IRC-010e

Chronic Cassava Toxicity

Proceedings of an interdisciplinary workshop
London, England, 29-30 January 1973

Editors: Barry Nestel and Reginald MacIntyre

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
CENTRE DE RECHERCHES POUR LE DÉVELOPPEMENT INTERNATIONAL

Ottawa, Canada

1973

IDRC-010e

CHRONIC CASSAVA TOXICITY

Proceedings of an interdisciplinary workshop
London, England, 29–30 January 1973

Editors: BARRY NESTEL AND REGINALD MACINTYRE

UDC: 615.9:547.49
633.68

Head Office: Box 8500, Ottawa, Canada. K1G 3H9
Microfiche Edition $1

Contents

The Cyanogenic Character of Cassava (*Manihot esculenta*)

G. H. DE BRUIJN[1]

Faculty of Agriculture
Brawijaya State University
Malang, Java, Indonesia

BRUIJN, G. H. DE. 1973. The cyanogenic character of cassava (*Manihot esculenta*), p. 43–48. *In* Chronic cassava toxicity: proceedings of an interdisciplinary workshop, London, England, 29–30 January 1973. Int. Develop. Res. Centre Monogr. IDRC-010e.

Abstract Results of a study on cassava toxicity carried out in the Ivory Coast are presented. The distribution of the cyanogenic glucosides in the plant has been studied. It is concluded that classifying clones for toxicity according to the glucoside content of the tuberous roots is not strictly correct for other parts of the plant. Glucoside concentration of the leaves and of bark of tuberous roots of less toxic clones tends to be, on average, only slightly lower than in the same organs of very toxic clones.

Environmental conditions have a very important influence on the cyanogenic glucoside content of the tuberous roots. Different clones do not react in the same way to changing ecological conditions. Nitrogen fertilization increases, and supply of potassium and farmyard manure decreases the glucoside content. The influence of phosphate, calcium, and magnesium does not seem to be important. Drought increases glucoside content. Shading young plants increased the glucoside content of the leaves, but decreased that in the roots. No relation was found between the glucoside content of tuberous roots and the age of the plant. Glucoside concentration of a clone appears to be positively correlated with water content of leaves and tuberous roots, and a slight positive correlation with productivity was found.

There may be transportation of the glucoside in the plant. Ringing of stems caused a considerable increase of the glucoside content in the bark above the incision, and such an accumulation was not found when the leaves were eliminated before.

Distribution in the plant of the enzyme linamarase was studied. Activity is highest in the very young expanding leaves. In the bark of tuberous roots the activity is relatively very high, but in the inner part of the roots activity is very low. A knowledge of the distribution of linamarase activity offers possibilities for developing more effective methods for elimination of the toxicity of cassava products. The process of breaking down the glucosides of the grated inner part of the tuberous roots can be accelerated considerably by the addition of leaves or bark of tuberous roots, after which the hydrogen cyanide can be driven off.

Résumé L'auteur fait connaître les résultats d'une étude sur la toxicité du manioc, effectuée sur la Côte d'Ivoire. Il a étudié la distribution des glucosides cyanogènes dans la plante. Il en conclut que les clones taxonomiques de toxicité basés sur la teneur en glucosides des racines tubéreuses ne sont pas strictement applicables aux autres parties de la plante. La teneur en glucosides des feuilles et de l'écorce des racines tubéreuses de clones moins toxiques a tendance, en général, à être seulement un peu plus faible que celle des mêmes organes de clones très toxiques.

[1]Department of Tropical Crops, Agricultural University, Wageningen, The Netherlands. The author is temporarily attached to the Institute of Social Development and the Faculty of Agriculture of the Brawijaya State University.

Les conditions du milieu ont une très grande influence sur le contenu en glucosides cyanogènes des racines tubéreuses. Les clones ne réagissent pas tous de la même façon à des conditions écologiques changeantes. La fertilisation à l'azote cause une augmentation, alors que le potassium et le fumier de ferme produisent une diminution de la teneur en glucosides. La protection des jeunes plants contre la la lumière résulte en une augmentation du contenu en glucosides des feuilles, mais en une diminution de celui des racines. On n'a pas trouvé de relation entre le contenu en glucosides des racines tubéreuses et l'âge de la plante. Il semble y avoir corrélation positive entre la teneur en glucosides d'un clone et la teneur en eau des feuilles et des racines tubéreuses, et, à un degré moindre, la productivité du clone.

Il peut y avoir transport des glucosides dans la plante. Une incision annulaire des tiges produit une augmentation considérable du contenu en glucosides de l'écorce au-dessus de l'incision. Il n'y a pas accumulation lorsque les feuilles ont été enlevées au prélable.

La répartition de l'enzyme linamarase dans la plante a été étudiée. L'activité est maximale dans les feuilles très jeunes en voie d'expansion. Dans l'écorce des racines tubéreuses, l'activité est relativement grande, mais elle est faible dans les parties internes des racines. Une connaissance de la répartition de l'activité de la linamarase permettra peut-être de développer des méthodes plus efficaces pour éliminer la toxicité des produits du manioc. Le processus de dégradation des glucosides de la partie intérieure râpée des racines tubéreuses peut être considérablement accéléré par l'addition de feuilles ou d'écorce de racines tubéreuses, après quoi l'acide cyanhydrique peut être enlevé.

THIS paper is based on an extensive study of cassava toxicity which the author carried out in the Ivory Coast during 1965–70 (de Bruijn 1971).

In the last 15 years a great deal of attention has been paid to cyanogenesis in different plants. An understanding of this subject in cassava is of particular importance in view of the role played by this plant in both human and animal nutrition. The chemical pathway of cyanogenic glucosides is now well known. We know that cyanogenesis is connected with protein metabolism, that amino acids can serve as precursors of cyanogenic glucosides, and that there is a structural relationship between the cyanogenic glucoside and the precursor amino acid. In cassava, Nartey (1968) proved that valine and isoleucine can serve as precursors of the aglycone moieties of its cyanogenic glucosides linamarin and lotaustralin.

The cyanogenic glucoside content of the tuberous roots of cassava depends on many factors. The most important of these is the genetic constitution of the clone. However, since environmental conditions also have a very important influence, and since different clones react differently to varying ecological conditions, it is very difficult to make a reliable prediction of the cyanogenic glucoside content of the tuberous roots of a specific clone in a specific location.

We have not attempted to study the genetic background of the probably polygenic character of cassava cyanogenesis, but have paid particular attention to the influence of ecological conditions.

In an effort to obtain a better understanding of the behaviour of clones with different cyanogen levels we selected for our studies two clones having a rather low (about 50 μg HCN/g fresh weight), and two clones having a rather high (about 200 μg HCN/g fresh weight) cyanogenic glucoside concentration in the peeled tuberous roots.

As far as we know, no noncyanogenic cassava clones have yet been found.

Distribution of the Glucoside

Results of a study on the distribution of the glucoside in the plant are presented in Table 1. As in other cyanogenic plants the glucoside concentration in the leaves decreases with age. In expanding leaves, the concentration in the leaf stalks is higher than that in the leaf blades, but in older leaves the reverse is true. In the bark of the leafless part of the stem, the glucoside concentration increases markedly from the top downwards. In the bark of tuberous roots, the concentration is much higher than in the inner part, this difference being relatively much more important in the less toxic clones than in the very toxic ones (Table 2).

From the standpoint of toxicity, clones are generally classified according to the glucoside content of the peeled tuberous roots, and it is often suggested that this classification is also valid for other parts of the plant. After analysing the glucoside concentration of the leaves and bark of

TABLE 1. Distribution of glucoside (*μg HCN/g fresh weight*) in different parts of plants of four clones.

Part of plant	Clones				
	Tabouca	A 13	Ta 25	461	Average
Leaf blades					
Very young, in expansion	330	330	490	790	490
Just full-grown	420	340	570	1040	590
Older	250	210	320	730	380
Leaf stalks					
Very young, in expansion	400	750	770	940	720
Just full-grown	210	350	350	460	340
Older	120	110	170	180	150
Stem bark					
Near oldest leaves	270	350	550	1330	630
At $\frac{2}{3}$ of leafless part	90	230	330	580	310
At $\frac{1}{3}$ of leafless part	190	420	430	650	420
Lowest part	550	680	900	970	780
Bark of cutting	190	370	810	390	440
Bark of tuberous roots	400	540	890	730	640
Inner part of tuberous roots	36	55	210	240	140

TABLE 2. Average glucoside concentration (*μg HCN/g fresh weight*) in leaves, and in bark and inner part of tuberous roots, of less toxic and very toxic clones.

	Tuberous roots		
	Bark	Inner part	Leaves
Less toxic (avg 8 clones)	690	73	–
Very toxic (avg 8 clones)	840	330	–
Less toxic (avg 15 clones)	–	60	770
Very toxic (avg 15 clones)	–	340	1040

tuberous roots, and of the inner part of these roots, from a large number of clones we found that this suggestion is not strictly correct. The glucoside concentration of the leaves and bark of tuberous roots of less toxic clones tends to be only slightly lower than that in the same organs of very toxic ones, although this is not so in the case of the glucoside concentration of the inner part of the tuberous roots.

It appears that the less toxic clones have a higher rate of degradation of the glucoside than do the very toxic ones, the rate of formation of the glucoside being more or less equal for both types of clones. Although the glucoside concentration may vary greatly between the tuberous roots of one plant, there is no correlation between glucoside concentration and tuber size.

There is also considerable variation in the distribution of the glucoside within a tuberous root. In almost every case the highest concentration is found at the proximal end of the root. In a horizontal section there is an increase in glucoside concentration from the centre outwards.

Factors Influencing Cyanogenesis

In studying the influence of fertilizers, we found that nitrogen increased and potassium and farmyard manure decreased the glucoside content of leaves and roots. In general, the influence of phosphate, calcium, and magnesium was not important.

The supposition that glucoside concentration is positively correlated with the availability of amino acids in the plant may explain the influence of nitrogen and potassium, because manuring with nitrogen increases, and with potassium decreases,

the amino acid content in the leaves of various plant species (Ozbun 1965; Mengel and Helal 1968; Helal and Mengel 1968).

Serious drought increased glucoside content; short drought periods generally had little effect as the plant adapted by abscission of some leaves.

Somewhat surprisingly there seems to be a relationship between the glucoside content of a clone and its dry-matter content. We found that the glucoside concentration of the roots was negatively correlated with the dry-matter content of the leaves ($r = -0.33$) and also with that of the tuberous roots themselves ($r = -0.40$). Leaf glucoside concentration was also negatively correlated with the dry-matter content of the tuberous roots ($r = -0.34$) and with that of the leaves themselves ($r = -0.29$).

Shading young plants caused an increase in the glucoside concentration of their leaves and a decrease of that in the roots.

We did not find any relation between the glucoside content of tuberous roots and the age of the plant. We think it is more likely that differences in the glucoside content of tuberous roots at successive samplings are due to changing ecological conditions, rather than to changes in the age of the plant itself.

In Indonesia people believe that planting cuttings upside down will increase the toxicity of the tuberous roots of the resulting plants. Bolhuis (1939) did not find convincing evidence supporting this belief, nor did we in our experiments.

It is often said that there is a positive correlation between the productivity of a clone and its toxicity. Comparing 67 clones we did find a small positive correlation between the glucoside content of peeled tuberous roots and the amount of leaf ($r = 0.20$), stem ($r = 0.24$), and tuberous root ($r = 0.20$) per plant (significance level: for $P = 0.05$, $r = 0.24$).

Possible Transport of Glucoside

The results of our studies on the distribution of the glucoside in the plant suggest there might be some transport of glucoside occurring within the plant.

Ringing of stems caused a considerable increase (more than 100%) of glucoside concentration in the bark above the incision, especially during the first few days. This accumulation was maintained for at least 2 months. Such an increase was not observed when leaves were eliminated. The effect of stem ringing, after 3 days, was more important in young plants (165%) than in older plants (65%). But we did not find an increase of the glucoside in the leaves above the incision.

Stem ringing caused a decrease in the glucoside content of the tuberous roots which fell by about 20% in 2 weeks.

Although these experiments indicate a transport and accumulation of the glucoside, it could be the precursors of the glucoside (e.g. amino acids) that are being transported. The use of radioisotopes would help to resolve this issue.

Activity of the Enzyme Linamarase

Because the enzyme linamarase is of great importance for the breakdown of the glucoside we thought it necessary to study the activity of this enzyme in the plant. The results of a study of the distribution of linamarase in the plant are presented in Table 3.

The enzyme activity in the plant is highest in the very young expanding leaves and lowest in the lowest part of the stem bark and in the inner part of the tuberous roots. In the bark of the stem there is a marked decrease in activity from the top downwards. In the bark of the tuberous roots the activity is many times higher than in the inner part of these roots.

Very little is known about the role of linamarase in the plant. Our results do not indicate any evidence of a direct relationship between the concentration of enzyme in the plant and the level of glucoside. Linamarase activity in both the bark and the inner part of the tuberous roots of very toxic clones differed little from that found in less toxic clones.

A knowledge of the distribution of linamarase activity is important for an understanding of the process of eliminating the glucoside after harvesting. It also offers possibilities for developing more effective methods to eliminate toxicity of cassava products used for human and animal food.

Elimination of the Glucoside

Tuberous roots of cassava, which contain a high level of glucoside, have to be specially treated

TABLE 3. Distribution of linamarase activity (*μg HCN liberated/g fresh weight per min*) in different parts of plants of four clones.

Part of plant	Clones				
	Tabouca	A 13	Ta 25	461	Average
Leaf blades					
Very young, in expansion	450	1000	600	850	730
Just full-grown	400	600	100	100	300
Older	200	150	10	40	100
Leaf stalks					
Very young, in expansion	650	1150	350	800	740
Just full-grown	200	550	300	400	360
Older	250	600	300	350	380
Stem bark					
Near oldest leaves	160	170	130	130	150
At $\frac{2}{3}$ of leafless part	140	110	20	70	90
At $\frac{1}{3}$ of leafless part	80	160	30	45	80
Lowest part	0	15	0	10	6
Bark of cutting	10	120	0	15	35
Bark of tuberous roots	140	480	160	280	270
Inner part of tuberous roots	9	13	6	7	9

to eliminate the toxic substances before they can be eaten. Many treatments for eliminating toxicity are known, but confusion still exists about their adequacy. Though cassava consumers generally know how to get rid of the toxic substance, intoxication accidents do still occur.

Treatments to reduce the danger of intoxication can be divided into two groups:

1. The glucoside is eliminated. This elimination can be direct (e.g. soaking) or by enzymic breakdown.

2. The glucoside is only partly eliminated, if at all, but the enzyme is broken down (e.g. heating). Combinations of both groups also exist.

The best way to obtain a safe product is to break down the glucoside by the enzyme and then to eliminate the HCN by drying or heating. The speed and adequacy of this process depends on the intensity of the contact between the glucoside and the enzyme, thus on the rate of grating of the tuberous roots, and, self-evidently, on the enzyme activity. In the leaves and in the bark of tuberous roots linamarase activity is so high that in a very short period after crushing or grating all the glucoside is broken down and the HCN can be driven off. But in the inner part of the tuberous roots linamarase activity is very low and it may take a long time before all the glucoside has been broken down. To eliminate toxicity, tuberous roots are often grated and fermented for one or more days. However, according to Adriaens (1955) this decreases the quality of the product.

We found that it was possible to considerably accelerate the process of breaking down the glucoside of the grated inner part of the tuberous roots by the addition of juice prepared from the leaves or bark of tuberous roots. This caused all of the glucoside present to be broken down within 1 h. Thus, when preparing food for animal consumption, it was sufficient to grate tuberous roots as a whole, including the bark. For human consumption this method might render the taste less pleasant. More research is necessary in this area.

The addition of leaves or of bark of tuberous roots to preparations of food from the inner part of the roots is also important from a nutritional point of view, since the protein content of cassava leaves is very high, and the nutritive value of the

root bark is also much higher than that of the inner part of the tuberous roots (Barrios and Bressani 1967).

When the glucoside is not eliminated, but only the enzyme is broken down, questions arise about the safety of the product. A good example is cooked tuberous roots. It has often been stated that cooking is a safe method for the elimination of toxicity of cassava roots. In fact, cooking destroys the enzyme at 72°C (Joachim and Panditteseker 1944), but not the glucoside. When the tuberous roots are cooked, the glucoside cannot get out of the starchy product and we found that up to 90% of the original quantity may remain. The safety of eating this product depends on the quantity of glucoside present, and of the quantity that can be safely consumed by man. According to Montgomery (1965) it has never been proved that the glucoside itself is toxic to man. Part of the glucoside consumed will be broken down by stomach acids. It is a moot point what part of this glucoside will be broken down and what conditions are important for this to take place.

References

ADRIAENS, L. 1955. Recherches sur la composition en acides amines des proteines d'aliments vegetaux du Congo Belge et du Ruanda Urundi. Mem. Acad. r. Sci. Colon. Cl. Sci. Nat. Med. 8. T. III, fasc. 3. 102 p.

BARRIOS, E. A., AND R. BRESSANI. 1967. Composicion quimica de la raiz y de la hoja de algunas variedades de yaca, *Manihot*. Turrialba 17: 314–320.

BOLHUIS, G. G. 1939. Omgekeerd geplante stekken van cassave. Landbouw, Buitenz. 15: 141–151.

BRUIJN, G. H. DE. 1971. Etude du caractere cyanogenetique du manioc (*Manihot esculenta* Crantz). Meded. Landbouwhogeschool Wageningen 71–13; 1–140.

HELAL, M., AND K. MENGEL. 1968. Der Einflusz einer variierten N-und K-Ernährung auf den Gehalt an löslichen Aminoverbindungen und auf die Ertragsbildung bei Sommerweizen. Z. Pfl. Ernähr. Bodenk. 120: 89–98.

JOACHIM, A. W. R., AND D. G. PANDITTESEKERE. 1944. Investigations of the hydrocyanic acid content of manioc (*Manihot utilissima*). Trop. Agri. Mag. Ceylon Agric. Soc. 100: 150–163.

MENGEL, K., AND M. HELAL. 1968. Der Einflusz einer variierten N-und K-Ernährung auf den Gehalt an löslichen Aminoverbindungen in der oberirdischen Pflanzenmasse von Hafer. Z. Pfl. Ernähr, Bodenk. 120: 12–20.

MONTGOMERY, R. D. 1965. The medical significance of cyanogen in plant foodstuffs. Amer. J. Clin. Nutr. 17: 103–113.

NARTEY, F. 1968. Studies on cassava, *Manihot utilissima* Pohl, I. Cyanogenesis: the biosynthesis of linamarin and lotaustralin in etiolated seedlings. Phytochemistry 7: 1307–1312.

OZBUN, J. L. 1965. Effect of light and potassium nutrition on photosynthesis and respiration. Diss. Abstr. 26: 611.