Working together to eliminate cyanide poisoning, konzo and tropical ataxic neuropathy (TAN).

CCDN News

Cassava Cyanide Diseases Network

Issue Number 7, June 2006

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Transgenic Strategies for Reducing Cyanogen Levels in Cassava Foods.

Cassava is the major source of calories for many people living in sub-Saharan Africa.[1] According to the FAO, cassava ranks fourth in caloric intake among all crops directly consumed by humans. Cassava is preferred in many areas of Africa for the food security it provides, its ability to grow in poor soils and drought-prone areas, and the low labour inputs required to produce a crop. The roots of cassava can serve as a food bank against future food shortages; they can remain in the soil for 1-2 years before harvest.

All parts of the cassava plant contain potentially toxic levels of the cyanogenic glucoside, linamarin. Linamarin is stored in the vacuoles of leaf and root cells. When the cells are crushed during food processing linamarin is released from the vacuole and interacts with the cell-wall and laticifer localized enzyme, linamarase.[2] Linamarase deglycosylates linamarin yielding acetone cyanohydrin, the immediate precursor of cyanide.[3-7] Linamarin in cassava is thought to protect the plant against herbivory. Recent evidence from transgenic plants, in which linamarin production was blocked, indicates that linamarin also serves as a transportable form of reduced nitrogen. Linamarin is synthesised in leaves and transported to roots where it serves as a source of nitrogen for protein synthesis.[8,9]

While the linamarin levels of roots may vary more than 30-fold (15-500 mg CN equivalents/kg fresh weight), the linamarin content of leaves generally varies much less and is quite high (200-500 CN equiv./kg fresh weight) regardless of cultivar.[2,10] In contrast to roots, cassava leaves have an additional cyanogen metabolizing enzyme, hydroxynitrile lyase (HNL), that converts acetone cyanohydrin into cyanide. HNL is localized in the plant cell wall and similar to linamarase is a very stable enzyme.[11] As a result of the high activity of linamarase and HNL in leaves, leaves can be more efficiently detoxified during processing (crushing followed by soaking and cooking) than roots.

In contrast, poor processing (limited incubation time of linamarin with linamarase and lack of heat treatment to spontaneously convert acetone cyanohydrin into cyanide) of cassava roots may lead to the accumulation of acetone cyanohydrin.[12] The residual acetone cyanohydrin then spontaneously converts into cyanide once it is ingested.

Recently, we have over-expressed the gene encoding HNL in transgenic cassava roots.[12] Processing experiments with greenhouse-grown transgenic plants indicated that acetone cyanohydrin is very efficiently converted into cyanide which is then lost by volatilization creating a safer food product. Importantly, plants over-expressing HNL have normal levels of linamarin in their roots. Thus, transgenic plants still have the protective benefits of cyanogenmediated herbivore deterrence. Plants over expressing HNL are currently in field trials in Puerto Rico and will be introduced into Africa if the results of these trials are positive. HNL over-expressing plants will be made freely available to farmer organizations in Africa.

In addition, to reducing cyanogen levels in processed roots by over-expressing HNL, we are attempting to engineer cyanogen-free roots using transgenic strategies. Information on this project is available at the BioCassava Plus web site; http://biocassavaplus.org/ .

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Cassava Consumption in South Kivu Province of DR Congo : For Survival or Death ?

Cassava is the fourth most important human food source worldwide after wheat, rice and maize. In 1999, nearly 55% of cassava production came from Africa. Nigeria and the Democratic Republic of Congo (DR Congo) are the two major consumers of cassava in Africa, using one third of the cassava produced for human food.[1]

Cassava is the staple food of nearly 70% of the people of DR Congo with approximately 10% of world cassava production. It is the biggest food reserve in DR Congo. The roots are "natural stock hidden under the ground". The main food sources are cassava leaves and roots which are processed into flour that is cooked in boiling water to make a porridge (ugali). Cassava roots are also boiled, fried and grilled. Cassava leaves are a good source of protein.

Although cassava offers several possibilities as far as human nutrition is concerned, there are two negative factors to prevent people from eating it. These factors are the toxicity of the edible parts of the plant, and the poor nutritional quality of cassava roots.[2] Cassava contains two cyanogenic glucosides, linamarin and lotaustralin. Both glucosides are hydrolysed to form hydrogen cyanide, catalysed by the enzyme linamarase, as it is liberated after cassava cell rupture. Hydrogen cyanide is lethal if present at a dosage between 50 and 100 mg in food swallowed by an adult.[1] The enzymatic activity is higher in the young fresh leaves as well as in the skin (peel) of the roots[2].

The cyanide content in cassava is surely one of the major handicaps of the plant for human food. Unless the roots and leaves are processed for man and animal, cassava can become the cause of destruction and damage. The danger may vary from temporary problems such as stomach aches to sudden death or other irreversible alterations of the organism. These dangers include impact on the function of the thyroid that can result in goitre or cretinism. In the sixties and seventies already, relying upon the findings of the survey conducted in eastern and western DRCongo (formerly Zaire), a causal relationship has been established between cassava consumption and the occurrence of these disorders.[3,4,]

In eastern DR Congo cyanide intoxication was widely reported mainly in the rural areas. Severe and deadly intoxications affecting the elderly and children are actually occurring until now. As farmers cope with indescribable misery, cassava processing techniques are no longer applied. Cassava crops that have not yet reached harvest season are uprooted and used the same day for flour production. The cassava porridge (ugali) which is made by cooking the flour (made from prematurely harvested cassava roots) in boiling water, is usually eaten with poorly cooked cassava leaves (sombe).

Konzo (tied legs) is a disease due to cyanide overload which causes irreversible paralysis of both legs, particularly in children and women of child bearing age. It has occurred in the western part of DR Congo for many years and exists in the east as well, though no serious study has been carried out by specialists.

Today, most researchers are interested in the use of cassava varieties that are resistant to mosaic virus, which is a good thing. Nevertheless, it would be even better to introduce sweet varieties of cassava and to explain to the communities how to use simple processing methods, less expensive and less demanding, to decrease the cyanide toxicity rate in cassava products.[5] It will be very useful and important for researchers to concentrate some of their work on the cyanide poisoning and cyanide diseases issue.

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Kits to Determine Cyanide in Cassava, Thiocyanate in Urine and to Remove Cyanide from Cassava Flour

It is nearly ten years since a simple picrate kit for determination of cyanide in cassava flour[1] was successfully field tested in Nampula Province of Mozambique.[2] Subsequently the picrate kit was further improved and adapted for analysis of cyanide in cassava root.[3] By using different chemistry the picrate method was used for the analysis of thiocyanate in urine.[4] This analysis is important because ingested cyanide is converted to thiocyanate in the body and the urinary thiocyanate level indicates the consumption of cyanide from cassava over previous days.

Additional kits have been developed to determine total cyanide in cyanogenic leaves (kit E), in bamboo shoots (kit F) and in flax seed meal (kit G). A new kit AA has recently been developed which describes the new wetting method for the removal of cyanide from cassava flour[5,6] and allows measurement of the fraction of cyanide remaining in the wet flour after using the wetting method.

Characteristics of picrate method It is simple to use and requires only a small amount of water to operate the kit. The stepwise procedure (protocol) is available in four different languages and is understandable by those with a high school training. The plastic kit has no breakable parts and can be sent worldwide in a padded envelope by Courier in 1-3 weeks. The picrate papers gradually darken at room temperature, but there is very little darkening over the normal transport time. They must be stored at 0° C or in the deep freeze, where they are stable indefinitely. The cyanide colour chart has ten shades of colour from 0-800 ppm. Quantitative results comparable with those using other established methods[7] may be made by extracting the colour with water from the picrate paper and measuring its absorbance in a simple spectrophotometer. Kits normally allow 100 analyses to be made and contain standard papers so that periodical checks can be made of the methodology. Kit AA has material for 25 analyses.

Distribution of kits Since 1996 kits have been available free of charge to agricultural and health workers in developing countries. Distribution has been limited to those who are able to understand the protocol and use the kit effectively and who have a defined program for its use. Users must have a refrigerator in which to store the picrate papers, a postal address and telephone number for delivery. Kits are sold to workers in first world countries for AU$250. Since 1996, 416 kits have been produced of which 312 (75%) have been given away to workers in developing countries.

What are the achievements of this program?

1. It is now possible for agricultural and health workers in tropical Africa to monitor cyanide levels in their staple food cassava, its roots, leaves and processed products and to measure urinary thiocyanate.
2. The kits have been important in a collaborative study of konzo in Mozambique and in monitoring cyanide levels in gari in Nigeria, related to the occurrence of TAN.
3. The kits have aided agriculturalists in monitoring cyanide levels in cassava cultivars and for selection/breeding of low cyanide cultivars. Projects have been very diverse, including work on roots and leaves of cassava as a feed source for animals and also cyanide and urinary thiocyanate studies quite unrelated to cassava.
4. The kits have been an aid in research and development in 30 countries in the tropical world. I have not kept a record of projects that have resulted from use of the kits. **I would be grateful to receive information from users of kits on this matter.**

The future Apart from production of some kits in Indonesia, as far as is known there are no other picrate kits produced worldwide. All the information needed to produce the kits has been published,[1, 3-7] including methods for preparation of linamarase[8] and linamarin[9] used in the kits. The free offer of kits to workers in developing countries was supported until 2004 with funds from the Australian Centre for International Agricultural Research (ACIAR). However this free offer cannot continue indefinitely.

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IMPORTANT NOTICE

The Cassava Cyanide Diseases Network (CCDN) is now five years old and continues to grow steadily with present numbers approaching 300. Up to the present time hard copies of CCDN News have been sent by air mail to all members. About 70% of CCDN Members have e-mail addresses. For quicker communication it is proposed to send CCDN News by e-mail to all these members.

A hard copy will not therefore be sent by air mail to members who have e-mail, unless you specifically notify me that you still wish to receive a hard copy by air mail.

The 30% of members who do not have e-mail will continue to receive hard copies by air mail.

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20-26 November 2006
Central Tuber Crops Research Institute
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Theme: Roots and Tubers for Sustainable Development: Issues and Strategies

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CCDN News is the Newsletter of the Cassava Cyanide Diseases Network (CCDN). The CCDN is a free, worldwide network commenced in June 2001, which is working towards the elimination of konzo, TAN and other cassava cyanide diseases.
CCDN News will consider for publication short articles and letters (1-3 pages A 4 double spaced) written in English concerned with the following subjects:
1. Cyanide poisoning, konzo, TAN, goitre and cretinism facilitated by cyanide intake from cassava and any other cyanide diseases.
2. Reduction of cyanide intake from cassava through agricultural and nutritional means such as by broadening the diet of cassava consumers through introduction of new crops, pulses, vegetables and fruits, and by reducing the cyanide content of cassava varieties through selection and breeding. The effect of environmental factors such as drought on cyanide levels in cassava.
3. Processing methods for conversion of cassava roots to stable food products of low cyanide content.
4. Other relevant matters of interest.

Because CCDN News is a newsletter, full-size original papers or reviews cannot be considered for publication.

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