* Plot self-tie labels of the trial
* 1x Notebook for annotation
* 3x Pencil
* 1x Erasor
* 1x Pencil sharpener
* 3x Batteries 9 volts
* 1x Battery charger

Procedures

* Collect ~5Kg of medium to large sized roots
* Carefully remove dirt from root skin and the root extremities
* Take the weight of root samples with portable scale using basket in the air and in the container fully with water
* Estimate DMC on specific gravity using the following formula (Kawano *et al*., 1987):

Table 2. Standard Operating Procedures (SOP) and Critical Control Point for dry matter content determination (Oven method)

|  |  |  |
| --- | --- | --- |
| **s/n** | **Standard Operating Procedures** | **Critical Control Points** |
| 1 | Barcode labeling of all selected samples generating using cassavabase | Ensure the sticky labels are available |
| 2 | Receive the roots from the field in the laboratory | Avoid to lose labels and roots from the onion bags during the transportation |
| 3 | Change the roots from the onion bags to plastic bowls | Avoid to lose the plot labels |
| 4 | Roots should be washed from soil and the root stalk cut off | Ensure samples are relatively clean and that non suitable roots (rotted) and root parts (stalks, fibrous) are not used |
| 5 | Roots should be peeled, cut in pieces, and changed to aluminum bowls | Ensure samples are relatively clean and only the outer and inner skin were removed |
| 6 | Calibrate and level the scale before use and ensure the unit of measurement is in grams | Ensure that the scale properly calibrated, leveled, and set to measure in grams with “g” displayed |
| 7 | ~200 grams of cube sampled should be weighed and dry in the oven. | Take note of fresh weight in fieldbook app |
| 8 | Proper labeling the air-forced oven levels with the entry day of samples | Report cases of sample(s) without label |
| 9 | Set all samples in the oven at the temperature of 70°C for 72 hours | Always check oven temperature at intervals and ensure functionality. |
| 10 | Weigh samples in bathes from the oven for ease of traceability | Report any missing samples to supervisor immediately after weighing. |
| 11 | Ensure the sensitive scale is shielded from wind, in a table without vibrations, and tared the scale with an aluminum bowl before taking the measurements | Tared the scale before start to weight and check at intervals if the scale is tared correctly |
| 12 | Upload the data into Cassavabase |  |

Table 3. Standard Operating Procedures (SOP) and Critical Control Point for dry matter content determination (Specific gravity method)

|  |  |  |
| --- | --- | --- |
| **s/n** | **Standard Operating Procedures** | **Critical Control Points** |
| 1 | Barcode labeling of all selected samples generating using cassavabase | Ensure the extreme of the plots self-tie labels are available |
| 2 | Collect ~5Kg of medium to large sized roots, put these roots in a onion bag, and staple the label at the onion bag | The roots selected should represent the plot, check if there is staples in stapler to avoid lose labels |
| 3 | Roots should be cleaned from sand, cut the roots extremities (Figure 1A and 1B) | Ensure samples are relatively clean and that non suitable roots (rotted) and root parts (stalks, fibrous) are not used |
| 4 | Assemble the iron structure (Figure1D), then tie the two portable scales in the structure, put the barrel fully of water below one of the scale, and calibrate scale before use and ensure the unit of measurement is in grams | Ensure that you are using the correct scale, if the scale is properly calibrated, and configured to measure in grams |
| 5 | Weigh the ~5kg of root sample in air (Figure 1E), and in water (Figure 1F) | Ensure that the root samples is totally immersed and suspended in water |
| 6 | Change the water if the water is getting turbid sand and dirt, this will influence the result | Ensure removal of foreign bodies (sand and dirt) from samples before weighing |
| 7 | Upload the data into Cassavabase |  |



Figure 1 - Standard operating procedures for dry matter content estimation by specific gravity methodology. A – cut roots per plot; B – root samples ready to be measured; C – root samples in the basket; D – Iron structure used to support the scales; E – Weigh the ~5kg of root sample in air; F - Weigh the ~5kg of root sample immersed in water

# Hydrogen cyanide content determination by Picrato methodology

Standard operation procedures for hydrogen cyanide content using picrato methodology

Outline

* Objective of hydrogen cyanide content determination
* Sampling procedure
* Picrato Methodology
* Standard Operating Procedures and Critical Control Points

Objectives of hydrogen cyanide content determination

* To determine the main purpose for the clone if it will be recommended for starch industry or fresh root consumption

**Sampling procedures**

1. Picrato methodology:

Materials needed

* 3-4x medium sized root samples per plot
* 1x Roll of plastic bags with 1,000 units
* 2x rolls of masking tape
* 2x knifes
* 2x cutting tables
* 1x Petri dish
* 3-4x Test tubes per plot
* 3-4x Test tubes caps per plot
* 3-4x Test tubes grids
* 3x Bunch of paper towel
* 3x Reels of filter paper Whatman n° 1
* 1x Scissor
* 1x Tweezer
* 1x Medicine dropper bottle
* 1 liter of toluene
* 1 liter of picrato solution
* 1x Tablet or mobile with fieldbook app
* 1x Notebook for annotation
* 3x Pencil
* 1x Erasor
* 1x Pencil sharpener

Picrato solution preparation

Materials needed for 200mL of picrato solution

* Mix 0.5 grams of picric acid (C6H3N3O7) in 50mLiter of water
* Mix 2.5 grams of sodium carbonate in 50mLiter of water
* Then mix the previously solutions to prepare the picrato solution

Procedures

* Receive the root samples from the field
* Remove the soil adherent to the roots
* Cut the filter paper in small pieces of 1cm x 6cm, it should enter easily in the test tube
* Cut 1-2 small root pieces from the middle of root per root replicate per plot
* Put 1-2 root pieces per test tube, add 5 drops of toluene to test tube
* Immerge one filter paper in the picrato solution
* Insert and attach the filter paper in the top of the test tube with the test tube cap
* Leave the samples in a light temperature protect room for 24 hours
* Evaluate the final color of each filter paper with color palette for picrato methodology (Figure 2)
* Clean the test tubes wearing face mask

Table 2. Standard Operating Procedure (SOP) and Critical Control Point for hydrogen cyanide content determination by Picrato methodology

|  |  |  |
| --- | --- | --- |
| **s/n** | **Standard Operating Procedures** | **Critical Control Points** |
| 1 | Receive 3-4 roots per plot from the field in the tent cover | Avoid to lose labels and roots during the transportation |
| 2 | Write previously the plot information in test tubes | Trained personnel should be involved in writing plot information |
| 3 | Enhance that there is enough toluene, picrato solution before start the activities | Report any missing materials to supervisor immediately before finishing it |
| 4 | Add a small amount of picrato solution to the petri dish, and toluene to the medicine dropper bottle |  |
| 5 | Each root should be cut in 1-2 small pieces | Ensure samples are relatively clean |
| 6 | Put the 1-2 root pieces with 5 drops of toluene into the test tube | Use carefully the test tube identified with the correct plot information |
| 7 | Immerge the filter paper at the picrato solution using the tweezer | Enhance to completely immerge the filter paper at the solution |
| 8 | Remove the excess of picrato solution in filter paper with a paper towel |  |
| 9 | Attach the filter paper with the cap while closing the test tube | Do not allow the contact of toluene with the filter paper |
| 10 | Leave the samples in a room without light and room temperature for 24 hours | During the transportation do not allow the contact of toluene with the filter paper, and the light exposure |
| 11 | Evaluate the final color of each filter paper with color palette for picrato methodology (Figure 2) and fill fieldbook trial template with the notes | Make the comparison always with the same room light pattern to avoid light inference in your evaluation |
| 12 | Upload the data into Cassavabase |  |



Figure 2 – Color palette for picrato evaluation, 1 corresponds to a sweetest cassava while 9 to a bitterest cassava

Appendix 1. Trait classification for data collection at harvest

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| s/n | Trait | Trait ID - Cassavabase | score | Description and scoring scale |
| 1 | Plants harvested | CO\_334:0000434 | count | Number of plants harvested and counted |
| 2 | Root number | CO\_334:0000011 | count | number of roots harvested and counted |
| 3 | Marketable root number counting | CO\_334:0000169 | count | Count of the number of storage root sizes visually adjudged to be big and weigh above one (1) kilogram |
| 4 | Non-marketable root number counting | CO\_334:0000168 | count | Count of the number of storage root sizes visually adjudged to be small and less than one (1) kilogram |
| 5 | Fresh root weight *(Kg)* | CO\_334:0000012 | weighing | Total fresh weight of storage roots harvested per plot measured in kilogram (kg) |
| 6 | Shoot Weight*(Kg)* | CO\_334:0000016 | weighing | Total fresh weight of harvested foliage and stems in kilograms per plot |
| 5 | Root length | CO\_334:0000450 | measure | Root length in cm |
| 6 | Root shape | CO\_334:0000020 | visual | 1 = Conical  2 = Conical-cylindrical  3 = Cylindrical  4 = Fusiform  5 = Irregular  6 = Combination of shapes |
| 7 | storage root periderm color | CO\_334:0000064 | visual | 1= White or cream, 2= Yellow, 3 = Light Brown, 3 = Dark brown |
| 8 | storage root cortex color | CO\_334:0000115 | visual | 1=White or Cream, 2 = Yellow, 3 = Pink, 4 = Purple |
| 9 | storage root pulp color | CO\_334:0000021 | visual | 1 = White or cream, 2 = Yellow, 3 = Pink |
| 10 | ease of peeling root cortex | CO\_334:0000308 | visual | 1 = Easy to peel  2 = moderately difficult  3 = Difficult to peel |
| 11 | rotted storage root counting | CO\_334:0000084 | count | A count of the number of rotted storage roots per plot at the time of harvest |
| 12 | Plant height | CO\_334:0000018 | measure | Vertical height of plants from the ground to top of the canopy measured in centimeter (cm) |
| 13 | Plant vigor | CO\_334:0000220 | visual | 1= Very little vigor  2 = Little vigor  3 = Fair  4 = Vigorous  5 =Very vigorous |
| 14 | Plant architecture | CO\_334:0000099 | visual | 1 = Excellent, upright plants with no branching  2 = Good, upright plants with high branching (>= 1m)  3 = Fair, plants with medium branching (>0.5m <= 1m)  4 = Bad, plants with low branching (<0.5m)  5 = Very bad, plants with low branching (<0.5m) and thinner stems |
| 15 | Stem color | CO\_334:0000062 | visual | 1Visual scoring of stem color with 1 = silver green, 2 = light brown or orange, 3 = dark brown, 4 = dark green, and 5 = golden |
| 16 | Stem cortex color | CO\_334:0000261 | visual | Observe from the middle third of the plant. Make a small shallow cut and peel back the epidermis, score as:  1 = orange, 2 = light green, 3 = dark green, 4 – purple2 |
| 17 | Stem epidermis color | CO\_334:0000262 | visual | Visual scoring of stem color. Peel epidermis back and look at underside of epidermis (skin). Scored as:  1 = cream, 2 = light brown, 3 = dark brown, 4 = orange |

1 Brazilian stem color characterization has the following visual scoring: 3 - orange; 4 - yellowish green; 5 - golden; 6 – light brown; 7 - silver; 8 - gray; 9 – black brown.

2 Brazilian stem cortex color has the additional color Purple.