

FREE CYANIDE PRODUCTION IN FIELD CASSAVA UNDER CHANGING WATER STATUS DURING GROWTH

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Abstract

The capacity of cassava cultivars (TMS 30572, TMS 4(2)1425, the local cultivar, and TMS 50207) to produce free cyanide during drought and thereafter was investigated under field conditions. Usually, leaves contained more cyanide than did roots. During the seedling stage, total cyanide increased more under drought than under irrigation. Free HCN was greater under drought than under irrigation. Drought favoured the formation of the cyanohydrin component from the cyanoglucoside, explaining the lower content of the latter under drought than under irrigation. During tuberization, all cyanide fractions were considerably lower under both conditions; but these fractions did not vary significantly between treatments, except when totalled. The results indicated that cassava plants have the inherent ability to minimize deposits of cyanide in its underground storage organs.

Introduction

Jones (1959) showed that cyanide content in cassava increased most under unfavourable conditions. Lancaster and Brooks (1983) also found that cassava plants exposed to long periods of drought responded with increased cyanide concentration. This effect can be produced more rapidly in stressed pot plants (de Bruijin 1973). Recently, Nwosu (1992) reported that the increased cyanide content of cassava seedlings derived from the fact that linamarase enzyme activity was inhibited under water stress, resulting in the accumulation of cyanoglucoside. Furthermore, this effect persisted after rehydration, indicating an inductive effect. As with most potted experiments, the leaves were used to assess the stress imposed, and plants were monitored at young ages. Even so, these results serve as tools for further research in the field, where the plant is exposed to other environmental factors.

This paper reports the levels of free cyanide production by field cassava when the crop is subjected to changing water status at various stages of growth.

Materials and Methods

Source of planting materials

Stem cuttings of the improved cassava cultivars TMS 30572, TMS 4(2)1425, and TMS 50207 were obtained from the Rivers State Agricultural Development Programme. Those of the local cultivar came from the village of Alikahia in the Akpor Local Government Area of Rivers State. The study was conducted in the botanical garden of the University of Port Harcourt, Nigeria.

Experimental design

The study was carried out during the dry season, from November 1991 to April 1992. The land was cleared and ridged, and had been cropped in previous years. The experiment was laid out in a split-plot design, with irrigated and non-irrigated treatments assigned to the main plots. These plots were subdivided into subplots to which the cassava cultivars were randomly allocated. The main plots were 2 m apart; the subplots (5 x 5 m) had five ridges. The blocks were replicated three times. Stem cuttings, 8-10 cm long, were planted manually at a slant and at distances of 1.0 m. Ridges were watered to ensure sprouting. Seven cuttings were planted per ridge, but were thinned down to five uniform plants. Plants on the first and fifth ridges, and those at the extreme ends of all ridges, were treated as border plants to prevent competition between subplots. All plots received uniform agricultural maintenance.

Treatments

Treatments began 30 days after planting (DAP). Sprinkler irrigation provided supplementary water to all plots from 1 DAP to enable the cuttings to sprout. Thereafter, the non-irrigated portion was kept under rainfed conditions. This meant that plants grew under water stress as the experiment was carried out during the dry season. The irrigated portion was supplied with the required amount of water to individual plots, using a hose. The amount of water was calculated from knowing the delivery rate. The source of water was a bore with an overhead tank for water storage. Each irrigated plot received 30 mm irrigation weekly until the rains arrived in April.

Sampling

Specimens were collected by harvesting one plant at random per plot during the seedling stage, tuberization, and at maturity. Plants were dug out and the roots carefully removed. After detaching the roots and leaves (youngest fully expanded) of the various cultivars, the following parameters were taken:

- (1) Cyanide content of leaves and roots, estimated according to the Nambisan and Sunderasan method (1984);
- (2) Cyanide fractions (i.e., free cyanide [HCN], non-glucosidic cyanide [cyanohydrin], and cyanoglucosides), determined by the method developed by O'Brien et al. (1991); and
- (3) Total cyanide (the sum of the foregoing cyanide fractions).

Results were subjected to ANOVA and the LSD test.

Results

Seedling stage

During this stage, non-irrigated plants experienced drought from November to January. The cyanogenic potential (Table 1) shows that, under drought, all cultivars responded with increased total cyanide for the whole plant. Except for the local cultivar, leaves had higher cyanide contents than the roots, confirming Onwueme's report (1978) that the leaves of cassava plants usually contain more HCN than do the tuberous roots. With respect to varietal differences, the sweet cv. TMS 4(2)1425 accumulated the largest amount of cyanide in the leaves, while the roots contained the least. It was followed by the hardy cv. TMS 30572 for leaves and the local cultivar for roots. TMS 50207 had the lowest cyanide content in the leaves under both treatments.

Overall, free HCN constituted the highest proportion among the cyanide fractions in both shoots and roots. Values for leaf cyanoglucoside were usually higher than for cyanohydrin in irrigated cassava plants. In drought-stressed seedlings, however, the cyanohydrin component differed according to cultivar. The hardy cultivars (TMS 30572 and TMS 50207) had equal contents of cyanohydrin and cyanoglucoside. The low-cyanide cv. TMS 4(2)1425 had a significantly higher proportion of cyanohydrin than cyanoglucoside. The local cultivar retained more of its cyanoglucoside than cyanohydrin. In drought-stressed roots, the cyanohydrin fractions were generally higher than those of cyanoglucoside.

These results indicate therefore that drought tends to favour the formation of the cyanohydrin component from the cyanoglucoside, especially in roots. The cyanohydrin can be lysed by hydroxynitrile-lyase or sometimes split non-enzymatically; hence, its degradation is faster. Understandably, therefore, overall accumulation of free HCN in the drought treatment occurred. Although the cyanogenic potential in the roots was lowered considerably (except for the local cultivar), the relative concentrations of the cyanoglucoside to cyanohydrin and/or free HCN were maintained within reasonable limits.

Tuberization

This stage coincided with the arrival of the rains and spanned March to May. Cassava seedlings that had previously been exposed to drought received ample water supply. Irrigation was also stopped. Table 2 shows that all cyanide fractions, including total cyanide, were significantly lower than the amounts during the seedling stage. This situation prevailed whether the plants were irrigated or not. The leaves maintained a higher production of cyanide than did the roots.

Cassava plants that had previously suffered drought still maintained higher total cyanide concentrations than did the irrigated ones. This observation agrees with those of Nwosu (1992), who found that seedlings accumulated cyanide even after several weeks of rehydration. Overall, free HCN ranked highest in amount, but cyanohydrin and cyanoglucoside contents did not differ significantly, especially in the roots.

Maturity

This period (May-October) received continuous rainfall, and samples taken during this time followed trends in the concentrations of total cyanide and/or its fractions that were essentially similar to those observed during tuberization. The cyanogenic potential (Table 3) was further depleted in both roots and leaves. Although the cyanide quantities in the drought-stressed treatments maintained generally higher quantities than did the irrigated ones, the differences were not significant. Furthermore, total cyanide values in the leaves of TMS 50207 and roots of TMS 30572 were actually lower than those for their irrigated counterparts. This shows that the long period of adequate water supply in the form of rainfall mitigated the adverse effect of the drought.

Discussion

The increase in cyanide formation during water stress is an established phenomenon (de Bruijn 1973; Lancaster and Brooks 1983; Nwosu 1992). With the leaves as the source, the concentration of cyanide in the roots was minimized, indicating that part of the cyanide was lost during the downward translocation. Furthermore, the development of the sink during tuberization and its enlargement during maturity did not affect the concentration at the source, that is, the plants still retained large quantities of cyanide in the leaves. The 'sweet' cultivar was a case in point: under stress, most of the cyanide produced remained at the top, with very

little in the roots (which is probably why they were 'sweet'). This will be understood once the manner in which cyanide is transported is known, that is, whether as free HCN, cyanoglucoside, cyanohydrin, or as all three.

Root and shoot tissues usually contained less cyanide during tuberization and maturity than in the seedling stage, regardless of treatment. That is, as plants get older, the HCN concentration in the various parts of cassava plants increases to a peak before declining (Sinha and Nair 1968; Onwueme 1978). The time of peaking varies according to cultivar. This phenomenon suggests that the cassava plant has an inherent ability to minimize the concentration of this toxic substance in its tissues.

The study of the pattern of accumulation of cyanide in plant parts or across time and of the physiology involved would be relevant to efforts to lower cyanide content in the roots, thereby increasing safety in cassava consumption.

The persistence of higher cyanide levels in drought-stressed cultivars synchronizes with the inductive ability of the plant's metabolic mechanism. Although linamarase enzyme activity was not reported here, results are in line with those of previous reports (Nwosu 1992).

At maturity, the effect of stress on cyanide levels virtually disappeared, showing that the level of water stress imposed was elastic in nature (Levitt 1980). Nartey (1978) stated that the normal range of cyanide in cassava is 15-400 mg/kg. The results of this research fall within this range. This means then that water stress at the seedling stage has insignificant effect on the final cyanide content of the roots. Products derived from cassava plants exposed to such water stress are therefore also unaffected.

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Table 1. Cyanide content ($\mu\text{g/g}$ dry wt) of various cassava cultivars in the seedling stage under irrigated and drought conditions.

Cultivar	Irrigated				Drought-stricken			
	Free HCN	Cyano-glucoside	Cyanohydrin	Total	Free HCN	Cyano-glucoside	Cyanohydrin	Total
Leaf sample								
30572	12.5 ± 0.15	15.5 ± 0.24	6.2 ± 0.24	34.1 ± 0.62	39.8 ± 1.15	5.8 ± 1.21	5.8 ± 1.62	51.4 ± 0.42
4(2)1425	26.1 ± 0.13	20.4 ± 0.32	5.6 ± 0.50	52.1 ± 0.30	38.1 ± 0.10	9.4 ± 0.92	29.0 ± 0.69	76.6 ± 0.34
Local	7.6 ± 0.40	18.1 ± 0.20	6.3 ± 0.20	32.2 ± 0.10	18.8 ± 0.20	9.5 ± 0.30	3.8 ± 0.60	32.1 ± 0.50
50207	18.1 ± 0.81	1.5 ± 0.78	3.0 ± 0.75	22.5 ± 0.27	17.4 ± 1.64	5.7 ± 1.64	6.9 ± 2.36	30.1 ± 1.50
Root sample								
30572	34.2 ± 0.05	9.5 ± 0.17	1.1 ± 1.48	44.7 ± 1.47	21.4 ± 1.19	4.7 ± 1.3	7.6 ± 1.55	33.6 ± 1.48
4(2)1425	7.0 ± 0.43	1.0 ± 0.20	1.6 ± 1.40	9.6 ± 0.60	2.0 ± 0.03	0.4 ± 0.8	1.6 ± 0.04	4.0 ± 0.04
Local	10.3 ± 0.20	1.5 ± 0.30	3.3 ± 0.30	15.1 ± 0.20	23.8 ± 0.81	1.8 ± 0.5	15.9 ± 0.71	41.4 ± 0.70
50207	7.7 ± 0.22	7.2 ± 0.87	6.1 ± 0.49	21.0 ± 0.67	12.4 ± 0.71	2.1 ± 0.36	2.2 ± 0.56	16.7 ± 0.08

Table 2. Cyanide content ($\mu\text{g/g}$ dry wt) of irrigated and drought-stressed cassava cultivars during the tuberization stage.

Cultivar	Irrigated				Drought-stricken			
	Free HCN	Cyano-glucoside	Cyanohydrin	Total	Free HCN	Cyano-glucoside	Cyanohydrin	Total
Leaf sample								
30572	4.4 ± 0.13	0.7 ± 0.09	1.0 ± 0.48	6.1 ± 0.11	3.9 ± 0.03	1.0 ± 0.06	2.5 ± 0.07	7.7 ± 0.04
4(2)1425	3.6 ± 0.10	1.1 ± 0.10	1.8 ± 0.10	6.6 ± 0.10	4.9 ± 0.10	3.0 ± 0.30	1.7 ± 0.61	9.6 ± 0.30
Local	6.3 ± 0.10	2.2 ± 0.10	1.0 ± 0.10	9.4 ± 0.10	11.7 ± 0.30	3.7 ± 0.03	2.8 ± 0.10	18.2 ± 0.00
50207	7.7 ± 0.19	1.6 ± 0.53	0.8 ± 0.19	10.1 ± 0.04	5.3 ± 0.26	1.0 ± 0.18	6.9 ± 0.90	13.2 ± 0.65
Root sample								
30572	0.6 ± 0.01	0.3 ± 0.05	0.2 ± 0.05	1.0 ± 0.05	0.5 ± 0.01	0.1 ± 0.00	0.1 ± 0.01	0.6 ± 0.01
4(2)1425	0.4 ± 0.03	0.1 ± 0.01	0.3 ± 0.60	0.8 ± 0.03	0.8 ± 0.02	0.7 ± 0.00	0.3 ± 0.02	1.8 ± 0.00
Local	0.8 ± 0.01	0.1 ± 0.04	0.2 ± 0.03	1.1 ± 0.02	1.0 ± 0.00	0.1 ± 0.01	0.1 ± 0.01	1.3 ± 0.02
50207	0.7 ± 0.07	0.5 ± 0.07	0.3 ± 0.05	1.5 ± 0.03	1.4 ± 0.02	0.2 ± 0.05	0.3 ± 0.10	1.8 ± 0.16

Table 3. Cyanide content ($\mu\text{g/g}$ dry wt) of various cassava cultivars at maturity under irrigated and drought-stressed conditions.

Cultivar	Irrigated				Drought-stricken			
	Free HCN	Cyano-glucoside	Cyanohydrin	Total	Free HCN	Cyano-glucoside	Cyanohydrin	Total
Leaf sample								
30572	4.1 ± 0.04	0.9 ± 0.04	0.9 ± 0.04	5.8 ± 0.03	3.5 ± 0.04	1.3 ± 0.18	1.8 ± 0.06	6.6 ± 0.16
4(2)1425	3.0 ± 0.03	1.5 ± 0.02	1.5 ± 0.02	5.9 ± 0.06	4.4 ± 0.12	2.2 ± 0.07	1.5 ± 0.13	8.1 ± 0.03
Local	4.1 ± 0.10	1.7 ± 0.10	2.2 ± 0.10	8.0 ± 0.01	10.2 ± 0.02	2.6 ± 0.01	2.6 ± 0.01	15.3 ± 0.00
50207	6.7 ± 0.06	0.7 ± 0.01	1.0 ± 0.05	8.3 ± 0.02	3.8 ± 0.11	2.0 ± 0.28	1.5 ± 0.05	7.23 ± 0.22
Root sample								
30572	0.4 ± 0.01	0.2 ± 0.01	0.1 ± 0.02	0.7 ± 0.02	0.3 ± 0.01	0.1 ± 0.00	0.1 ± 0.00	0.6 ± 0.00
4(2)1425	0.3 ± 0.01	0.1 ± 0.02	0.3 ± 0.01	0.7 ± 0.01	0.6 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	1.0 ± 0.02
Local	0.7 ± 0.02	0.1 ± 0.01	0.2 ± 0.02	1.0 ± 0.01	0.8 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	1.2 ± 0.01
50207	0.4 ± 0.01	0.1 ± 0.00	0.1 ± 0.01	0.7 ± 0.01	1.1 ± 0.01	0.3 ± 0.03	0.2 ± 0.03	1.6 ± 0.01