Ife Journal of Agric. Vol. 7 Nos. 1 & 2 (1985)

The metabolism of Dhurrin in Sorghum seedlings 1: Distribution, biosynthetic site and function

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Abstract

The variation of dhurrin in various parts of sorghum seedlings was monitored over a period of 28 days. The results showed that a peak of 2.8 umol was reached in the shoot on the 8th day while in the root, it was only 0.24 umol on the 7th day. Dhurrin concentration reduced with age and size of seedlings.

Labelling studies with ^{14}C-shikimic acid showed that all the tissues synthesized and stored dhurrin; above 90% of the radioactivity was found in the dhurrin of the root. Different parts of sorghum seedlings showed ability to synthesize dhurrin from ^{14}C-tyrosine. *In vitro* synthesis of dhurrin by microsomal fractions of the different parts also confirmed this.

Analysis of the dhurrin content of the first, second and third leaves showed a fair distribution in the first leaf. The second and third leaves however showed a gradient from the apex to the stalk.

The results are discussed on the basis of dhurrin distribution, biosynthetic capability of the parts and function.

Introduction

Sorghum seedlings were first known to be cyanogenic in the 19th century when the transport animals of General Kitchener passing through the Sudan got poisoned after eating freshly sprouting seedlings later shown to be 'Sudan grass' cultivar of *Sorghum spp.* Since that period, intensive research has been carried out on different aspects of cyanogenesis in sorghum and other plants.

The precursor product relationship of dhurrin in sorghum has been worked out by Conn (1980). The *in vitro* biosynthsis was demonstrated by McFarlane *et al* (1975) while the cellular and subcellular localizations and the metabolic enzymes involved in the synthesis, were shown by Kojima *et al* (1979), Thayer and Conn (1981) and Wurtele *et al* (1982). Agronomists have investigated the cyanide potential and the influence of environmental factors on it, in many cultivars of sorghum. Geneticists have also studied the genetic basis for the production of dhurrin (Loyd and Gray, 1970; Harms and Tucker, 1972; Conn, 1980).

Despite these intensive research activities, some questions still remain unanswered. For instance, is the biosynthesis of dhurrin centralised and the product distributed to other parts for storage as in *Manihot esculentus Crantz?* What is the synthetic capability of the different parts of the sorghum seedling, and finally what is the role of dhurrin in the seedlings? This investigation attempts to answer some of these questions.

Materials and Methods

Plant culture

Two varieties of sorghum *(Sorghum bicolor (Linn) Moench)* were used. Seeds of some were obtained from Northrup King and Co, Lubbock, Texas U.S.A. and the other from the local market at Ile-Ife, Nigeria. The seeds of the Texas variety were soaked in aerated water for 24 hours before being planted in vermiculite. They were then transferred into a growth chamber and left to grow at 26 : 22^0 C(Day/Night temperature) and 16:8 hr (light: dark) photoperiod. The local cultivar was planted on a wire mesh covered with a cotton gauze, kept in a tray of water, and allowed to germinate in the green house.

Various parts of sorghum seelings were analysed for dhurrin content. The various leaves were cut into approximately equal sections, weighed and analysed for dhurrin content. Dhurrin was estimated by the method of Gorz *et al* (1977).

Administration of radioactive compounds.

^{14}C-shikimic acid (specific activity 84 mCi) was obtained from Radiochemical Centre, Amersham while L-(U-^{14}C)- tyrosine (specific activity 410 mCi) was from ICN pharmaceuticals.

(i) 2 uCi ^{14}C-shikimic acid in 5 ml 400 uM cold shikimic acid was fed to 25 4–day old sorghum seedlings through the root for 48 hours. Thereafter, the roots were washed thoroughly and the radioactivity in the solution was counted by liquid scintillation. The different parts of the seedling were analysed for radioactive dhurrin.

(ii) 2 uCi ^{14}C-tyrosine in 5 ml 0.1– cold tyrosine was fed to 25 4-day old sorghum seedlings through the root for 48 hr. Both shoot and rot were analysed for radioactive dhurrin. In another experiement, 25 4-day old sorghum seedlings were derooted under water and fed with 1 mCi^{14}C-tyrosine for 48 hours after which the seedlings were analysed for radioactive dhurrin.

(iii) Different parts of 4-day old sorghum seedlings were excised under water and incubated with 0.2 uCi ^{14}C-tyrosine for 6 hours. These were washed thoroughly in water and analysed for radioactive dhurrin.

(iv) Microsomal fractions of different parts of 4—day old seedlings were prepared by the method of McFarlane *et al* (1975) and incubated with 0.20 uCi ^{14}C-tyrosine for 1 hr. Labelled dhurrin, its intermediate metabolites: p-hydroxyphenylacetaldoxime and p-hydroxyphenylacetonitrile, and products: p-hydroxybenzaldehyde and p-hydroxybenzoic acid, were determined.

Dhurrin in sorghum seedlings and parts was heat-hydrolysed by the method of Gorz *et al* (1977). The solution was concentrated in a rotary evaporator and extracted 3 times with 2 ml diethyl ether (1 ml in the case of the microsomal fractions). The extract was reduced in volume and spotted on Bakerflex Silica gel 1B and chromatographed in 5:1 benzene: ethyl acetate solution for 3—4 hours. The TLC plates were air-dried, sprayed with 0.2% solution of 2',7'dichlorofluorescein and visualized under UV-light. The plates were also radio-scanned using a Packard model 7201 radiochromatogram. The radioactive peaks were cut, eluted and counted by liquid scintillation.

All experiments were repeated at least once and average values presented.

Results

Figure 1 shows the variation in dhurrin content of sorghum seedlings. The shoot reached a peak of 2.80 umol on the 8th day, decreased gradually to 2.12 umol on the 18th day and later increased to 2.73 umol on the 24th day. The stalk reached a peak of 1.40 umol dhurrin on the 6th day and decreased gradually to 0.72 umol on the 28th day. Dhurrin content of the first leaf increased gradually to 0.65 ± 0.05 umol on the third day and remained fairly constant thereafter. A peak of 0.95 umol was reached in the second leaf on the 7th day, decreased gradually till the 10th day and then remained constant. Fig. 2 shows the variation of dhurrin in the root. It increased gradually from the second day to reach a peak of 0.24 umol on the 7th day and decreased to 0.20 umol on the 10th day.

Table 1 shows the distribution of dhurrin in leaves of the Texas variety of sorghum seedlings while Table 2 shows that of the Nigerian cultivar on the 9th day. In both cases, the concentration of dhurrin was fairly constant in the first leaf but varied in the second and third leaves. Table 3 shows the distribution of labelled dhurrin in sorghum

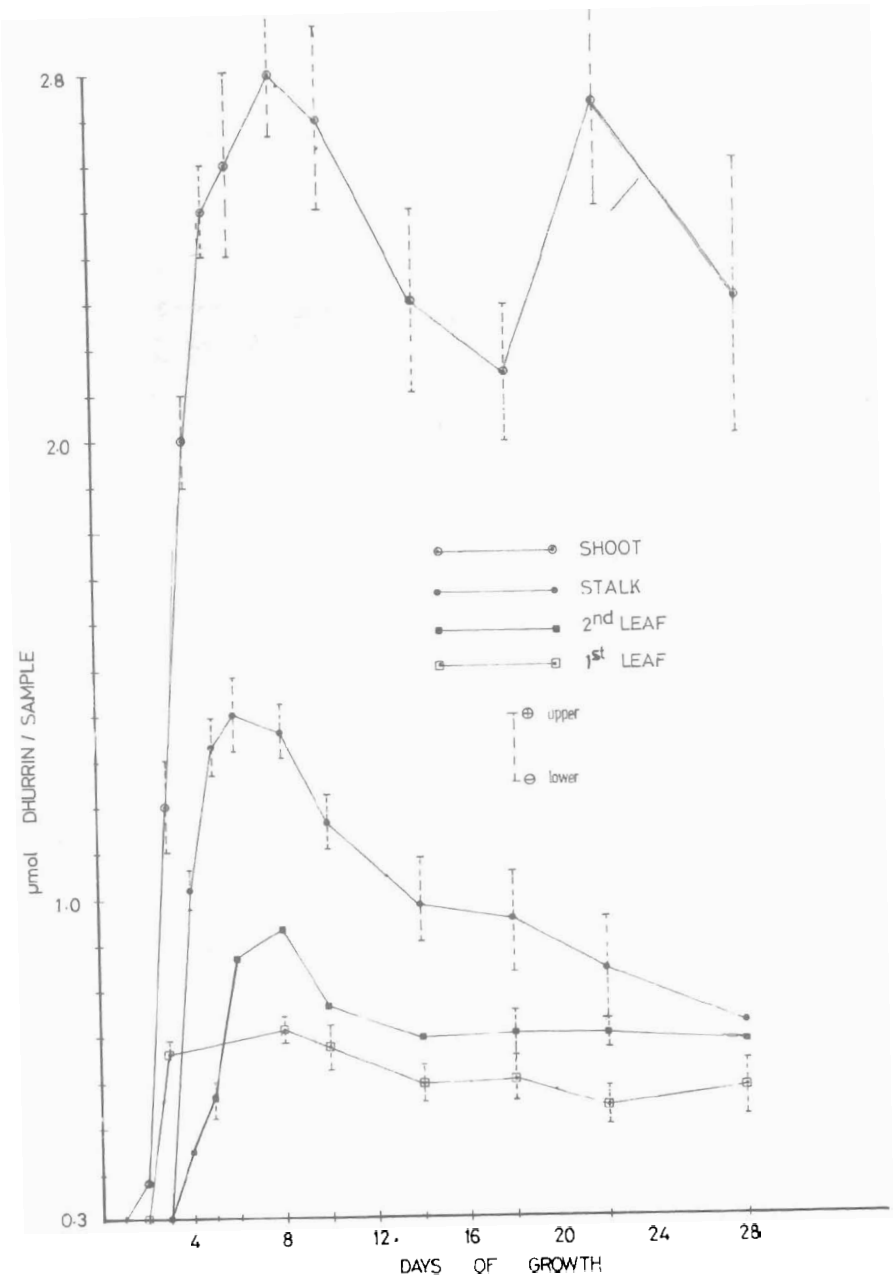

FIG. 1: Variation of the dhurrin content of *Sorghum bicolor* seedlings with age.

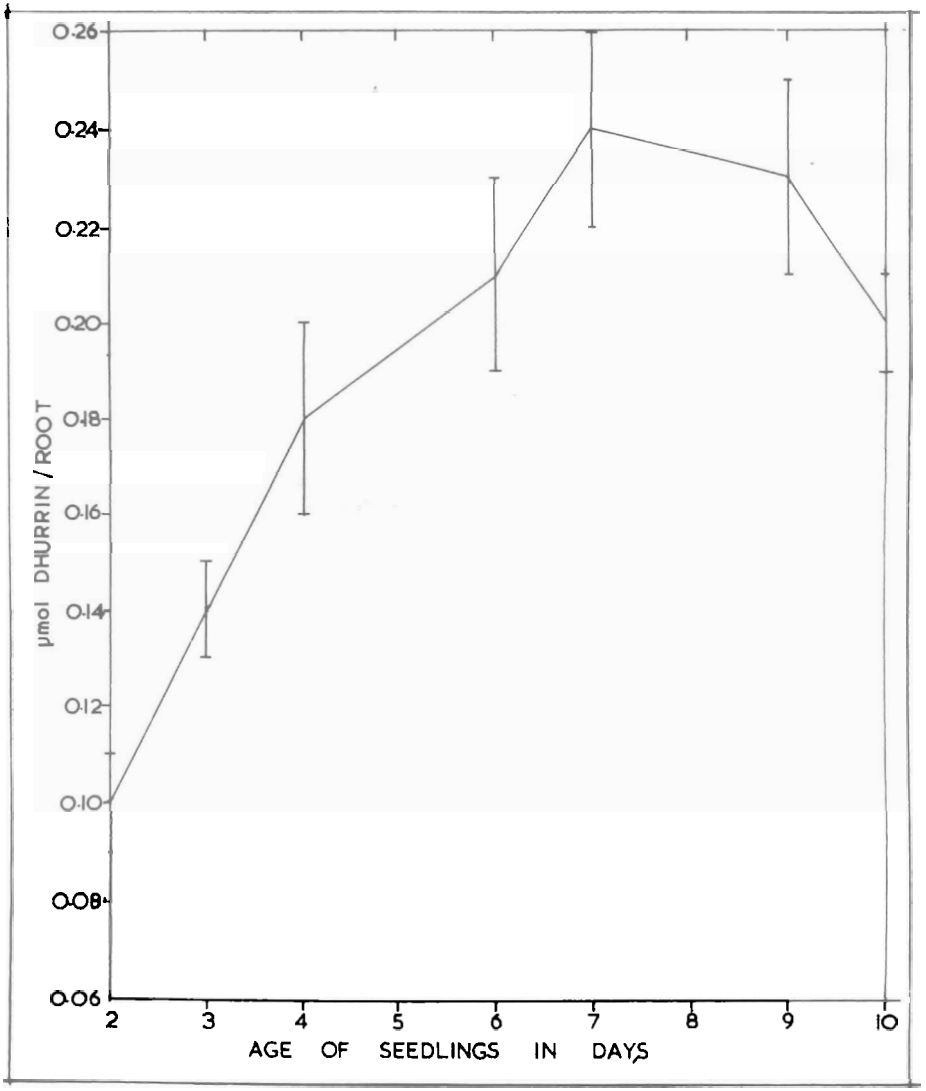

FIG. 2: Variation of the dhurrin content of the root of *sorghum bicolor* seedlings with age.

TABLE 1: DISTRIBUTION OF DHURRIN IN THE LEAVES OF SORGHUM BICOLOR SEEDLINGS*
HCN (ppm) at different samples dates ± SD

Plant part sampled		Days 4		5		6		8		14	
1st leaf	Apex section	1,650	52	1,697	200	1,643	130	1,628	150	1,670	100
	Leaf stalk section	1,526	106	1,530	144	1,550	90	1,540	100	1,562	80
2nd leaf	Apex section	–	–	1,007	82	1,043	41	1,065	69	922	88
	Leaf stalk	–	–	713	74	767	74	696	76	589	70
3rd leaf	Apex section	–	–	–	–	–	–	–	–	323	11
	Leaf stalk section	–	–			–			–	108	5

*1 The leaves were divided into 2 roughly equal parts. in duplic

TABLE 2: DISTRIBUTION OF DHURRIN IN THE FIRST, SECOND AND THIRD LEAVES OF 9-DAY OLD SORGHUM SEEDLINGS.

Leaf Section	HCN (ppm) ± SD					
	1st leaf		2nd leaf		3rd leaf	
1 (Apex)	2,848	200	2,125	100	2,073	80
2	2,905	150	1,545	98	1,889	120
3	2,551	250	1,277	150	1,819	60
4	2,650	120	756	60	1,200	80
5 (Base)	2,460	100	524	40	984	30

TABLE 3: DISTRIBUTION OF RADIOACTIVITY IN DHURRIN FROM DIFFERENT PARTS OF SORGHUM SEEDLINGS FED ^{14}C SHIKIMIC ACID THROUGH THE ROOTS.

	(Dpm total)
Roots	130,390
2nd leaf blade	8,971
1st leaf blade	4,860
Coleoptile + hypocotyl	4,681
3rd leaf (still embedded in the coleoptile)	1,548

seedlings fed 2 uCi ^{14}C-shikimic acid. The root contained over 90% of the ^{14}C-labelled dhurrin followed by the second leaf, first leaf and the stalk while the unexposed third leaf contained a little but significant amount of labelled dhurrin. Table 4 shows the conversion of ^{14}C-tyrosine to dhurrin by different parts of sorghum seedlings. All parts have about the same ability to synthesize and accumulate dhurrin. Table 5 shows that the mirosomal fractions of the seedlings' different parts (except the root) are capable of synthesizing dhurrin and its intermediates *in vitro*.

TABLE 4: CONVERSION OF ^{14}C TYROSINE TO DHURRIN BY DIFFERENT PARTS OF SORGHUM SEEDLINGS

Plant tissue	^{14}C-tyrosine taken up (Dpm)	Dhurrin (Dpm)	% Conversion
Whole shoot	2.23×10^5	2,808	1.3
1st leaf + sheath	10.3×10^5	5,455	0.5
1st leaf blade only	3.3×10^5	1,545	0.5
Coleoptile + hypocotyl	0.86×10^5	430	0.5
2nd leaf + sheath	6.36×10^5	338	0.5

TABLE 5: IN VITRO SYNTHESIS OF DHURRIN BY MICROSOMAL FRACTIONS OF VARIOUS PARTS OF THE SHOOT OF SORGHUM SEEDLINGS

	Whole seedling	Coleoptile + hypocotyl	1st leaf	2nd leaf
P-hydroxyphenylacetaldoxime*[2]	14,738	34,991	50,796	8,774
P-hydroxyphenylacetonitrile*[2]	8,878	10,556	16,775	1,845
P-hydroxybenzaldehyde*[3]	47,658	52,691	29,999	2,371
P-hydroxybenzoic acid*[4]	20,123	10.276	11,592	626

*[2] P-hydroxyphenylacetaldoxime and p-hydroxyphenylacetonitrile are established intermediate metabolites in the biosynthesis of dhurrin in Sorghum seedlings.

*[3] P-hydroxybenzaldehyde is the hydrolytic product of dhurrin.

*[4] P-hydroxybenzoic acid is the oxidation product of p-hydroxybenzaldehyde. This reaction occurs spontaneously *in vitro* (Ref. 2).

Discussion

The result of the present study on dhurrin content of sorghum seedlings is in agreement with the findings of Loyd and Gray (1970) who worked on 3 cultivars of sorghum. Our result shows that the concentration of dhurrin in the shoot decreases with age while it remained fairly constant in the first leaf. A similar result was also obtained by Gorz *et al* (1977).

Analysis of the first, second and third leaves shows that dhurrin is only uniformly distributed in the first leaf but decreases from the apex to the base in other leaves. The distribution of dhurrin in this pattern may be serving a protective role against grazing. This cyanogenic glycoside – bitter and poisonous – is concentrated in the leaf apices which are easily accessible to grazing animals. Jones (1962) has also shown that the cyanogenic glycoside of *Lotus corniculatus L.* has a deterrent effect on grazing molluscs and voles.

The level of cyanogenic glycoside in parts of sorghum seedling does not necessarily indicate the organs that are responsible for dhurrin production. Results presented in Tables 4, 5, and 6 show that ^{14}C-shikimic acid and ^{14}C-tyrosine were converted to dhurrin in all tissues.

TABLE 6: FRESH WEIGHT AND HCN-POTENTIAL OF THE SHOOT, FIRST AND SECOND LEAVES OF SORGHUM SEEDLINGS WITH AGE

Age Days	Shoot Fresh Weight (mg)	Shoot HCN (ppm) ±	SD	1st leaf Fresh weight (mg)	1st leaf HCN (ppm) ±	SD	2nd leaf Fresh weight (mg)	2nd leaf HCN (ppm ±	SD
2	7.5	1371	123	–	–		–	–	
3	23.9	1405	108	10.5	1696	74	–	–	
4	47.9	1138	41	10.8	1517	66	8.8	1386	81
5	70.1	967	31	10.4	1450	115	17.1	891	32
6	75.5	943	68	9.5	1724	49	25.3	929	91
8	106.5	716	36	10.9	1772	67	31.8	786	39
10	113.0	655	44	10.6	1707	124	27.9	733	70
14	140.3	450	42	9.7	1641	120	25.9	723	28
18	176.4	329	23	9.4	1719	139	26.9	705	47
22	217.1	340	32	7.5	1935	127	23.0	828	31
28	292.6	204	27	8.6	1813	198	22.8	671	50

The microsomal fractions of these tissues also synthesized dhurrin from ^{14}C-tyrosine *in vitro*. Clegg *et al* (1979) reported that the linamarin content of the seeds of wild Costa Rican lima beans was translocated to the seedling during germination and growth. This was confirmed by th presence of linamarin in the various tissues and the inability of these tissues to carry out *de novo* synthesis. Bediako *et al* (1980) observed that all tissues of cassava convert ^{14}C-valine to linamarin. The rate of conversion by the root is very small compared to its labelled linamarin content. They found that translocation of linamarin occurred from the leaves to the tuber and from senescent leaves to young ones in the absence of a tuber. Does translocation of dhurrin occur from the shoot to the root in sorghum seedling as in cassava? This cannot be so, for the following reasons:

1. The total dhurrin content of the root is small: 0.24 umol compared to 2:8 umol in the shoot.
2. The root synthesizes more dhurrin from ^{14}C-shikimic acid thar. the shoot.

Does translocation then take place from the root to the shoot? Again, this is not likely because:

1. The shoot is capable of synthesizing dhurrin *in vivo* and *in vitro*.
2. Biosynthesis of dhurrin is a highly channelled process in sorghum seedlings and only 2-3% of the exogenously administered precursors is converted to dhurrin by the shoot (Bough and Gander, 1971; Adewusi, 1983). This may account for the low ^{14}C-labelled dhurrin found in the shoot.
3. Derooted seedlings synthesized dhurrin from ^{14}C-tyrosine.
4. Compartmentalization of dhurrin and its metabolic enzymes demonstrated by Conn, (1980) and Wurtele *et al,* (1982) tend to imply that dhurrin is stored where it is synthesized.

Acknowledgement

Part of this work was carried out in the Department of Biochemistry and Biophysics, University of California, Davis, under the auspices of Dr. E.E. Conn to whom I am very grateful. Part of the investigation was supported by grant USPHS GM-05301 (to E.E.C.). I acknowledge the invaluable help of Dr. E. S. Wurtele in the preparation of microsomal fractions and Dr. Kunle Adelusi for a critical review of the manuscript.

References

Adewusi, S.R.A. 1983. Some aspects of cyanogenic glycoside metabolism in plants and animals. Ph.D. thesis, University of Ife. 242pp.

Bediako, M.K.B., Tapper, B.A. and Pritchard, G.G. 1980. Metabolism, synthetic site and translocation of cyanogenic glycosides in cassava. pp. 143-148 *In* E. R. Terry, K. A. Oduro and F. Caveness (Eds.) *Tropical root crops: Research strategies for the 1980s. Proceedings of the first triennial root crops Symposium of the International Society for Tropical Root Crops, Africa Branch.* IDRC Ottawa Canada.

Bough, W.A. and Gander, J. E. 1971. Exogenous L-tyrosine metabolism and dhurrin turn-over in sorghum seedlings. *Phytochem., 10: 67-77.*

Clegg, D. O., Conn, E.E. and Janzen, D.H. 1979. Developmental fate of the cyanogenic glucoside—linamarin - in Costa Rican wild lima bean seeds. *Nature,* 278: 343-344.

Conn, E.E. 1980. Cyanogenic glycosides pp. 461-492 *In* E.A. Bell and B.V. Charlwood (Eds.) *Encyclopedia of Plant Physiology New Series Vol. 8. Secondary Plant Products.* Springer-Verlag, Berlin.

Gorz, H. J., Haag, W.C., Specht, J E. and Haskins, F.A. 1977. Assay of p-hydroxybenzaldehyde as a measure of hydrocyanic acid potential in *Sorghum. Crop Sci.*, 17: 578–582.

Harms, C. L. and Tucker, B.B. 1973. Influence of nitrogen fertilization and other factors on yield, prussic acid, nitrate and total nitrogen concentration of Sudan grass cultivars. *Agron. J.* 65: 21–26.

Jones, D. A. 1962. The selective eating of the acyanogenic form of the plant *Lotus corniculatus* L. by various animals. *Nature,* 193: 1109–1110.

Kojima, M., Poulton, J.E., Thayer, S.S. and Conn, E.E. 1979. Tissue distributions of dhurrin and of enzymes involved in its metabolism in leaves of *Sorghum bicolor. Plant Physiol.*, 63: 1022–1028

Loyd, R.C. and Gray, E. 1970. Amount and distribution of hydrocyanic acid potential during the lifecycle of plants of three sorghum cultivars. *Agron. J.*, 62: 394–397.

McFarlane, I. J., Lees, E.M. and Conn, E.E. 1975 The *in vitro* biosynthesis of dhurrin, the cyanogenic glycoside of *Sorghum bicolor. J. Biol. Chem.*, 250 . 4708–4713.

Thayer, S.S. and Conn, E.E. 1981. Subcellular localization of dhurrin β-glucosidase and hydroxynitrile lyase in the mesophyll cells of *sorghum* leaf blades. *Plant Physiol., 67: 617–622.*

Wurtele, E.S., Thayer, S.S. and Conn, E.E. 1982. Subcellular localization of UDP-Glucose: Aldehyde cyanohydrin B-glucosyl transferase in epidermal plastids of sorghum leaf blades. *Plant Physiol., 70: 1732–1735.*