THE BIOGENESIS AND METABOLISM OF CYANOGENIC GLUCOSIDES IN GERMINATING CASSAVA SEED AND SEEDLINGS

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SUMMARY

Analyses of the storage lipids (47%) and proteins (34%) of cassava seeds indicate that these could be nutritionally and industrially useful sources of vegetable fats and proteins. Seedlings have high lipolytic and proteolytic activities. In seedlings storage lipids are converted to carbohydrates. Valine and Isoleucine are incorporated respectively into the cyanogenic glucosides linamarin and lotaustralin. Seedlings of both bitter and sweet cultivars contain large amounts of cyanogenic glucosides. Electronmicroscope and tracer studies showed that the biosynthesis and metabolism of cyanogens, cyanide, proteins and lipids are associated with specific organelles and microbodies which become apparent after 10 days active seed germination.

RESUME

Les analyses du contenu de lipides (47%) et de proteines reservées (34%) ont montré que les graines de la cassave forment une source de graisses et de proteines végétales utilisables en industrie. Les germes portent une grande activité de lipolyse et de proteolyse, reforment les lipides reservées en carbohydrates, et ils peuvent incorporer la valine et l'isoleucine dans les glycosides cyanogénes linamarine et lotaustraline, respectivement. La microscopie électronique et l'utilisation des substrats radioactifs ont montré que la biosynthèse et la metabolisme de substances cyanogénes, du cyanure, des proteines et des lipides sont liées a des organelles specifiques et a des 'microbodies' qui se développent clairement au cour de 10 jours de germination active de graines.

RESUMEN

Los análisis de las reservas de lípidos (47%) y de proteínas (34%) en semillas de yuca, indican que podrian ser nutricional e industrialmente fuentes útiles de grasas vegetales y proteínas. Las plantas tienen actividades lipolíticas y proteolíticas elevadas; en ellas las reservas de lípidos son convertidas en carbohidratos. La Valina y la Isoleucina se incorporan respectivament a los glucósidos cianogénicos linamarina y lotaustralina. Las plántulas tanto de cultivos agrios como dulces, contienen grandes cantidades de glucósidos cianógenicos. La microscopía electrónica y los estudios con trazadores demostraron que la biosíntesis y metabolismo de cianógenos, ciaminas proteínas y lípidos seasocian con organelos y corpúsculos específicos que se hacen aparentes a los 10 días de la germinación de la semilla activa.

INTRODUCTION

The potential, and ultimate usefulness of cassava and all its products are expressions of genetic, biochemical and physiological processes and mechanisms we are investigating in Denmark.

CONSTITUENTS OF CASSAVA SEED

Localization of lipids and proteins

The cassava seed kernel contains 47% lipids and 34% proteins as major reserves, and 0.13% soluble nitrogenous compounds, 0.3% starch and 3.8% soluble carbohydrates as minor constitutents[17]. Thus the major constituents of the cassava seed compare favourably with those of typical useful oil seeds such as those of *Ricinus, Sesamum, Elaeis* and *Glycine*[3].

The bulk of cassava seed storage lipids and proteins are localized in the endosperm, although cotyledonary and radicle tissues show a similar distribution of storage materials. Figures 1 and 2 are electronmicrographs of thin sections through the endosperm and radicle tissues of a dry cassava seed. They show

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cells filled with large lipid bodies (spherosomes) and protein bodies (aleurone grains), as well as some cytoplastic organelles.

Lipid and protein composition

Triglycerides comprise 98% of the total cassava seed lipids, with di- and monoglycerides, phospholipids, glycolipids and steroids constituting only 2%. This is a similar distribution to that in most important oil-bearing seeds[18].

Cassava seed fats are characterized by a high content of linoleic, oleic and palmitic acids which occur as their triglycerides. Table 1 shows the fatty acid composition of the total lipids and triglycerides of cassava seed kernel[20].

Cassava seed proteins are largely salt-soluble, and may readily be obtained as concentrates or meals free from lipids, and with high amino acid content. Table 2 shows the concentrations of total amino acids (protein-bound and free) of cassava seed kernels expressed as mg amino acid per 100g dry tissue, and as g

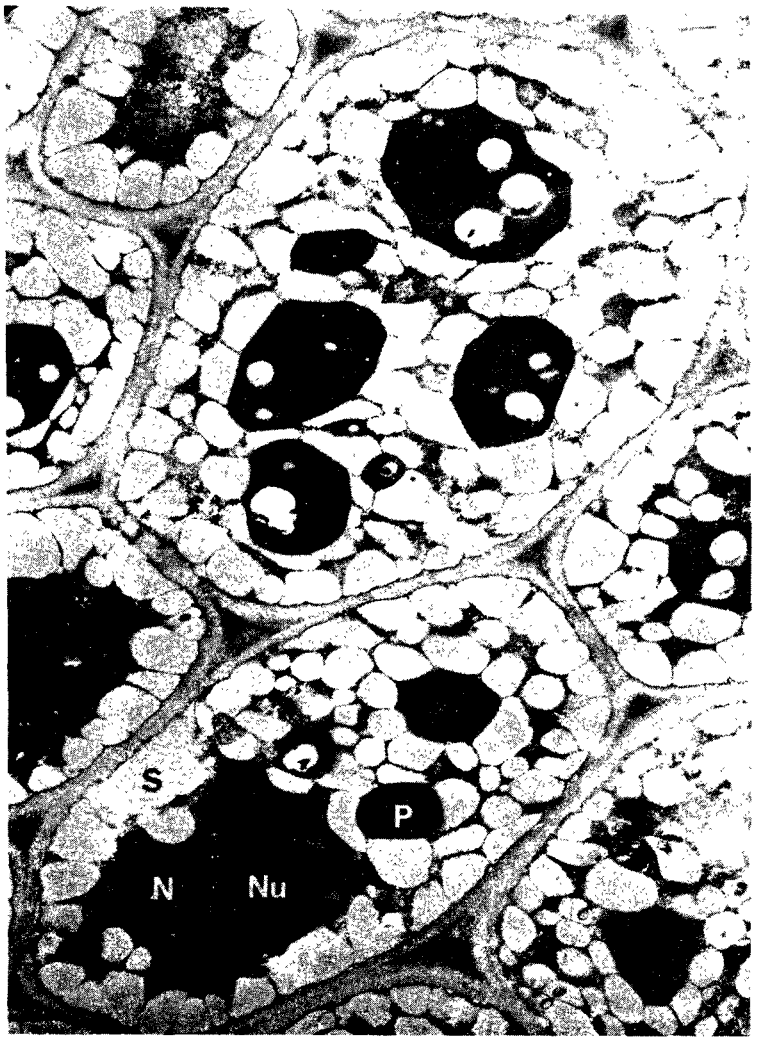

Figure 1. *Electronmicrograph of a thin section through the endosperm of a dry cassava seed. The cells are filled with lipid bodies (Spherosomes, Sp) and protein bodies (Aleurone Grains, AG). Nucleus (N), Nucleolus (Nu). Sections of seed tissues were fixed in 6% glutaraldehyde in phosphate buffer pH 7.2, and post-fixed in 2% osmium tetroxide. x 14,000.*

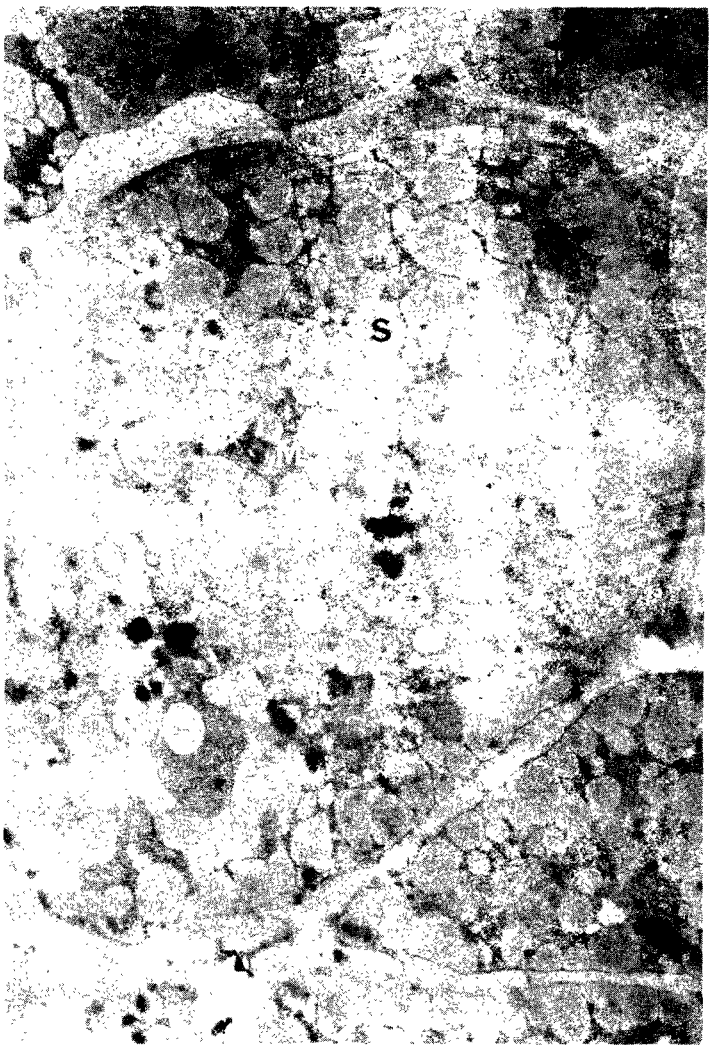

Figure 2: *Electronmicograph of a thin section through the radicle of a dry mature cassava seed. The cells contain large amounts of lipid bodies (Spherosomes, Sp). Cytoplasmic and Organelle Membranes are poorly defined. Nucleus (N), Plastid (Pl), Mitochondrion (M). x 14,000.*

amino acid per 16g nitrogen. A comparison of the amino acid profiles of cassava seeds, leaves and tubers[19,23] shows that seeds contain higher levels of both essential and non-essential amino acids, and therefore better quality proteins. Cassava seed proteins may therefore be of potential nutritional use in cassava-growing countries.

The low starch content of the cassava seed kernel is accompanied by a relatively high level of soluble sugars almost entirely composed of sucrose.

Cyanogenic glucosides

Generally, variations in the concentration of cyanide in cassava root tubers, as well as the morphological characteristics of the plants, form the basis of a taxonomic differentiation between the bitter (high cyanide content) and the sweet (low cyanide content) cultivars[22]. This basis for delineation does not offer an adequate means for differentiating all cassava cultivars; it implies that tissues of all cassava cultivars are potentially cyanophoric. Seeds of cassava cultivars generally characterized as sweet do not contain cyanogenic glucosides, whereas those of bitter cultivars contain low levels of cyanogenic materials. Table 3 shows the concentrations of cyanogenic glucosides in seeds and other tissues of sweet and bitter cultivars of cassava.[16]

CHANGES IN THE MAJOR CONSTITUENTS DURING GERMINATION AND GROWTH

Conversion of lipids to carbohydrates

In seeds beginning to germinate in the dark, total cassava seed storage lipids remains largely unchanged. After about 4 days of germination, total lipids decrease gradually, and then rather steeply. Dormant cassava seeds have weak lipase activity which increases several fold during active germination, with the resultant formation of free fatty acids. As free fatty acids accumulate, the activities of enzymes involved with the B-oxidation of the fatty acids also increase several fold. The over-all results are that large amounts of storage lipids are mobilized and converted to non-lipid materials. The rate of lipid degradation is remarkably higher in light-grown seedlings than in etiolated seedlings[18]. Figure 3 shows the changes in the concentration of total lipids as a function of growth condition and period. These changes are accompanied by both qualitative and quantitative variations in the composition of fatty acids (Table 4). From Table 4 it can be seen that although etiolated and light-grown seedlings degrade storage lipids at different rates that no specific fatty acids are preferentially metabolized[20].

As the lipid concentration falls, the carbohydrate levels undergo striking changes. Figures 4 and 5 show variations in the concentrations of starch and soluble sugars as a function of growth condition and period. The variations in the concentrations of starch and soluble sugars are more pronounced in etiolated seedlings, where sucrose is the major carbohydrate. This provides evidence for the operation of a fat to carbohydrate conversion in cassava through sucrose. A similar mechanism operates in some other oil seeds such as *Ricinus* through the glyoxylate cycle in which cassava seedlings rapidly convert storage lipids to sucrose, which is readily channelled into starch synthesis[1,8,9].

Mobilization of amino acids from storage proteins

One of the earliest metabolic events in germinating cassava seeds involves the degradation of storage proteins. Electronmicroscopic studies indicate that reserve proteins disappear at a faster rate than lipids. This is illustrated in Figure 7 which shows variations in the concentration of proteins in dark and light-grown seedlings. Data in Figure 6 show that imbibed seeds rapidly lose proteins within 8 days of germination. During this period, there is a high increase in the activities of proteolytic enzymes, as shown by experiments with seedling extracts. Except for a transient period, light-grown seedlings generally contain higher levels of proteins.

CYANOGENSIS IN CASSAVA

As shown in Table 3, seeds of sweet cassava cultivars do not contain cyanogenic glucosides, and are therefore non-cyanophoric. On the other hand, seeds of bitter cassava cultivars contain low levels of cyanogenic glucosides, and are therefore cyanophoric. However, 10-day-old seedlings of both bitter and sweet cultivars synthesize and accumulate large amounts of cyanogenic glucosides, namely, linamarin and lotaustralin (methyllinamarin). Linarmarin, 2(B-D-glucopyranosyloxy) isobutryronitrile, accounts for 93%, while lotaustralin, 2(B-D-glucopyranosyloxy) 2-methylbutyronitrile, accounts for 7% of the total cyanogens in cassava. Cyanogenesis in cassava therefore involves enzyme-catalysed hydrolysis of linamarin and lotaustralin with the resultant release of hydrogen cyanide from these glucosides. The structures and hydrolytic products of linamarin and lotaustralin are illustrated in Figure 7.

The hydrolysis of linamarin and lotaustralin is catalysed by linamarase, a β-glucosidase which is very active in all tissues of the growing plant; young cassava seedlings and leaves are particularly rich sources of the enzyme[13]. Other cyanophoric plant genera such as *Trifolium*, *Linum*, *Lotus* and *Phaseolus* which also contain linamarin and lotaustralin, contain the same enzyme; however, some *Trifolium* species may contain the glucosides but not the enzyme[6,10].

ULTRASTRUCTURAL DEVELOPMENTS ASSOCIATED WITH METABOLIC CHANGES

As shown in Figures 1 and 2, the endorsperm, cotyledonary and radicle tissues of resting cassava seeds are filled mainly with large fat bodies (spherosomes) and protein bodies (aleurone grains), both of which are membrane-bound reserves utilized during germination and growth. The fat bodies are electrontransparent, while the protein bodies are electrondense. Few cytoplasmic organelles such as mitochondria, proplastids, Golgi apparatus, endoplasmic reticulum and microbodies are present. This state is one of low cellular organization and low metabolic activity, and it is typical of the state of dormancy.

The onset of active germination (ca. 4 days after imbibition) is accompanied by a gradual concurrent increase in metabolic activities and ultrastructural development. Whilst proteolysis is initiated immediately after imbibition, lipolysis, fat to carbohydrate conversion and cyanogenesis are detectable 4 days after germination. Figures 8a and 8b are electronmicrograph of a thin section through the cotyledon of a 10-day-old etiolated cassava seedling. Both fat and protein bodies are now in the process of degradation, and cytoplasmic organelles are more discernible. Figure 11 illustrates the ultrastructural and metabolic characteristics of root cells. Tightly packed fat bodies of the resting radicle are electron-dense, suggesting a higher level of unsaturation. Profiles of the endoplasmic reticulum, Golgi apparatus, mitochondria, plastids, lomasomes and microbodies are well developed. Figures 10 and 11 are electronmicrographs of thin sections through the leaf and root tissues of a 17-day-old etiolated seedling, showing the metabolic state and the fine structure of cells apparent at this time. Storage proteins are absent, and only a few fat bodies are present. The ultrastructure of the cells is much more elaborate. Several cytoplasmic organelles with well defined profiles are present, indicating a high level of organization and metabolic activity.

Figures 12 and 14 are electromicrographs of thin sections through the leaf and root tissues of a 17-day-old seedling which has received light over the last 7 days. The leaf cells contain well developed chloroplasts and some electron-dense lipid bodies. The ultrastructure and metabolic state of leaf, stem and root tissues of a mature cassava plant are illustrated in Figures 14, 15 and 16. All cells contain several cytoplasmic organelles with well defined membranes. Leaf and stem cells contain well developed chloroplasts filled with starch, and root cells contain large vacuoles also filled with starch grains.

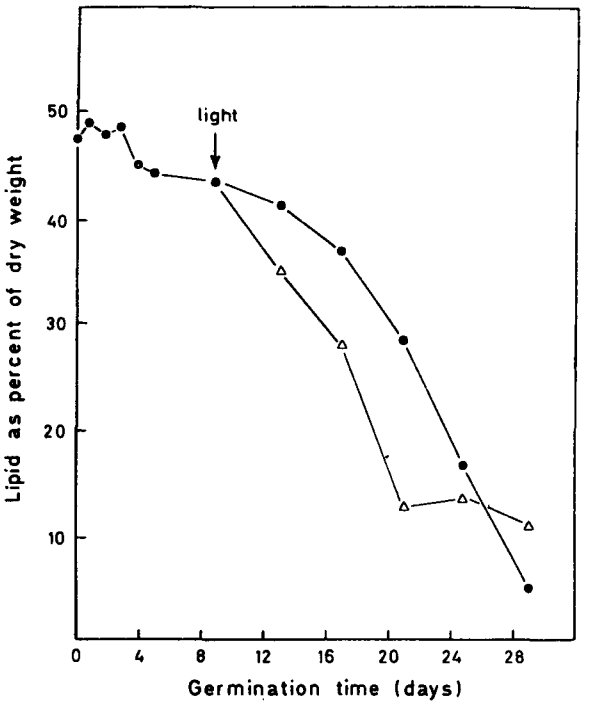

Figure 3. *Changes in the concentration of total lipids in cassava seedlings as a function of germination and growth condition and period.*

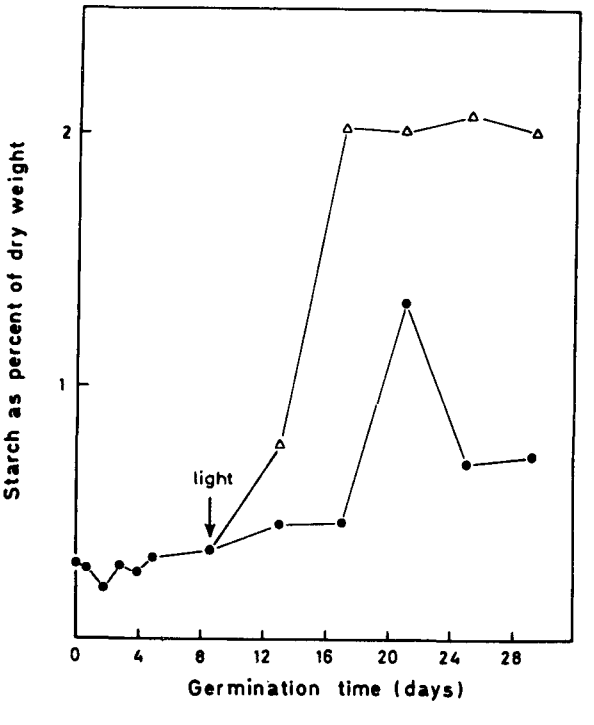

Figure 4. *Changes in the concentration of starch in cassava seedlings as a function of germination and growth condition and period.*

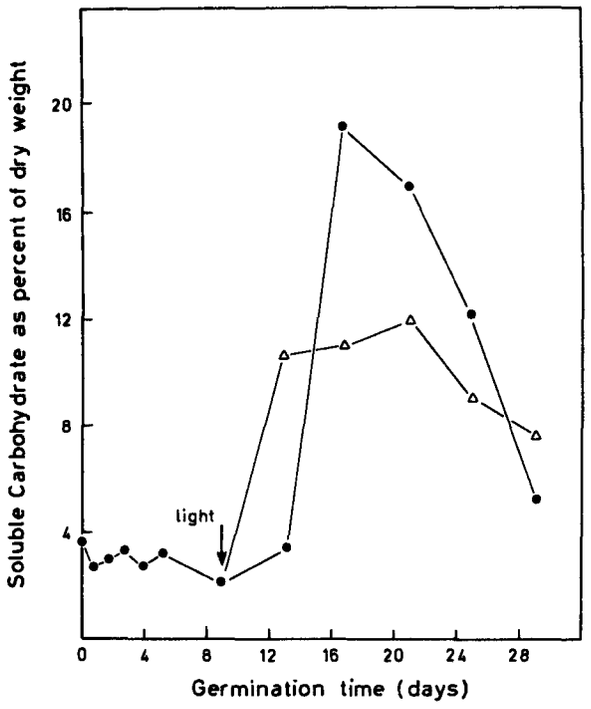

Figure 5. *Changes in the concentration of soluble carbohydrates in cassava seedlings as a function of germination and growth condition and period.*

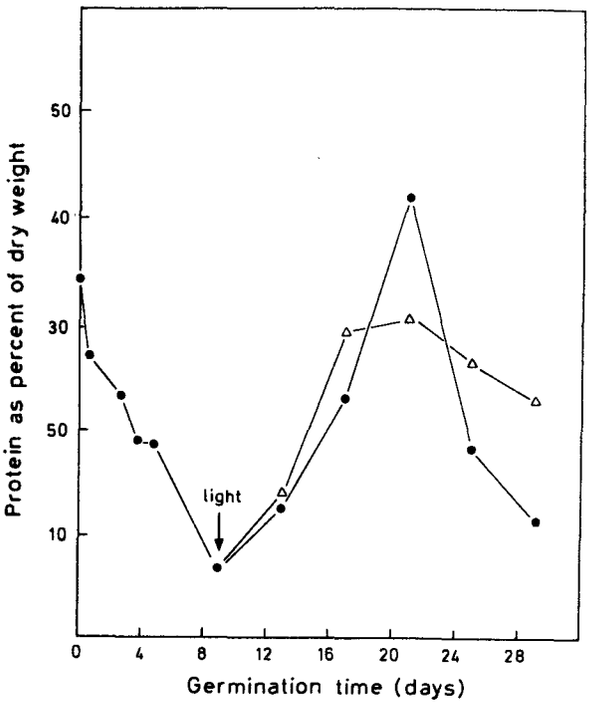

Figure 6. *Changes in the concentration of proteins in cassava seedlings as a function of germination and growth condition and period.*

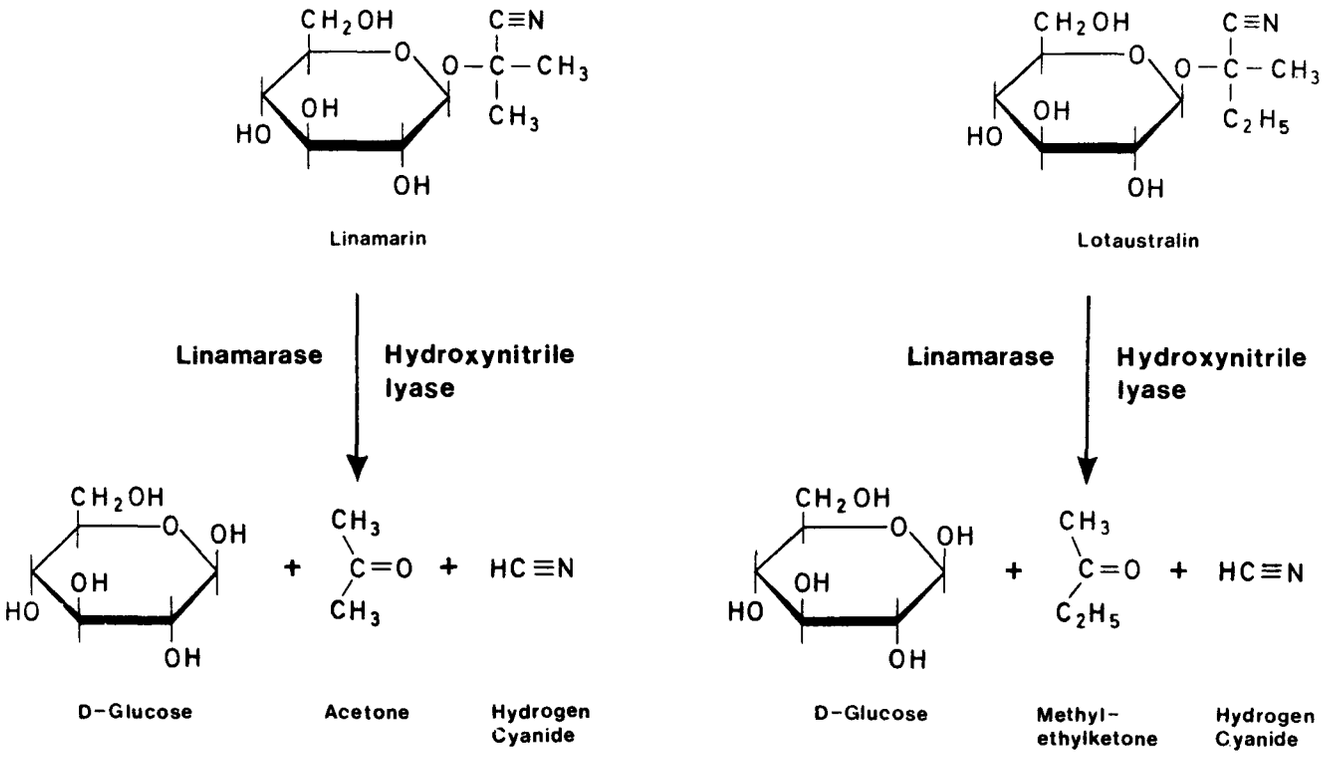

Figure 7. *The structures of linamarin and lotaustralin and their hydrolytic products.*

The overall changes in the ultrastructure and metabolic state of the cells of cassava seeds during germination and growth show striking similarities with changes that occur in the seed of castor, *Ricinus communis*. Thus, in the early germinative period, aleurone grains are rapidly hydrolysed; the active degradation of spherosomes is concurrent with the appearance of well defined organelles such as mitroghondria and glyoxysomes in endosperm tissue. The latter organelles contain enzymes of the glyoxylate cycle as well as the enzymes for the β-oxidation of fatty acids, and are therefore directly involved with the conversion of storage lipids to carbohydrates in the endosperm of germinating oil-seeds[1,8,9,24].

ULTRASTRUCTURAL DEVELOPMENT AND CYANOGENESIS

The active accumulation and degradation of linamarin and lotaustralin can be detected between 10—14 days after seed germination. Electronmicroscopic studies reveal that in 10-day-old seedlings the root cells are more highly organized than the cotyledonary cells, indicating a more active metabolic state. This suggests that the biosynthesis and degradation of cyanogenic glucosides in actively germinating cassava seeds may occur initially in the roots and be localized in specific cells or cell organelles. Autoradiographic studies are now being conducted to investigate this.

BIOSYNTHESIS OF LINAMARIN AND LOTAUSTRALIN

From structural considerations, it can be inferred that the aglucone moieties of linamarin and lotaustralin may be derived from the amino acids valine and isoleucine, respectively. Similarly, the aglucone moiety of acacipetalin may be derived from leucine, those of prunasin, sambunigrin, amygdalin and vicianin from phenylalanine, dhurrin and taxiphyllin from tyrosine, and zierin from m-hydroxyphenylalanine. These inferences have been tested by several tracer studies[6,7,14]. The mechanism of the biosynthesis involves decarboxylation of the precursor amino acid, the conversion of its α-amino group and α-carbon to a nitrile function and the hydroxylation of the β-carbon, followed by glycosylation. Figure 17 illustrates the structural relationships of some cyanogenic glucosides and their amino acid precursors[6,7].

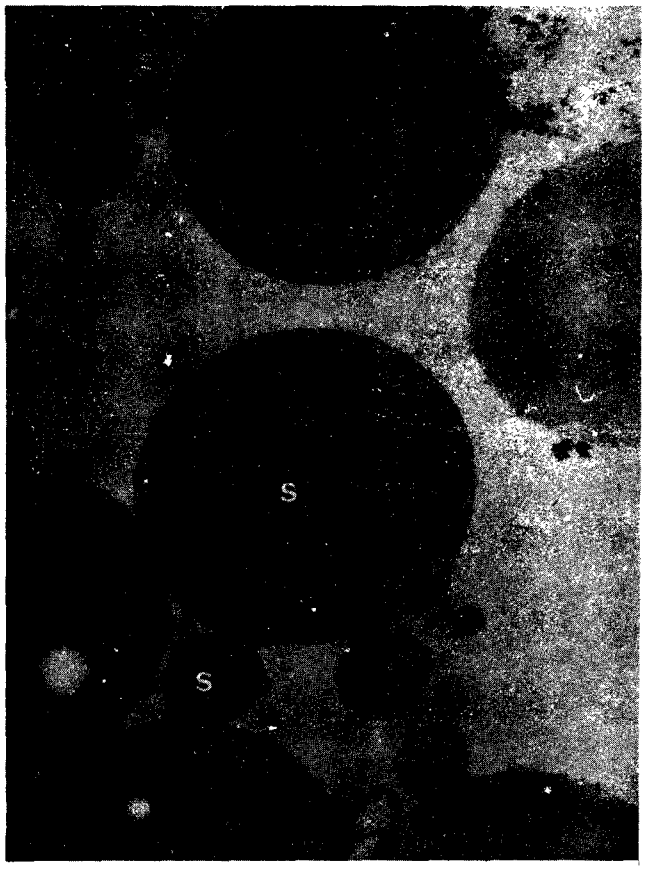

Figure 8a. *Electronmicrograph of a thin section through the endosperm of a 10-day-old etiolated cassava seedling. Spherosomes and Aleurone Grains are being rapidly degraded. Several microbodies (glyoxysomes) are present. x 28,000.*

Figure 8b. *Electronmicrograph of a thin section through the cotyledon of a 10-day-old etiolated cassava seedling. The cells are packed with lipid (Sp) and protein (P) bodies. x 14,000.*

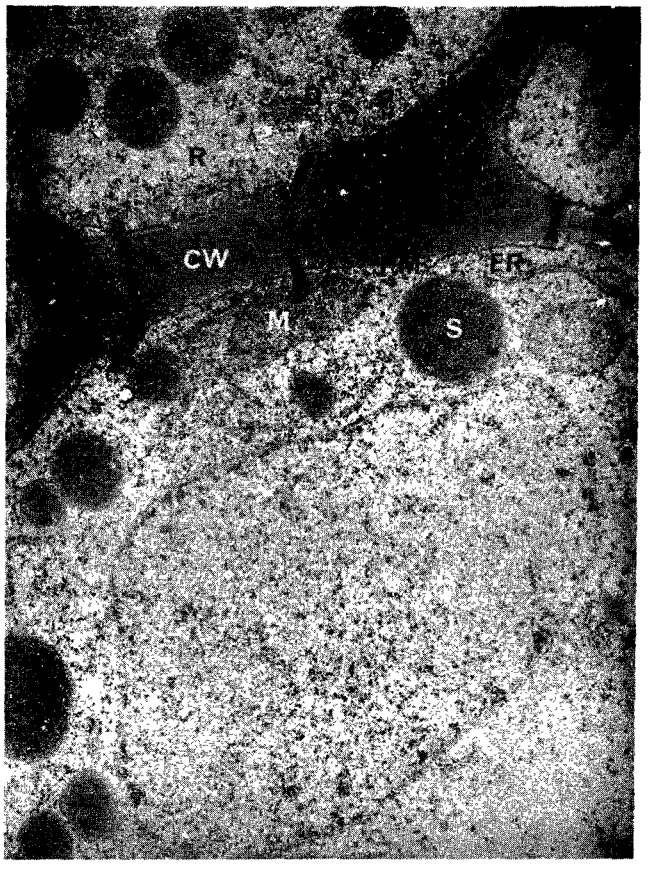

Figure 9. *Electronmicrograph of a thin section through the root of a 10-day-old cassava seedling. Lipid bodies (Sp) are scattered through the cytoplasm. Profiles of the endoplasmic reticulum (Er) and golgi apparatus (G) are well defined. Plastid (Pl), spherosome (Sp), mitochondrion (M). x 32,000.*

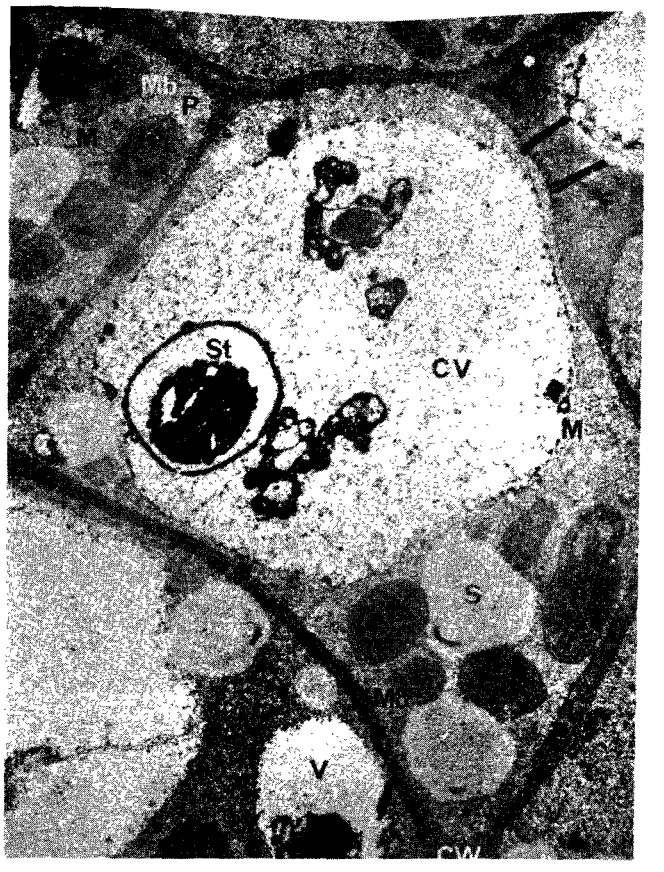

Figure 10. *Electronmicrograph of a thin section through the leaf of a 17-day-old etiolated cassava seedling. Cytoplasmic organelles are well defined, few lipid bodies are present. Spherosome (Sp), mitochondrion (M), endoplasmic reticulum (Er), golgi apparatus (G), cell wall (CW). x 10,000.*

Figure 11. *Electronmicrograph of a thin section through the root of a 17-day-old cassava seedling. Cytoplasmic membranes are well defined. Lipids bodies are absent. Mitochondrion (M), Cell Wall (CW), Crystalloid containing microbody (Cr). x 10,000.*

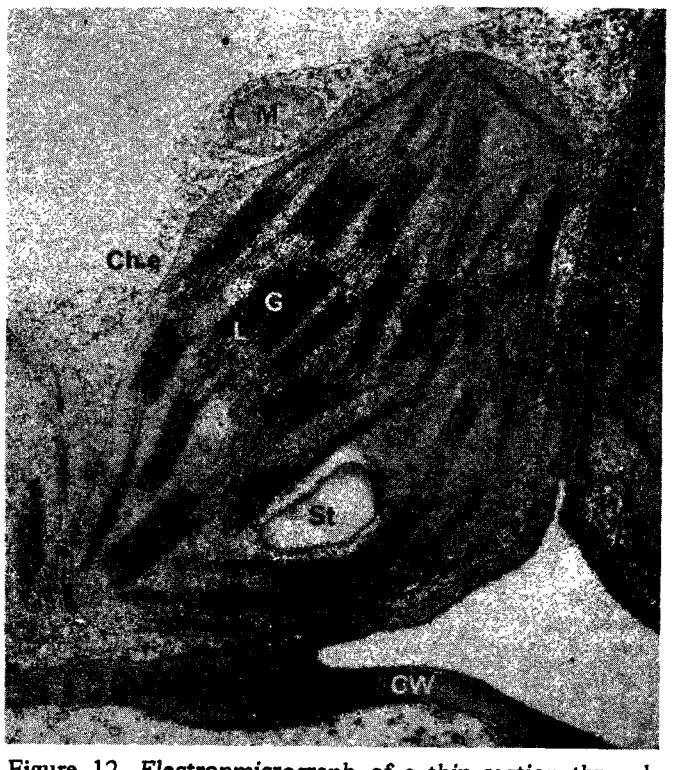

Figure 12. *Electronmicrograph of a thin section through the leaf of a 17-day-old cassava seedling which had light over the last 7 days. The cells contain well-developed chloroplasts with starch granules. Chloroplast (Ch), Starch (S), Mitochondion (M). x 28.000.*

Seeds of both sweet and bitter cassava cultivars contain low levels of free valine and isoleucine[14,19]. However, on germination, the activation of proteolytic enzymes leads to the accumulation of high levels of free amino acids, notably valine and isoleucine. Concurrent with this accumulation, the concentrations of linamarin and lotaustralin increase sharply. When uniformly labelled L-valine^{-14}C and L-isoleucine^{-14}C are administered to seedlings during the period of active cyanogen synthesis (10—14 days after germination), large amounts of radioactivity are incorporated in the aglucone moieties of linamarin and lotaustralin, respectively[13,14]. Table 5 illustrates the incorporation of radioactive valine and isoleucine into the aglucone moieties of cassava cyanogens, but also into asparagine; the latter is significant with respect to the mechanism for cyanide detoxification and metabolism in cassava.

The biosynthesis and accumulation of cassava cyanogens are influenced by such factors as protein degradation and synthesis, and photosynthesis[14]. Other factors such as the activities of the enzymes involved in the biosynthesis of cyanogens, the concentrations and availability of substrates, as well as the rates of transport, storage and degradation of cyanogens in specific tissues, influence variations in the concentrations of cyanogenic glucosides in tissues of different cultivars[21].

The concentrations of cyanogens in cassava plants generally fluctuate, indicating enzyme catalysed degradation and resynthesis, and therefore, the dynamic nature of these compounds. The biosynthesis of linamarin and lotaustralin is illustrated in Figure 18. This pathway is operative in a variety of cyanophoric plant species which accumulate linamarin and lotaustralin during germination and growth[7].

The concentration of cyanogenic glucosides in cassava plants fluctuates with growth period and condition. If cassava is grown in a closed container, decreases in cyanogen content do not lead to the release of HCN into the surrounding air. Furthermore, cassava plants kept in closed systems containing high levels of HCN appear to grow normally, in spite of the fact that cyanide normally inhibits plant respiration. Investigations on the fate of HCN released intracellularly from linamarin and lotaustralin show that cassava plants possess two enzyme catalysed systems for the detoxication of cyanide.

DETOXIFICATION AND METABOLISM OF HCN

Cassava seedlings metabolize ^{14}C—labelled HCN, CO_2 and acetate equally efficiently. However, the labelling patterns found in metabolites from seedlings fed these compounds are different. Whereas small frac-

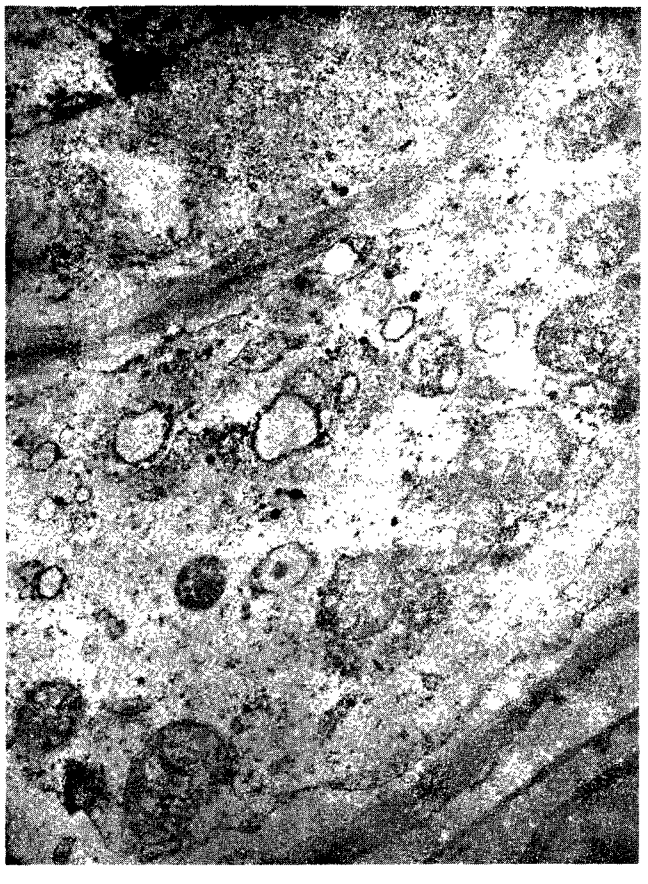

Figure 13. *Electronmicrograph of a thin section through the root of a 17-day-old cassava seedling which had received light over the last 7 days. Cytoplasmic membranes are well defined. Lipid bodies are absent. x 32,000.*

Figure 14. *Electronmicrograph of a thin section through the leaf of a 5-month-old cassava plant. The cells contain well developed chloroplasts and other cytoplasmic organelles. x 5,000.*

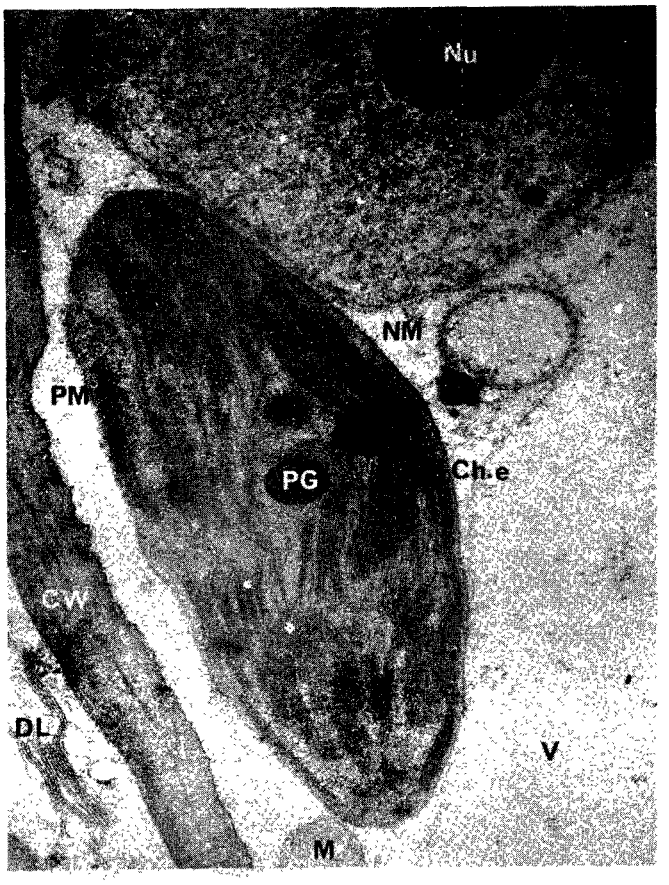

Figure 15. *Electronmicrograph of a thin section through the stem tissue of a 5-month-old cassava plant, showing the presence of a chloroplast containing starch, mitochondrion and other cytoplasmic organelles. x 10,000.*

Figure 16. *Electronmicrograph of a thin section through the root of a 5-month-old cassava plant. Many cells are vacuolated. Several lomasomes and other cytoplasmic organelles are present. x 5,000.*

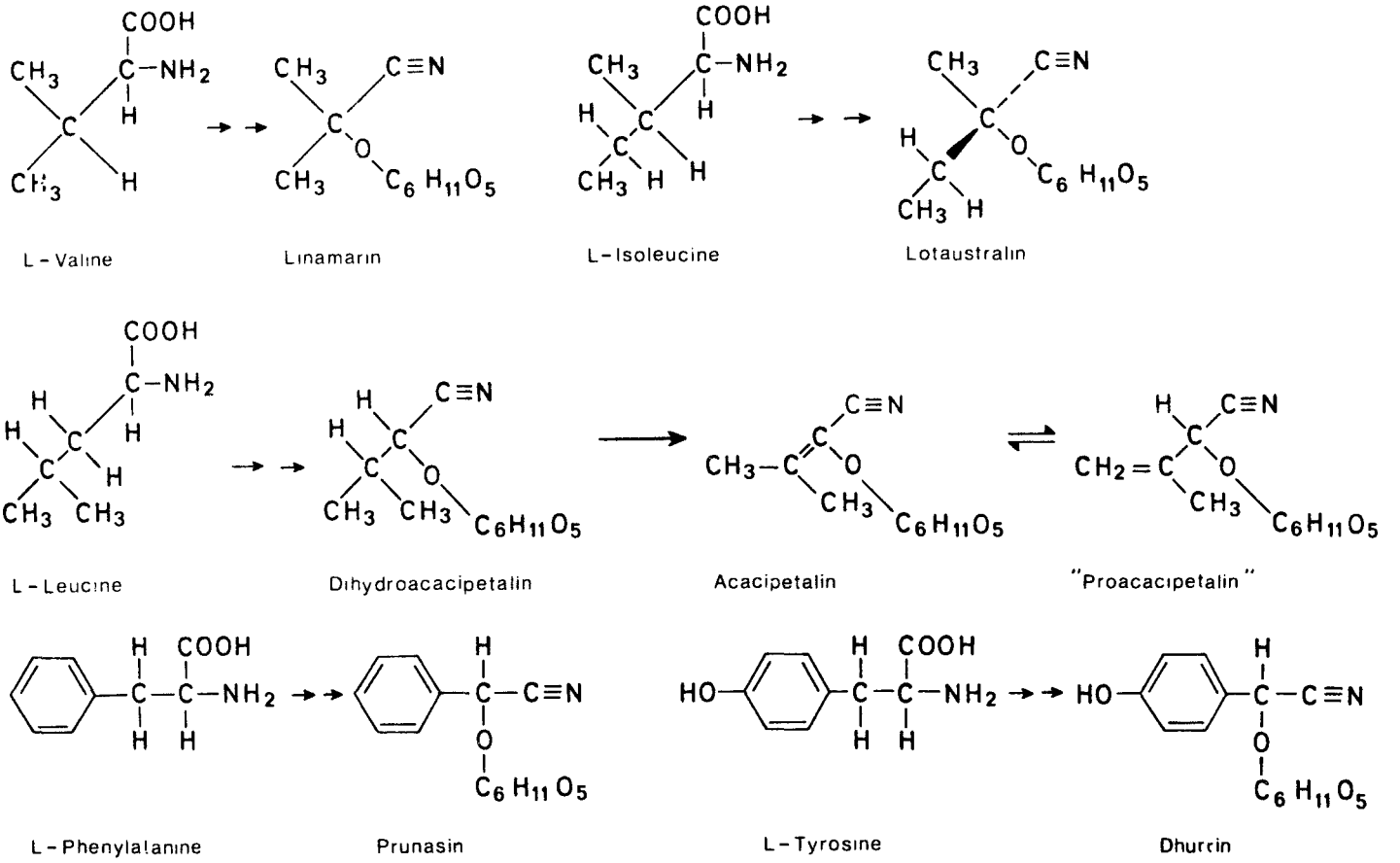

Figure 17. *The structural relationships between some cyanogenic glucosides and their precursor amino acids.*

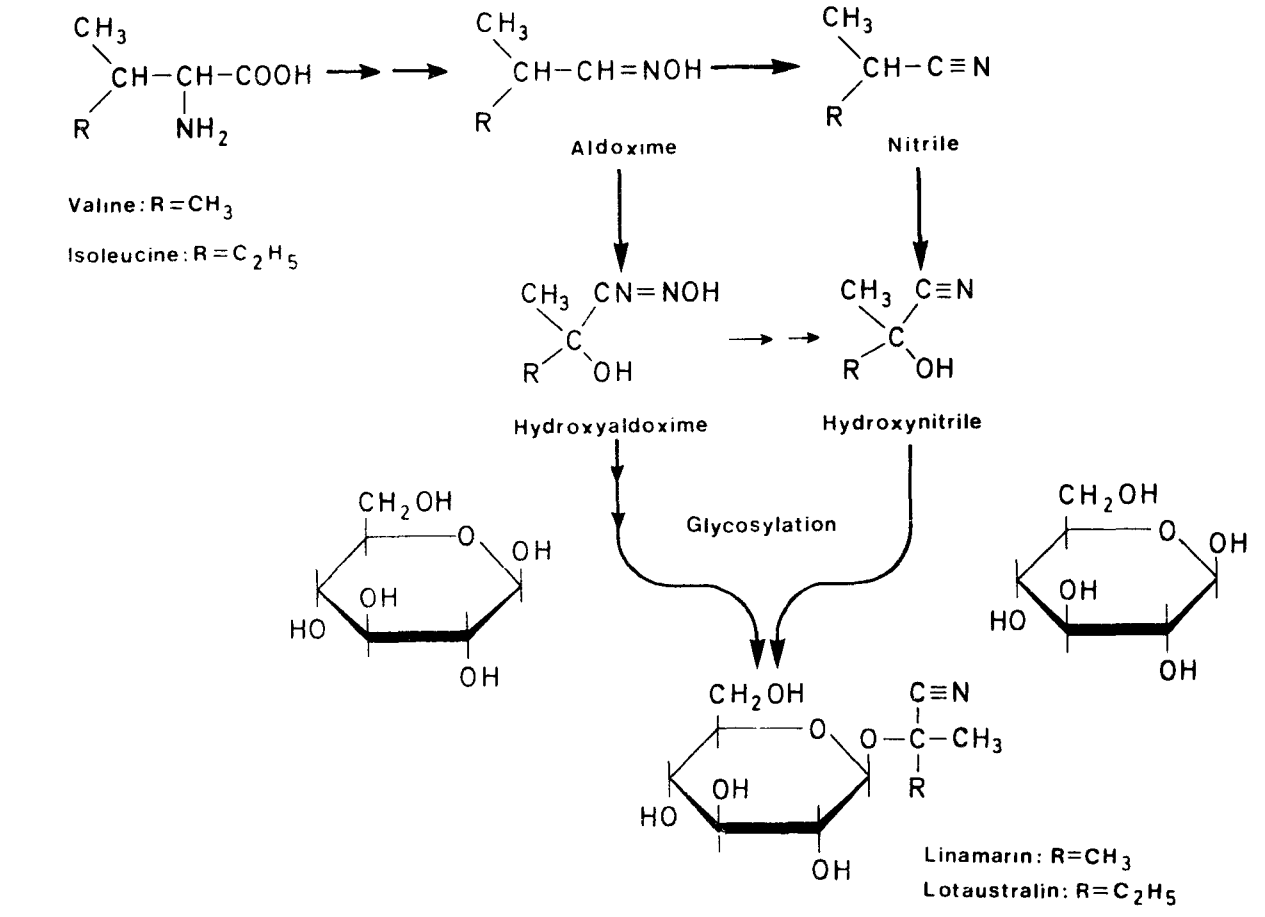

Figure 18. *The biosynthesis of linamarin and lotaustralin in cassava plants.*

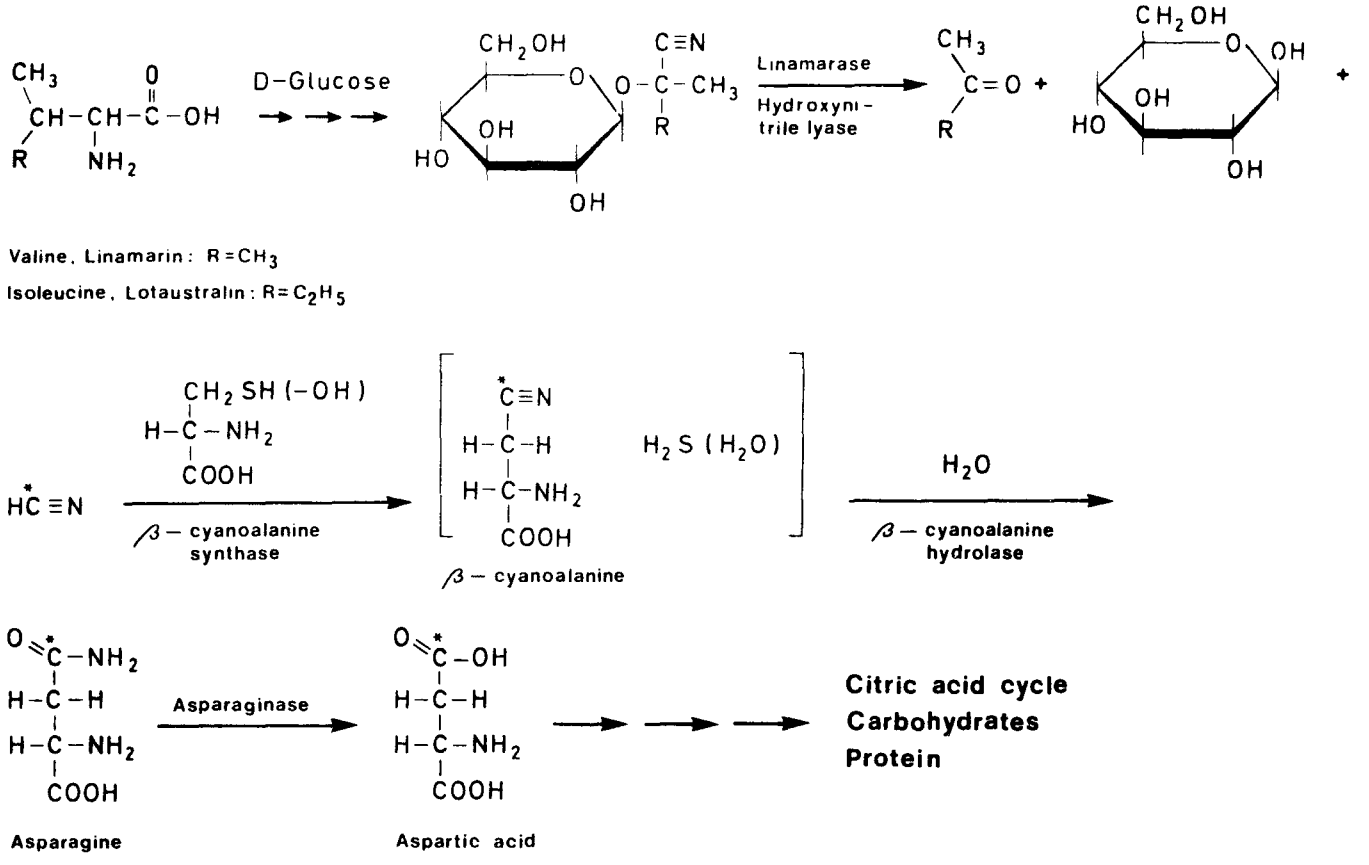

Figure 19. *The biosynthesis and degradation of linamarin and lotaustralin, and the detoxication and assimilation of hydrogen cyanide by cassava plants.*

tions of radioactivity from $^{14}CO_2$ and ^{14}C-acetate are found in the free amino acid pools, large amounts of radioactivity from $H^{14}CN$ are incorporated in the free amino acid pools. A most striking feature of the labelling patterns is that although 49% of the total radioactivity from $H^{14}CN$ is located in the free amino acid fraction of seedling extracts, over 95% of the total radioactivity in this fraction is located in asparagine, aspartic acid, glutamine and glutamic acid. Table 6 shows that asparagine is the major labelled product of cyanide metabolism in cassava. Furthermore, well over 97% of the total radioactivity in asparagine is located in its amide-carbon[14]. This mechanism for cyanide detoxification operates in a variety of cyanophoric plant species[2,15], and involves the enzyme catalyzed reaction of cyanide with serine or cysteine, with the resultant formation of B-cyanoalanine as the primary reaction product[7,14,11].

Quantitative data from in vivo and in vitro studies on cyanide metabolism by cassava seedlings and seedling extracts show that the natural acceptor of cyanide in cassava is serine[14]. B-cyanoaline is not accumulated in cassava tissues, which contain highly active B-cyanoalanine synthase, B-cyanoalanine hydrolase and asparaginase[16]. The collective operation of these enzyme systems ensures that HCN evolved from cassava cyanogens by the action of linamarase and 2-hydroxynitrile lyase (or by non-enzyme catalysed dissociation of the hydroxynitrile moieties) is rapidly converted to amino acids, proteins, carbohydrates, lipids and other cellular materials. Figure 19 illustrates the detoxification and assimilation of hydrogen cyanide by cassava plants[14].

Cassava plants possess also rhodanase activity. Rhodanase catalyses the reaction of cyanide with inorganic and organic sulphur compounds with the resultant formation of thiocyanate[4,5,16]. Cassava rhodanase activity is inhibited by cysteine, while B-cyanoalanine synthase activity is inhibited by thiosulphate. Thus only one cyanide detoxification system in cassava may operate at any particular time. Both enzyme systems are localized in the metochondria, and their functions may well be the reduction of high intracellular concentrations of cyanide for the preservation of electron transport and oxidative phosphorylation in cassava plants[16].

ACKNOWLEDGEMENTS

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REFERENCES

1. Beevers, H. (1961) Metabolic production of sucrose from fat. *Nature* 191, 384–6.

2. Blumenthal-Goldschmidt, S. *et al.* (1965) Incorporation of hydrocyanic acid labelled with carbon-14 into asparagine in seedlings. *Phytochem.* 4, 127–31.

3. Butt, V.S. *et al.* (1966) The plant lipids. In. *Plant Physiology, a treatise.* (Ed. F.C. Steward) 1VB, 226–414. Academic Press, New York.

4. Chew, M.Y. *et al.* (1972) Rhodanase in tapioca leaf. *Phytochem.* 11, 127–31.

5. – – – – – (1973) Rhodanase in higher plants *Phytochem.* 12, 2365–7.

6. Conn, E.E. (1973) Cyanogenic glycosides: their occurrence, biosynthesis, and function. In *Chronic cassava toxicity: proceeding of an interdisciplinary workshop.* pp. 55–63. Int. Develop. Res. Centre Monogr. IDRC-010e.

7. Conn, E.E. *et al.* (1969) The biosynthesis of cyanogenic glycosides and other simple nitrogen compounds. In *Perspectives in Phytochemistry.* p. 47–74. J.B. Harbourne and T. Swain (Ed). Academic Press, London.

8. Cooper, T.G. *et al.* (1969) Mitochondria and glyoxysomes from cartor bean endosperm. *J. Biol. Chem.* 244, 3507–13.

9. – – – – – (1969) Oxidation in glyoxysomes from castor bean endosperm. *J. Biol. Chem.* 244, 3514–20.

10. Corkill, L. (1940) Cyanogenesis in white clover (*Trifolium repens* L.) 1. Cyanogenesis in single plants. *N.Z.J. Sci.* 22B, 65–7.

11. Fowden, L. *et al.* (1965) Cyanide metabolism by seedlings. *Nature* 206, 110–12.

12. Hitchcock, C. *et al.* (1971) *Plant Lipid Biochemistry.* Academic Press, London.

13. Nartey, F. (1968) Studies on cassava, *Manihot utilissima* Pohl. 1. Cyanogenesis: the biosynthesis of linamarin and lotaustralin in etiolated seedlings. *Phytochem.* 7, 1307–12.

14. Nartey, F. (1969) Studies on cassava, *Manihot utilissima.* 11. Biosynthesis of asparagine –^{14}C from ^{14}C-labelled hydrogen cyanide and its relations with cyanogenesis. *Physiol. Plant* 22, 1085–96.

15. – – – – – (1970) Cyanide metabolism in higher plants. *Z. f. Pflanzenphysiol.* 62, 398–400.

16. – – – – – (1973) Biosynthesis of cyanogenic glucosides in cassava (*Manihot* spp.). In *Chronic cassava toxicity; proceedings of an interdisciplinary workshop.* pp. 73–87. IDRC-010e.

17. – – – – – *et al.* (1973) The major constituents of cassava seeds. *Trop. Sci.* 15, 273–277.

18. – – – – – (1974) Changes in the major constituents of *Manihot esculenta* Crantz). *Econ. Bot.* 28, 145–154.

19. – – – – – (1976) Amino acid profiles of cassava seeds (*Manihot esculenta* Crantz). *Econ. Bot.* 30, 419–423.

20. – – – – – (1973) Fatty acid profiles in germinating *Manihot esculenta. Phytochem.* 12, 2909–2911.

21. Nestel, B. (1973) Current utilization and future potential for cassava. In *Chronic cassava toxicity: Proceedings of an interdisciplinary workshop.* pp. 11–26. Int. Develop. Res. Centre Monogr. IDRC-010e.

22. Rogers, D.J. (1965) Some botanical and ethnological considerations of *Manihot esculenta. Econ. Bot.* 19(4), 369–77.

23. – – – – – (1959) Cassava leaf protein. *Econ. Bot.* 13, 261–3.

24. Vigil, E.L. (1970) Cytochemical and developmental changes in microbodies (glyoxysomes) and related organelles of castor bean endosperm. *J. Cell Biol.* 46, 435–54.

TABLE 1

Fatty acid composition of total lipids and triglycerides of cassava seeds

Fatty acid	% Composition	
	Total lipids	Triglycerides
$C_{14:0}$ (Myristate)	0.07	0.00
$C_{16:0}$ (Palmitate)	10.34	9.90
$C_{16:1}$ (Palmitoleate)	0.09	0.00
$C_{18:0}$ (Stearate)	4.10	3.90
$C_{18:1}$ (Oleate)	22.40	23.00
$C_{18:2}$ (Linoleate)	61.60	62.00
$C_{18:3}$ (Linolenate)	1.40	1.20
$C_{20:0}$ (Arachidate)	0.00	0.00

TABLE 2

Amino acid profiles of cassava seeds, expressed as mg amino acid/100 g dry seed kernel, and as g amino acid/16 g N

Amino acid	mg/g dry seed kernel	g/16g nitrogen
Aspartic acid	3600	10.85
Threonine	1345	4.05
Serine	1340	4.03
Glutamic acid	5815	17.51
Proline	1595	4.80
Glycine	1405	4.24
Alanine	1460	4.40
Valine	2775	8.36
Isoleucine	1015	3.06
Leucine	2110	6.36
Tyrosine	1005	3.02
Phenylalanine	1415	4.26
Lysine	1040	3.13
Histidine	765	2.31
Ammonia	460	1.39
Arginine	4590	13.83
Methionine	570	1.71
Cystine	545	1.64
Tryptophan	540	1.62

TABLE 3

Concentration of cyanogenic glycosides in tissues of sweet and bitter cultivars of cassava

Cultivar	Tissue	Cyanogenic glycosides (mg HCN/kg fresh wt tissue)
Sweet (3 cvs)	Seeds	0.00
	Seedlings (10 day old)	285.00
	Leaves (mature)	468.00
	Roots	126.50
	Tubers	402.00
Bitter (3 cvs)	Seeds	7.50
	Seedlings (10 day old)	245.00
	Leaves (mature)	310.00
	Roots	185.00
	Tubers	395.00

TABLE 4

Net changes in the fatty acid composition of cassava seedlings as a function of growth condition and period

	% composition					
Age (days)	3	9	13	13	17	17
	Dark	Dark	Dark	Light	Dark	Light
Fatty acid	(49.2)	(43.8)	(41.0)	(35.7)	(37.2)	(28.5)
$C_{14:0}$ (Myristate)	0.04	0.10	trace	0.00	0.00	trace
$C_{16:0}$ (Palmitate)	10.20	11.60	11.80	10.20	11.00	12.50
$C_{16:1}$ (Palmitoleate)	0.05	0.11	trace	trace	trace	trace
$C_{18.0}$ (Stearate)	4.10	5.10	4.60	3.40	4.10	4.60
$C_{18:1}$ (Oleate)	21.80	22.70	23.00	21.60	21.60	22.60
$C_{18:2}$ (Linoleate)	62.20	59.20	58.70	63.30	60.20	58.70
$C_{18:3}$ (Linolenate)	1.60	1.30	2.00	1.60	3.00	1.60
$C_{20.0}$ (Arachidate)	0.11	trace	0.00	0.00	0.00	0.00

TABLE 5

Incorporation of radioactivity from L-Valine^{14} –C(U) and L-Isoleucine^{14}C (U) into the aglucone moieties of Linamarin and Lotaustralin, and into Asparagine, by cassava seedlings

Label administered	Incorporation (%) into Linamarin,	Lotaustralin,	Asparagine
L-Valine^{14}-C(U)	13.20	-	1.10
L-Isoleucine^{14}-C(U)	-	2.40	0.53

TABLE 6

Incorporation of radioactivity from H^{14}CN into free amino acids by 10g cassava seedlings exposed to H^{14}CN released from 2.3 mole Na^{14}CN, 125 Ci. 8.38 x 10^6cpm. in a closed system for the periods specified

Period of feeding(min)	Radioactivity (cpm x 10^{-5}) incorporated in Asparagine	Aspartic acid	Glutamine	Glutamic acid
10	0.86	0.11	0.16	0.07
60	24.91	5.73	0.89	0.18
180	42.03	6.68	0.65	0.43