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Leaf sampling for hydrogen cyanide determination in cassava leaves

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Matema LE Imakumbili[1]

[1]Sokoine University of Agriculture

Matema LE Imakumbili
Sokoine University of Agriculture

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We use this protocol and it's working

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Abstract

This protocol describes how leaf samples from cassava plants are to be collected for total hydrogen cyanide determination in cassava leaves. The protocol was originally described by Essers (1994). It has however been rewritten to include some additional insights.

Guidelines

Leaf sampling time is an important factor for cassava leaf sampling. If possible ensure that all samples are collected within the early morning hours, like around 07:00 hours, while ambient temperatures are low.

Materials

- Cool box
- Ice packs
- Plastic bags
- Labels
- Marker pen

Plant selection before leaf sampling

1 Four plants of the same cassava variety must be selected on a field or experimental plot. This is why pot experiments need to be replicated at least four times, to produce a minimum of four plants per treatment for cyanide determination in cassava leaves. Four plants of the same variety are recommended due to the high variability of leaf HCN levels between plants of the same variety and also between leaves of the same cassava plant growing on a field. Make sure that the selected plants are all healthy and of the same age.

Note:
All sampled leaves from the four cassava plants growing on an experimental plot of field give the leaf cyanogenic glucoside content of that cassava variety under the respective field conditions.

Leaf sample collection

2 The first fully-expanded leaf from the top of each cassava plant plus two leaves below it must be picked during leaf sample collection. The first-fully expanded leaf should not have any of its lobes still in a folded state. The leaf may still appear as a rather small leaf yet much like the other older leaves in appearance. Slowly examine the plant to identify which leaf it is. The next two leaves after the first-fully expanded leaf are much easier to identify. Leaves must be picked together with their petioles to avoid damage to the leaf blade, which could prematurely release cyanide. It is the leaf blade that is of interest during cyanide determination in cassava leaves. The three picked leaves from each plant should be placed in labelled plastic bags. Note that if leaves are collected from a field or experimental plot then 12 leaves would have been collected and placed in plastic bag together. However if the experiment is a pot experiment then only 3 leaves would have been collected and placed in a plastic bag, as each plant in a pot is a treatment on its own. The leaves in the plastic bags must be immediately placed and temporarily stored in a cool box until all plants in the experiment have been sampled.

Note:
- In an experiment, sampling must be carried out on the same day if you can manage to prepare and have all leaves incubated (if using picrate paper method for cyanide determination in fresh cassava leaves), ideally within the first 8 - 10 hours after they have been all picked. If this cannot be done, sample each experimental block or two blocks of the experiment consecutively over the next 2 - 3 days and not more. This is because differences in plant age can introduce changes in a plants leaf cyanogenic glucoside content. Make sure you set-up manageable experiments taking into consideration the time needed to carry-out sample collection and analysis.

- Once collected, leaves can however be kept frozen in a deep freezer, below 0^{o}C, but they then become more susceptible to cyanide loss when defrosting, as cell walls are burst by the freeze and thaw action and all cell contents get mixed. Extra speed and care will be needed to prepare the frozen leaf samples for cassava cyanide analysis.

Before cyanide analysis

3 Once back at the laboratory where you shall carry out the cyanide analysis, quickly place the leaf samples in a refrigerator at 4^{o}C to keep the leaves cool and fresh. Only remove each sample right before you begin preparing it for total hydrogen cyanide (HCN) analysis.

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