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THE METABOLISM OF GALACTOSE AND THE RAFFINOSE OLIGOSACCHARIDES BY GERMINATING BAMBARRA GROUNDNUT SEEDS

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Abstract—Stachyose is present in the highest amount in the soluble sugar fraction of dry bambarra groundnut cotyledons, followed in descending order by raffinose, sucrose and verbascose. During germination in the dark, the stachyose and raffinose content decrease rapidly, but there is little change in the relatively small amount of verbascose present. The sucrose content increases rapidly during the first two weeks and decreases thereafter. Free glucose and fructose were present in the cotyledons after the 7th day and gradually increased in amount with time of germination. Free galactose and other galactose-containing oligosaccharides were not detected in either the dry or germinated bambarra seeds. During germination, galactose was the only identifiable sugar, aside from traces of sucrose, glucose and fructose, in the extracted soluble sugar fraction in the embryonic axes of all ages when the tissue was incubated with D-[1^{14}C] galactose. With the cotyledons, however, most of the radioactivity was in glucose and fructose during the early period of germination and in sucrose later. A small fraction of the radioactivity was lost as CO_2.

INTRODUCTION

The raffinose oligosaccharides serve as reserve carbohydrates in a number of seeds [1–3]. During germination they disappear and there is a concomitant increase in the amount of glucose and fructose present. The sucrose content increases or remains fairly constant initially, before eventually decreasing. Neither free galactose nor other galactose-containing oligosaccharides are observed [2, 4–10]. The fate of the galactose moiety of the oligosaccharides of these seeds remains obscure. In contrast, free galactose and mannose appear and increase in amounts in separated endosperms during germination of certain endosperm-containing legume seeds as a consequence of the hydrolysis of chacteristic reserve galacto-mannans. The amounts of both sugars decline eventually and exogenous radioactive mannose and galactose are incorporated into various products by the cotyledons, indications that they are further metabolized [11].

Other pertinent studies on the general metabolism of galactose in plant tissues include the following: Radioactive galactose infiltrated into *Canna* leaves and wheat seedlings was rapidly converted to radioactive sucrose (12). When *Avena* coleoptile segments were incubated with galactose-[^{14}C], the sugar was neither respired as CO_2 nor incorporated into cellulose as rapidly as glucose-[^{14}C] [13]. In a similar study, when decapitated *Avena* coleoptiles were incubated with galactose-[^{14}C] only a small fraction was respired as CO_2, while 90% of the introduced radioactivity was incorporated into cell wall materials; compounds which were neither amino acids nor sugars were also labeled [14].

Radioactive galactose taken up by corn (*Zea mays*) roots and barley (*Hordeum vulgare*) coleoptiles was incorporated into 80% ethanol insoluble materials [15]. It was reported earlier that excised corn root tips take up galactose-[1-^{14}C] which was subsequently converted to cell wall polysaccharide. The radioactivity was found predominantly in galactose in hemicellulose and in pectin: only in α-cellulose was an appreciable amount of radioactivity found in glucose [16].

Cooper and Greenshields [7] concluded that the French bean (*Phaseolus vulgaris*) seedlings has a mechanism for utilizing galactose, since on germination the galactose-oligosaccharides disappear. No galactose was found although sucrose was found in the cotyledons, and glucose and fructose were found, additionally, in the embryo. Further, stachyose and raffinose were metabolized rapidly when they were infiltrated into dissected tissue but no free galactose was detected. Shiroya [6] concluded that intact cotyledons of cotton (*Gossypium herbaceum*) seedlings have an effective mechanism for utilizing galactose, only under aerobic conditions.

A possible shortcoming of some of the above investigations is that the pathway of galactose metabolism was studied in growing tissues in which galactose probably does not occur to a great extent or is of little metabolic significance. Accordingly, we pursued the present research on the fate of endogenous galactosyl-sucroses and exogenous radioactive galactose in this apparently typical germinating legume with the expectation that the results would more accurately reflect the utilization of these storage carbohydrates *in situ*.

RESULTS AND DISCUSSION

Changes in soluble sugars during germination

In the dry bambarra groundnut (*Voandzeia*

subterranea) cotyledons, stachyose is present in the highest amount (44.6%) in the soluble sugar fraction, followed by raffinose (31.1%), sucrose (22.0%) and verbascose (2.3%). This is unlike what has been reported in *Glycine max* [4, 8] and *Coffea* spp. seeds [5] where sucrose is present in the highest amount.

Fig. 1 shows that during germination the stachyose and raffinose contents decreased in the groundnut, similar to what has been reported for other seeds [2, 4–9]. The already low verbascose content remained fairly constant. Similar results were obtained with the soluble sugars of cotyledons from another cultivar during germination.

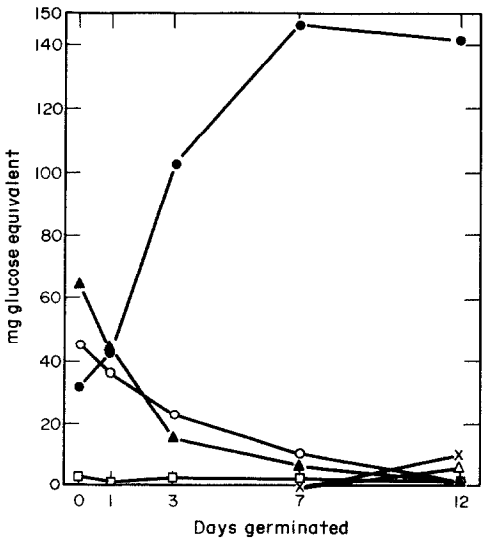

Fig. 1. Pattern of change of soluble sugar components in bambarra groundnut cotyledons during germination in the dark. Eight cotyledons at the different days of germination were extracted thoroughly with hot 80% ethanol. The alcohol extract was made to a known volume and an aliquot was evaporated to dryness. The residue was dissolved in water and the amount of the different sugars was determined after paper chromatography, elution of the areas and reaction with the anthrone reagent. ●—● Sucrose; ▲—▲ stachyose; ○—○ raffinose; □—□ verbascose; × — × fructose; and △—△glucose.

The sucrose content of the cotyledons increased about 4-fold up to the 7th day in this cultivar, while the increase was 2.5-fold in the other cultivar, for up to *ca* 18 days before decreasing. The increase in sucrose content in the cotyledons may be partly due to the hydrolysis of raffinose, stachyose and verbascose but not solely so since the increase in sucrose extends beyond the time when the amount of the raffinose oligosaccharides present is virtually nil. It is known that amylolytic activity increases in the cotyledons [17] and it would be expected that its action would supply glucose to the organ which could in turn be converted to sucrose.

Free glucose and fructose were not detected in the cotyledons until after the 7th day of germination, unlike the green gram (*Phaseolus radiatus*) where free fructose was detected after 6 hr germination [2]. Free galactose (or other galactose-containing oligosaccharides) was not detected in the bambarra cotyledons, unlike soybean (*Glycine max*) in which the dry seed was said to contain small amounts of free galactose in addition to glucose and fructose [4, 8].

The changes in sucrose, glucose and fructose during late germination are probably not due to invertase (β-fructofuranosidase) activity since none was detected in the cotyledons at any age (data not given). The enzyme could not be detected in crude extracts of embryonic axes; on dialysis against buffer for 18 hr, however, appreciable activity was observed. But neither crude preparations nor dialysed extracts of cotyledons hydrolysed sucrose.

Embryos from dry bambarra groundnut contained the same soluble sugars as the dry cotyledons; after germination for 18 to 26 days, however, only sucrose, glucose and fructose were detected in the seedlings.

The metabolism of galactose-[1-^{14}C] by bambarra seedlings

Table 1 shows the distribution of radioactivity in different fractions and components of the bambarra cotyledons and embryonic axes after incubating the tissues with galactose-[1-^{14}C] for 7 hr in the dark. In both tissues, a small amount of the introduced radioactive label was respired as CO_2. The amount respired by the embryonic axes at all ages studied (5–9% of the radioactivity taken up) was higher, as expected, than that evolved from the cotyledons (1–2%).

Table 1. Distribution of radioactivity in different fractions of bambarra groundnut cotyledons and embryonic axes incubated with 1 μCi of D-galactose-[1-^{14}C] for 7 hr in the dark

Fraction	Days germinated	cpm/cotyledon or embryonic axis					
		Cotyledons			Embryonic axes		
		6	11	26	6	11	26
CO_2		276	388	1650	590	2500	4490
80% ethanol extract		12600	31300	81800	7800	22700	73700
Residue		2060	2670	1970	2400	2300	9050
Total cpm		14900	34400	85400	10800	27600	87200

The CO_2 evolved during incubation was bubbled through barium hydroxide solution and the precipitated barium carbonate filtered, dried and counted. At the end of the incubation period, tissues were rinsed briefly with distilled water and placed in boiling 80% ethanol. The tissue was then homogenized and extracted thoroughly with hot 80% ethanol. The alcohol extract was evaporated to dryness, redissolved in water (80% ethanol extract) and an aliquot counted. The original 80% ethanol insoluble material (Residue) was also dried, weighed and counted. Radioactivity is expressed as cpm/cotyledon or embryonic axes.

Table 2. Distribution of radioactivity in various fractions of the 80% ethanol soluble materials extracted after exposure of bambarra groundnut cotyledons and embryonic axes to 1 μCi of D-galactose-[1-^{14}C] for 7 hr in the dark

Fraction	cpm/cotyledon or embryonic axis					
	Cotyledons			Embryonic axes		
Days germinated	6	11	26	6	11	26
Lipid	2710	2500	3050	1710	4450	20000
Compounds adsorbed on cation exchanger	558	2340	287	1000	1980	189
Filtrate from cation exchange resin (sugars and other compounds)	7980	17900	55000	3330	13700	33300

The 80% ethanol extract of Table 1 was evaporated to dryness and extracted with hot petrol (lipids). The residue was dissolved in water, treated with Dowex 50 for 1 min, filtered and counted (compounds absorbed on cation exchanger). The filtrate after the resin treatment was also counted (sugars and other compounds fraction). Radioactivity is expressed as cpm/cotyledon or embryonic axis.

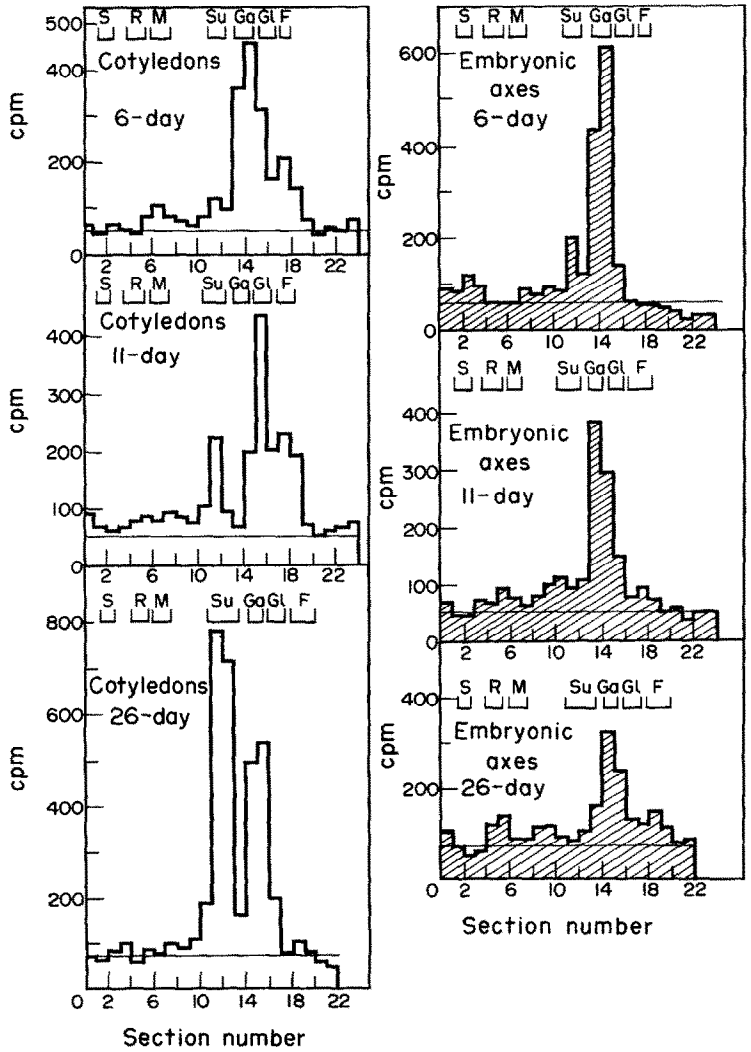

Fig. 2. Patterns of distribution of radioactivity on chromatograms of the soluble sugar fraction of cotyledons and embryonic axes exposed to 1 μCi D-(1-^{14}C) galactose for 7 hr. Chromatograms were developed in PrOH–EtOAc–H_2O 7:1:2) and 2 cm sections were counted in a liquid scintillation spectrometer. Areas on the chromatogram are: S—stachyose, R—raffinose, M—melibiose, Su—sucrose, Ga—galactose, Gl—glucose, and F—fructose. The horizontal line in this figure indicates the level of background radioactivity.

The amount of radioactivity from the galactose incorporated in the residue (probably mostly cell wall materials) was nearly the same in the 6-day, 11-day and 26-day old cotyledons. The amounts of label found in this fraction of the 6-day and 11-day embryonic axes were about the same but there was about a 4-fold increase in radioactivity in the 26-day-old tissue.

Further fractionation of the ethanolic extract revealed that some radioactivity was contained in lipids, some in sugars and other compounds and additionally some in compounds adsorbed on sulfonic acid cation exchange resin (Table 2). In the embryonic axes, the amount of radioactivity incorporated in lipids increases with age of tissue as expected, whereas the amount incorporated into lipids of cotyledons was fairly constant throughout germination. This probably reflects the rate of synthesis of cellular components in the two tissues. The pattern of incorporation of radioactivity in material(s) adsorbed on the cation exchange resin (most likely all positively charged compounds) was that of an increase at the 11th day over the 6th day with much less radioactivity being incorporated in the 26-day-old tissue.

Most of the radioactivity extracted by 80% ethanol was in the fraction referred to as sugars and other compounds (Table 2) and a pattern of increased incorporation with age in both the cotyledons and embryonic axes was noted. The distribution of radioactivity on chromatograms of extracts containing the soluble sugars and other compounds is shown in Fig. 2 for the 6-, 11- and 26-day-old tissue. (The chromatograms from which Fig. 2 was derived were developed in *n*-PrOH–EtOAc–H_2O (7:1:2) system).

There was no indication that radioactivity was present in the raffinose family of sugars in extracts from either tissue since there was no correspondence between the area of the color developed by the sugars with the diphenylamine reagent and the areas of radioactivity on the chromatograms.

In the embryonic axes, the radioactivity in the fraction containing sugars and other compounds was mainly due to residual galactose-[1-^{14}C]. There is, however, the indication that radioactivity was incorporated in sucrose in the 6-day and 11-day old axes. There was also label in glucose and fructose in the 11-day and 26-day old axes (Fig. 2). Essentially the same results were obtained, i.e. evidence that glucose and fructose were labelled, when another chromatographic system (*n*-PrOH–EtOAc–H_2O, 4:5:1) which gives a better separation of the monosaccharides was used.

In the cotyledons, on the other hand, most of the radioactivity in the fraction containing sugars and other compounds was in galactose, glucose and fructose during early stages of germination. The amount incorporated in glucose and fructose increased in the 11-day-old over the 6-day-old tissue and the 26-day-old tissue incorporated much less label in these sugars (Fig. 2). With increase in age of the tissue, there was an increased incorporation of label in sucrose (Fig. 2), and at the 26th day after germination, most of the radioactivity was found in sucrose. It was also observed that the 26-day-old cotyledons, but not younger tissue or embryonic axes, secreted radioactive sucrose into the incubation medium (data not given). Whether this is due to autolysis is not known although the tissue appeared healthy.

Thus, germinating bambarra groundnut cotyledons convert galactose mostly to sucrose, apparently increasingly so with time of germination. This is in contrast to its conversion mainly to cell wall materials by corn roots and *Avena* coleoptiles [14, 15]. These differences may be a consequence of the existence of metabolic determinants or controls of tissues that reflect their specific needs. a need for sucrose for transport to the embryo by the germinating cotyledons of hypogeal seedlings and a need for precursors of cell wall materials for meristematic tissue. The formation of sucrose can be accounted for by the sequential action of enzymes known to be present in legume seeds: galactokinase, UTP: galactose-1-phosphate uridyltransferase. UDP-galactose-4-epimerase and sucrose synthetase. The formation of glucose and fructose would involve additionally hexokinase. phosphohexose isomerase and phosphatase activities.

EXPERIMENTAL

Bambarra groundnut seeds were obtained from the Department of Crop Science, University of Ghana and from local markets in Ghana. Standards of raffinose, stachyose and melibiose were gifts from Dr. A. Kivilaan. Galactose-[1-^{14}C] (sp. act. 60 mCi/mmol) was purchased from Amersham/Searle. Bambarra seeds were planted in the dark in vermiculite which was kept moist with H_2O at 25°. Planting was staggered so that all tissues in a series were taken on the same day. Tissues were surface sterilized with 1% NaOCl for 3 min and rinsed thoroughly with H_2O before use. The extraction, chromatographic separation, detection and quantitative estimation of sugars were carried out as reported earlier [3]. For the incubations, germinated and surface sterilized bambarra cotyledons and embryonic axes were sliced and placed in a 10 ml soln of galactose-[1-^{14}C] (1 μCi) and kept in the dark for 7 hr in a 125-ml conical flask fitted with air inlet and outlet tubes. Air was constantly drawn through the chamber and bubbles through a gas scrubber containing a soln of $Ba(OH)_2$ to precipitate the respired CO_2 as $BaCO_3$. At the end of the incubation, tissue slices were rinsed briefly with H_2O, placed in boiling 80% EtOH and homogenized. Homogenate was centrifuged at 6620 *g* at room temp. Residue was extracted with EtOH several times, the alcoholic extracts pooled, subsequently evaporated to dryness under red. pres., and the solid remaining dissolved in a known vol. of H_2O. (This is referred to as the 80% EtOH extract.) An aliquot was then counted in liquid scintillation fluid containing 5 g 2,5-diphenyloxazole (PPO) and 0.3 g 1,4-bis-2-(4-methyl-5-phenyl-oxazoyl)-benzene (POPOP) in 1 l. toluene. The alcohol fraction was again evaporated to dryness and the lipid fraction dissolved in hot petrol. The extracts were pooled, evaporated to dryness, reconstituted in a known vol of petrol (Lipid fraction) and an aliquot counted. Residue remaining after the petrol extraction was dissolved in H_2O and was treated with Dowex 50 H+ for 1 min to extract any positively charged compounds. Resin was filtered and the filtrate evaporated to dryness. Residue was dissolved in a known vol of H_2O (Sugar and other compounds fraction) and an aliquot counted as given above. The filtered resin (compounds adsorbed on cation exchanger) was counted in Bray's counting fluid [18]. The material remaining after extracting the incubated slices with EtOH was dried, weighed and counted in the toluene-based counting fluid. This is referred to as the residue. Similarly, the precipitated $BaCO_3$ was filtered from the $Ba(OH)_2$ soln, dried and counted in the toluene-based fluid.

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