Laboratory Standard Operating Procedure

NIRS Acquisition on Fresh Cassava Roots Using FOSS DS2500 and ASD Quality Spec and Relating the NIRS Spectra to Fresh HCN Content by Picrate Method

High-Throughput Phenotyping Protocols (HTPP), WP3

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Ethics: The activities, which led to the production of this manual, were assessed and approved by the CIRAD Ethics Committee (H2020 ethics self-assessment procedure). When relevant, samples were prepared according to good hygiene and manufacturing practices. When external participants were involved in an activity, they were priorly informed about the objective of the activity and explained that their participation was entirely voluntary, that they could stop the interview at any point and that their responses would be anonymous and securely stored by the research team for research purposes. Written consent (signature) was systematically sought from sensory panelists and from consumers participating in activities.

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RTBfoods

WP3: High-Throughput Phenotyping Protocols (HTPP)

SOP: NIRS Acquisition on Fresh Cassava Roots Using FOSS DS2500 and ASD Quality Spec and Relating the NIRS Spectra to Fresh HCN Content by Picrate Method

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ABSTRACT

All cassava varieties are known to contain some level of cyanogenic glucosides (CGs). Linamarine and lotaustrine are the commonest CGs in cassava but linamarine is the most abundant. The linamarine is readily hydrolyzed to glucose and acetone cyanohydrin in the presence of linamarase enzyme, which is also inherently produced by the plant. Acetone cyanohydrin decomposes rapidly in neutral or alkaline solution, liberating cyanide gas. Production of free HCN in cassava roots is prevented by compartmentalization of linamerase in cell walls and linamarine and lotaustraline in the vacuole. Therefore, damage/rupture of root tissues is desired for this reaction to occur. This Standard Operating Procedure (SOP) documents steps taken during determination of cassava root HCN content using the picrate method at the National Crops Resources Research Institute (NaCRRI), under the auspices of the RTBfoods project. Furthermore, the RTBfoods project seeks to develop high-throughput phenotyping protocols to provide RTB breeders with fast, accurate, and reproducible procedures for rapid phenotyping of their populations. NIRS is one such technologies that has been widely deployed in plant phenomics. In order to build NIRS prediction models, reference data must be matched with NIRS spectra. This document provides an SOP for generating HCN reference data using the picrate method and matching this data to NIRS spectra. Calibration of HCN reference data and NIRS data is the object of a separate document.

Key Words: Cassava, HCN, Picrate method, NIRS

1 SCOPE AND APPLICATION

This sop applies to fresh cassava roots that are grated using a kitchen grater and loaded in the small cap of the FOSS DS2500 for spectral acquisition and to spectra acquisition from whole root using ASD Quality Spec. The portion of scanned root is then used for determination of HCN content, in order to develop a database of NIRS spectra and HCN reference data, for calibration purpose.

2 REFERENCES

1. Bradbury, M. E. (1999). Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *Journal of the Science of Food and Agriculture*, 79:593–601.
2. Fukuda, W. C. (2010). *Selected morphological and agronomic descriptors for the characterization of cassava.* Ibadan, Nigeria: International Institute for Tropical Agriculture (IITA).

3 PROCEDURE

3.1 Sample preparation/presentation

Preparation of Picrate Solution (100 mL)

Materials

1. Whatman™ 1 filter paper (125 mm pore size)
2. Picric Acid
3. Sodium Carbonate (2.5% w/v)
4. Distilled water

i. Prepare picrate solution by weighing 1.4 grams of picric acid (BDH Laboratories) and 2.5 grams of Sodium Carbonate
ii. Dissolve the sodium carbonate in 100 ml of distilled water
iii. Add picric acid to sodium carbonate solution
iv. Incubate mixture in dark at room temperature for 30 minutes, under stirring.

Preparation of Picrate papers

i. Dip Whatman™ filter paper in Picric acid acid solution until fully saturated
ii. Remove the soaked paper from solution and dry on a non-absorbent surface, in the dark overnight at room temperature
iii. Color of the filter paper after dipping may vary slightly from lime green to yellow, but lime green is preferred as it matches the scale perfectly for very low HCN (Figure 1)
iv. Cut and shape the filter paper into strips of 1cm diameter and 3 cm length

Note:

a. Picric acid is explosive when dry! Ensure all recommended laboratory and personal safety measures.
b. Prepare fresh picrate paper for each new batch of samples.
c. Ensure picrate paper does not come into contact with moisture

***Figure 1**: Freshly prepared picrate paper for qualitative determination of fresh root HCN content*

Root sampling

Fresh root HCN content is known to differ between environments, roots of the same accession, same plant, different root sizes and even within the same root. Care must be taken to control for this inherent variation as much as possible, e.g. by sampling several roots and plants of the same accession.

Field sampling (a plot represents an accession)

i. Harvest all plants in the middle rows of each accession, leaving border plants
ii. Take care not to physically damage roots during harvest
iii. Pool all roots from plot together
iv. Select 3 uniformly sized roots and take to the lab for analysis

Root sample preparation

i. Wash roots to remove debris and dry with a kitchen towel.
ii. Slice off extreme end of root (proximal and distal) and retain a middle 5 cm portion
iii. Peel and grate middle portion using a kitchen grater
iv. Weigh 5 grams of grated root into a glass tube / 50 mL Falcon tube containing 5 mL of phosphate buffer pH 7.
v. Suspend picrate paper above the sample and tightly seal the tube with a cork
vi. Let sample stand for 12 hours and match the final color of picrate paper with the 1 to 9 color scale where 1 and 9 represent extremes of low and high HCN content respectively (Figure 2)
vii. For ease of data curation, it is best that this data be entered in a Fieldbook layout generated from the field trial samples picked for the assay

Note:

a. Since HCN is a volatile gas, steps iii to v must be performed rapidly (within 60 seconds) for each sample to minimize loss of HCN gas
b. Be sure to analyze your samples in duplicate (repeat step iv – vi) for each root. In the end, you should have 6 readings per plot from which a plot mean HCN content can be computed.

c. In areas where cassava brown streak disease (CBSD) is endemic, **avoid sampling visibly necrotic roots.** Necrotic tissue is basically dead tissue. These will give false low readings given that the reaction leading to production of HCN is enzymatically catalyzed and both the enzyme and the CGs can only be present in living tissue.

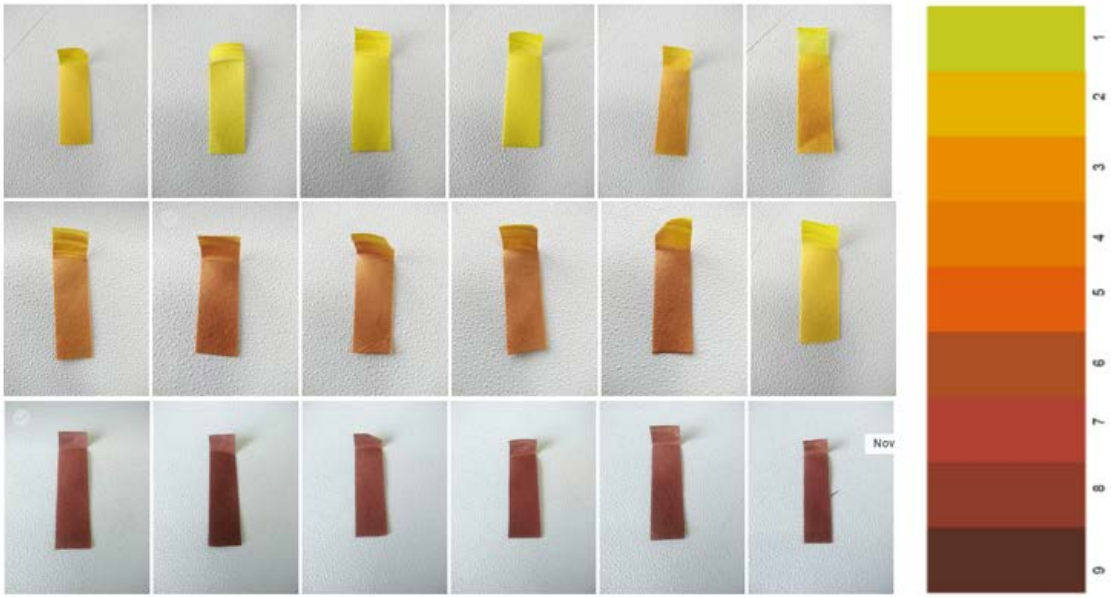


Figure 2: *Appearance of picrate papers after 12 hours. HCN Scores are generated by matching color of individual papers to the 1 to 9 HCN color scale by Fukuda et al (2010)*

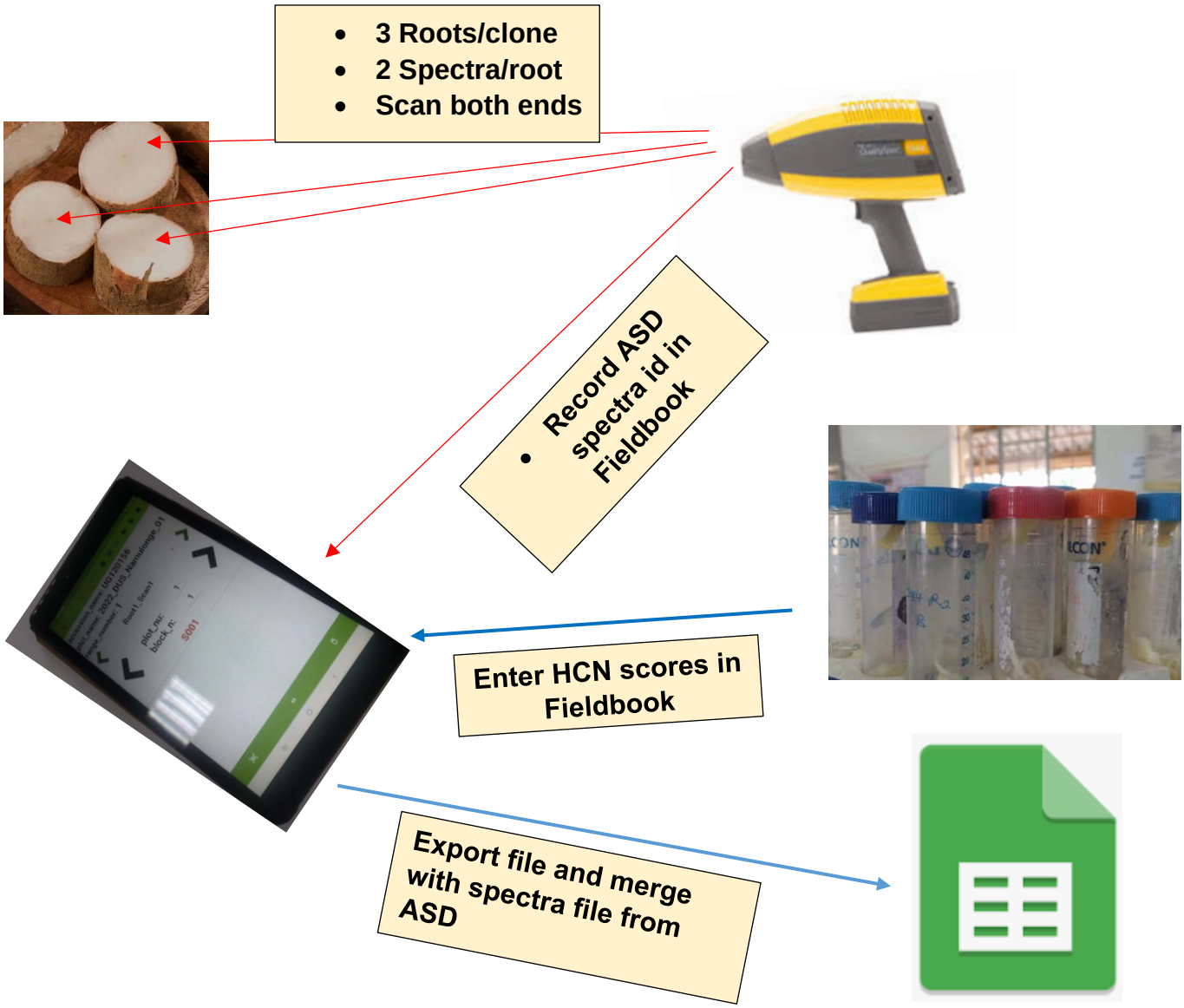
3.2 Protocol of spectral measurement and sample codification

A. ASD Quality Spec Trek

- After preparing root sample in step ii, take NIRS spectra by scanning one or both ends of the middle root section prior to grating.
- Be sure to maintain the same sample name for the Spectra as the sample for HCN determination (step iv – vi)

Sample coding with the ASD Quality Spec

- The ASD Quality Spec generates its own sample identities (id) (has no provision for sample entry)
- Hence, the operator needs to have an electronic tablet next to the Quality Spec, with the layout of the field trial and the list of the samples for HCN assay
- On the electronic tablet, record the ASD spectra id to the corresponding field plot or tissue sample (e.g. using Fieldbook)
- Export the spectra following the protocol provided by the manufacturer and match the spectra to field plots
- Merge the spectra file with the reference data file in vii above, ensuring accurate match of sample identities.



B. Foss DS2500

- After grating the root sample in step iii, immediately place ~20 grams into the small NIRS cap and collect NIRS spectra. It is critical to do this rapidly (within 60 seconds) for each sample to minimize loss of HCN gas.
- Be sure to maintain the same sample name for the Spectra as the sample for HCN determination (step iv – vi). If barcode labels are available, a barcode scanner can be connected to the Foss DS2500 and sample name (ids) scanned. However, the sample id should not exceed 12 characters, else it twill be truncated! A table of correspondence may be necessary, to match the full sample code (typically including the codes of the field trial, plot number, accession name, etc.), and a shorter sample code of maximum 12 characters.
- Export the spectra file and merge it with reference data in vii above, taking care to ensure accurate match of sample id

3.3 Procedures for spectra storage

For both ASD and FOSS DS2500, there is memory on the machine where exported spectra files can be stored. However, for long term storage, the spectra should be copied from the machines' memory, curated and uploaded to Cassavabase (www.cassavabase.org) under the corresponding field trial from which field samples were picked.

4 HCN REFERENCE REPEATABILITY

- Select two uniformly sized roots of the same accession
- Chop off extreme ends of root and retain middle 10 cm portion
- Bisect this portion. Process one 5 cm portion in your lab (NaCRRI) using two independent technicians. Send the second portion to a reference lab (NaRL-Kawanda) and have it evaluated for HCN by two independent technicians
- Aggregate data and compute lab error

4.1 Method of calculation and formulae

Score for HCN using the qualitative 1 to 9 scale (Figure 2; Fukuda et al., 2010).

4.2 Results and discussions

The fresh root HCN data is collated with the NIRS spectra and the resultant file used for NIRS model calibrations.

5 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- Ensure that the desktop NIRS device is mounted on a stable, shock and vibration free surface
- Keep the sample window of the NIRS clean at all times to avoid changes in NIRS spectra (parasite signals in the NIRS spectra)
- Ensure that the sample cap adapter of the DS2500 is mounted very well prior to loading your sample, to avoid leak of sample into the DS2500.
- When determining HCN with picrate method, take utmost care to ensure personal safety.

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