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ASPECTS OF THE METABOLISM OF *ADANSONIA DIGITATA* L.

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Abstract

Seeds of *Adansonia digitata* contain appreciable quantities of sucrose, starch, proteins and lipids as reserve food materials. The food reserves in the seeds decreased during the first two weeks of germination due to hydrolysis by enzymes, reflecting the increase in the activities of invertase and lipase enzymes. It is suggested that the initial reaction in the utilisation of sucrose in *A. digitata* seeds is probably the invertase hydrolysis of this disacchride to glucose and fructose. High lipid content in the seeds is converted into free fatty acids and glycerol by lipase enzymes which are then fed into the glyoxylate cycle to release sucrose which is further utilized to generate energy for the growth of the embryo.

Introduction

Adansonia digitata L., a member of the family Bombacaceae is widely distributed in the drier parts of Nigeria where savanna vegetation abounds. Its population density in the field is very low (Etejere & Osatimehin, 1984). Apart from studies on the economic importance of this plant (Pelgrave, 1977; Weiss, 1979) there has not been any investigation on aspects of its physiology. Analytical studies could assist in the understanding of successful growth of plant habit. This communication deals with aspects of the metabolism of *A. digitata.*

Materials and Methods

Seeds were obtained from mature pods of *A. digitata* that were harvested in January 1982. The seeds were randomly obtained from pods collected from 50 trees and then pooled together. The seeds were then scarified with conc. H_2SO_4 for 15 min (Etajere & Osatimehin, 1984) and germinated in 20 cm diam., plastic pots filled with loamy soil. The plastic pots were kept in a chamber with a 12 h day light and alternating 30°C day and 24°C night temperatures. The seedlings were harvested for analyses after 1, 2, 3 and 4 weeks.

(i) *Ethanol-soluble sugars:* Fifty ml of boiling 80% ethanol was used to extract 1.5 g of either the powdered dry seeds or 1, 2, 3 or 4 week old whole seedlings for 12 h. The crude extract was than purified and chromatographed on Whatman No. 1 chromatography paper (Olofinboba, 1969). Ethanol-soluble sugars were detected by the alkaline silver nitrate reagent method of Travelyan *et al* (1950) except that 5% sodium thiosul-

phate was used in place of ammonium hydroxude. Quantitative determination of sugars was made according to the method of Dubois, *et al.*, (1956) using glucose as the standard.

(ii) *Total Starch:* The insoluble residue 0.2 g of the extracted seeds and seedlings was hydrolysed with diastase according to Shriner (1972) and Barnel (1936). The hydrolysed starch was quantitatively determined by the method of Dubois *et al* (1956) and the weight of starch multiplied by 0.9 (Hassid & Neufield, 1964) to give the actual weight.

(iii) *Total Proteins:* Powdered seeds and seedlings 0.2 g were extracted with 20 ml of 2% NaCl solution for 30 min. The extract was repeated twice and then pooled. The protein was precipitated with trichloracetic acid as described by Fawole (1977) and then estimated by the Folin-Phenol method of Lowry, *et al* (1951).

(iv) *Total Lipids:* Marshed seeds or seedlings 0.3 g were extracted in 50 ml chloroform-methanol (Folch *et al.*, 1957). The non-lipid materials present in the extract were removed by washing with water in a separating funnel. The extract was evaporated in an extraction flask and the residue oven-dried to a constant weight at 100°C. The weight of the lipid was then obtained by difference in initial and final weight of flasks.

(v) *Total Amino-acids:* Powdered seeds or seedlings 0.3 g were extraced with 25 ml boiling 80% ethanol for 30 min. The extraction was performed three times and then pooled. The extract was concentrated in-vacuo and the residue dissolved in 1 ml NaOH solution. The total amino-acid content of the extract was measured by the method of Yemm & Cocking (1955) as modified by Rosen (1957).

(vi) *Invertase:* Marshed seeds or seedlings 2.0 g were extracted with 100 ml sterile distilled water for 20 min., and the solution filtered. The resulting solution was centrifuged for 5 min at 500 r.p.m. The filtrate was freeze-dried, stored in a dessicator over calcium chloride ($CaCl_2$) for 5 h and then transferred to a refrigerator. Invertase activity was assayed using the Hagedorn & Jensen (1923) method.

(vii) *Lipase:* Marshed seeds or seedlings 2.0 g were extracted with 10 ml of sterile distilled water and 5 ml of 0.1 NH_2SO_4. The flasks were then incubated at 37°C for 1½ h. Lipase activity was assayed using the method of Tietz & Fiereck (1966).

Results

Ethanol Soluble Sugars: Seeds of *A. digitata* contained sucrose as the only ethanol soluble sugar (Table 1). Two-week old seedlings contained sucrose, fructose, glucose and maltose. In 3 and 4 week old seedings, mannose was also detected. Sucrose content in the seeds one-week old seedlings were 5.81 and 1.39 mg g^{-1} dry weight of sample, respectively (Table 1). Glucose was the main sugar in 2 and 4 week old seedings while maltose was

Table 1. Changes in the food reserves during germination of *Adansonia digitata* seeds. Food reserves are expressed as mg g^{-1} dry weight of sample.

Food reserve	Resting Seeds	Age of seedlings (weed) 1	2	3	4
Glucose	–	3.54 ± 0.04	4.45 ± 0.04	3.40 ± 0.01	3.71 ± 0.03
Fructose	–	2.07 ± 0.06	2.76 ± 0.02	1.90 ± 0.03	2.21 ± 0.07
Sucrose	5.81 ± 0.05	1.39 ± 0.03	2.84 ± 0.09	4.53 ± 0.03	4.23 ± 0.05
Maltose	–	4.09 ± 0.07	4.31 ± 0.01	4.13 ± 0.04	3.29 ± 0.03
Mannose	–	–	–	2.50 ± 0.02	2.86 ± 0.03
Starch	2.63 ± 0.01	0.55 ± 0.03	5.23 ± 0.01	10.80 ± 0.15	11.22 ± 0.16
Amino Acid Pool	0.06 ± 0.0	0.08 ± 0.0	0.22 ± 0.0	0.23 ± 0.0	0.27 ± 0.0
Proteins	3.58 ± 0.04	2.2 ± 0.12	1.64 ± 0.02	1.20 ± 0.06	1.36 ± 0.02
Lipids	32.40 ± 1.18	28.20 ± 1.12	22.20 ± 1.15	18.19 ± 0.94	17.73 ± 0.92

highest in 2 and 3 week old seedlings (Table 1). The starch content in the seeds decresed from 2.65 to 0.55 mg g^{-1} dry weight during the early stages of seedling developemnt but increased significantly to 11.22 mg g^{-1} dry weight during the fourth week of seedling development (Table 1).

Total Protein: The fairly high protein content recorded in the seeds decreased during germination and subsequent growth of the seedlings.

Total Lipids: The seeds contain an abundance of lipids as shown by 32.0 mg g^{-1} dry weight of sample (Table 1). The lipid content decreased gradually from 28.2 mg in one-week old seedlings to 17.7 mg g^{-1} dry weight of sample in four-week old seedlings.

Total Amino Acids: The amino acid content of seeds (0.05 mg g^{-1} dry weight of sample) was observed to be the lowest when compared with the contents of the seedlings. The highest quantity of 0.27 mg g^{-1} dry weight of sample occurred in 4-week old seedlings.

Invertase Activity: Invertase activity in *A. digitata* seedlings increased during the first 2 weeks of growth and thereafter sharply decreased in the third week (Fig. 1). However there was an increase in the fourth week. The highest activity was observed during the second week of growth.

Lipase activity: Lipase activity in *A. digitata* seedlings significantly increased with an increase in seedling age during the first 2 weeks of growth but decreased thereafter (Fig. 2). The period of highest activity occured during the second week of growth.

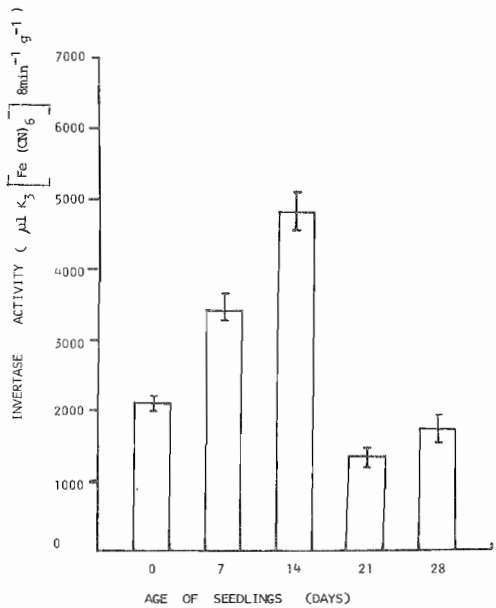

Fig. 1. Activity of invertase in seeds and seedlings of *Adansonia digitata.* Enzyme activity was expressed as µl potassium ferricyanide reduced 8 min^{-1} reaction time g^{-1} sample.

Discussion

The data show that resting seeds of *A. digitata* contain major food reserves for the resumption of growth of the embryo when environmental conditions become favourable. These major food reserves include carbohydrates, proteins and lipids. Lipid concentations were the highest in the seeds. Dalziel (1965) reported that the major food reserves which are stored in the cotyledons are utilised to generate energy for the development of the embryo. Sucrose was the only ethanol soluble sugar in the seeds of *A. digitata.* Miller (1973) observed that sucrose constituted the most widely distributed disaccharide in higher plants. During germination the sucrose is hydrolysed in fructose and glucose which become abundant in one and two-week old seedlings. This suggests that the initial reaction in the utilization of sucrose is the invertase catalysed hydrolysis of this disaccharide to fructose and glucose. This is confirmed by the high invertase activity in the one and two-week old seedlings.

As the seedlings increased in age, synthesis of food was possible as a result of the development of green foliage. This is reflected by subsequent increase in the quantity of ethanol soluble sugard despite utilisation during grwoth of the embryo. Maltose observed in three-week old seedlings wes probably generated through the hydrolysis of reserve starch during growth. Frendenberg (1966) observed that maltose did not occur randomly in nature but formed only when there was enzymic hydrolysis of starch. The

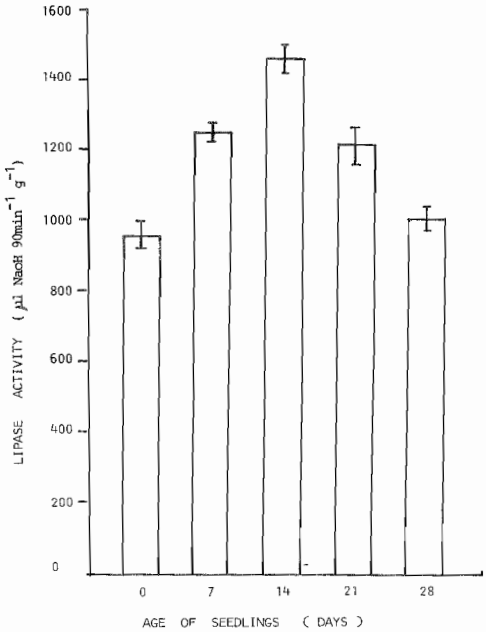

Fig. 2. Activity of lipase in seeds and seedlings of *Adansonia digitata*. Enzyme activity was expressed as µl NNaOH required to neutralise the free fatty acids 90 min^{-1} incubation time g^{-1} sample.

initial decrease in the starch content after one week is an indication that starch was being converted to some other materials necessary for growth and development of the seedlings. The subseqent gradual increase in the starch content of 2-3 and 4 week old seedlings is probably due to an accumulation of food reserve from photosynthesis.

The decrease in protein content during seedling growth could be attributed to hydrolysis which yields amino acids that are transported to form new proteins in the growing regions. This was reflected in the gradual increase in the total amino acid content as the seedlings aged. Similar observations were made by Nobumoro & Tamaki (1967) for tobacco leaf. Beevers & Splittstoesser (1968) noted that during germination the protein bodies and reserve proteins disappear from cotyledons and accumulated in the developing axis. Koller *et al* (1962) also report that as proteins are degraded there is often an increase in the amount of amino acids and amides followed by the synthesis of new proteins in the growing parts.

The lipid content in the seeds decreased sharply during germination and subsequent growth of the seedlings. This is an evidence that the lipids were being converted into fatty acids and glycerol by lipase. These are further converted into sucrose through the glyoxylate cycle. The sucrose is then transported to the growing regions where it is utilised to supply energy for growth. This sudden loss of lipids confirms the high lipase activity during the first 2 weeks of seedling growth. Goodwin & Mercer (1972) reported that fatty

acids are activated by conversion into Acyl co-A derivatives by a microsomal enzyme and later converted by solubel enzymes into Acetyl co-A. When the acetyl co-A is fed into the glyoxylate cycle it is converted into sucrose. The key enzymes in these conversions are rapidly synthesized during germination in seeds with abundant lipids but are not synthesized during germination in seeds with abundant lipids but are not synthesized in seeds which do not store large amounts of lipid.

A. digitata seeds and growing seedlings are equipped with the necessary food reserves for a healthy and rapid growth. The low population density of this species in the field could thus be attributed to the seed coat dormancy exhibited by the seeds (Etejere & Osatimehin, 1984) and the annual savanna bush fires which burn off the young seedlings. Since the seed coat dormancy can be overcome by acid scarification (Etajere & Osatimehin, 1984) perhaps raising of the young seedlings in nurseries and adequate protection from environmental hazards after transplant in the field would enhance population density and distribution of the species.

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