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Cassava Root Sampling for Cyanide Analysis

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We use this protocol and it's working

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Abstract

This protocol describes how to collect cassava root samples from a field or experimental plot for total cyanide (HCN) determination.

Materials

- A cassava harvesting tool e.g. a hand-hoe or spade
- Polypropylene sacks (4 per field to be sampled)
- Marking pen for labelling

Troubleshooting

Before start

Sampling time is an important factor for cassava root sampling. If possible ensure that all samples are collected within the early morning hours, while it is cool.



Plant selection

Randomly select four (4) plants from which cassava roots shall be sampled. The plants can be harvested from a field or an experimental plot. Select cassava plants that are representative of most plants on the plot. Also ensure that you sample plants of relatively the same age when sampling a farmer's field. All plants selected must belong to the same cassava variety. Avoid sampling boarder plants.

Note: Due to the high variability of root HCN levels between plants of the same variety and also between roots of the same cassava plant growing on the same field, four plants of the same variety are recommended to be selected on one field. Three cassava roots are then recommended to be selected from each sampled plant. This gives a total of 12 roots sampled from one field for root HCN analysis. The root HCN levels of the sampled roots will be determined and the mean calculated to represent the root HCN level of that cassava variety growing on that particular field or experimental plot.



Fig 1. Cassava plants selected for root sampling

Harvesting and selection of roots for HCN analysis

Uproot each selected cassava plant with all its roots as shown in Fig. 2. This should be done carefully, avoiding injury to the roots. Select three (3) roots from each plant (Fig. 2). As roots on cassava plants are of various sizes, divide the roots into three general sizes (large, medium and small) and then select one root from each sub-division. This gives



three roots per plant collected from a single cassava variety on a field. Don't rush to mix all the sampled roots together, as this depends on your interest (see step 3).





Fig 2. An uprooted cassava plant (left) and a representation of different sized cassava roots from one plant (right)

Sample labelling and packing

- 3 You have three sample labelling and packaging options as follows:
 - 1. You could mix up all collected roots from all plants on the field in one sack (i.e. all 12 roots from a field in one sack). This is ok as your final result is the average of the root HCN levels of all the 12 roots. This method only needs one sack per field when collecting root samples. Place the field name of the sampled field on the sack to identify the sampled roots. During analysis label the samples from 1 12 and include the field number (Table 1).
 - 2. If you would like to separately determine root HCN levels of roots from each of the four different plants sampled, then you will need four sacks (one sack for the roots of each plant) per field to pack up your cassava root samples. Only roots of the same plant will be mixed together here. Place the field name and the plant number on the label on the sack for roots collected from each plant on a field. Sacks will thus have the field name and the plant number on them (see Table 2). If you can manage to label the sampled roots in the field then one sack per field sampled is also possible to use here. Just make sure that the labels placed on the roots cannot fall off. Labelling cassava roots in the field may be challenging, but labelling can also be done in the lab during HCN analysis (see Table 2).



3. If you have an interest in knowing the HCN levels of each root sampled, i.e. the HCN content of roots between plants and within plants, this will affect your labelling. Each root sampled should have the field number, plant number and root number (Table 3). All roots can be packed in one sack in the field as full labelling will have to be carried out right in the field. You will have to find a reliable labelling option that does not involve damaging the roots.

Plant number	Plant label	Label on sack	Label on root sample
	1		F1
Plant 1	1	F1	F2
	1		F3
Plant 2	2		F4
	2	F2	F5
	2		F6
Plant 3	3		F7
	3	F3	F8
	3		F9
Plant 4	4		F10
	4	F4	F11
	4		F12
			7,00,20

Table 1: Simple labelling, differentiating between all roots sampled on a field

Plant number	Plant label	Label on sack	Label on root sample
	1		F1
Plant 1	1	F1	F1
	1		F1
	2		F2
Plant 2	2	F2	F2
	2		F2
	3		F3
Plant 3	3	F3	F3
	3		F3
	4		F4
Plant 4	4	F4	F4
	4		F4

Note: F is the field identification number/name.

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Table 2: Simple labelling, only differentiating between roots sampled from different plants



Plant number	Plant label	Root number	Label on sack	Label on root sample
	1	1		F1 ₁
Plant 1	1	2	F1	F12
	1	3		F13
	2	1		F21
Plant 2 Plant 3	2	2	F2	F22
	2	3		F23
	3	1		F31
	3	2	F3	F32
	3	3		F3 ₃
	4	1		F41
Plant 4	4	2	F4	F42
	4	3		F43

Note: F is the field identification number/name.

Table 3: Complex labelling, differentiating roots sampled from different plants and roots collected from the same plant

Sample storage while in the field

Once the roots are packed they must be quickly returned to the lab to begin cyanide analysis. If you will have to delay due to the number of samples to be collected or due to the distance to the laboratory, then place the sampled roots in a cool place right after harvest while in the field. This can be under the shade of some trees. Also keep the roots cool during their transportation. This is one reason for using polypropylene sacks, as they allow the cassava roots to still breathe and to stay cool, thus delaying deterioration. Never sample too many roots at a time as cyanide determination should be done at most within 6 - 12 hours after roots are harvested. Roots can rapidly deteriorate and have their composition changed. Beginning HCN analysis as soon as possible is hence essential for obtaining representative results (yes, even if it means a sleepless night). The state of the roots should be as close as possible to the state they were in right at harvest. Time spent in the field should thus take this factor into consideration.

Sample storage before HCN analysis



It may not be always possible to immediately analyse all roots or any of the roots. Samples hence need to be stored correctly before HCN analysis can begin. Some researchers advise that the middle section of the cassava roots can be cut and packed in an airtight plastic bag and kept frozen in a deep freezer until the time of HCN analysis. Others suggest that root samples should be freeze-dried. Think of the most efficient and cost effective method for the number of samples that you have. Also think of the equipment available. Its however best to determine HCN in cassava roots right from the field within a few hours after harvest. This eliminates variations that may be introduced by storage conditions and thereby gives more accurate results.

BIBLIOGRAPHY

Essers AJA. Further Improving the Enzymic Assay for Cyanogens in Cassava Products. In: Bokanga M, Essers AJA, Poulter N, Rosling H, Tewe O, editors. Int Work Cassava Safety 1 - 4 March, 1994. Ibadan, Nigeria, Nigeria: Working Group on Cassava Safety (WOCAS), International Society for Horticultural Science (ISHS), International Society for Tropical Root Crops (ISTRC); 1994. p. 97–104.