Studies on the enzyme activity in the haustorium of the germinating coconut

Part I. Preliminary Paper.

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INTRODUCTION

The process of germination in the coconut seed is an interesting one. Under conditions congenial for germination, the small embryo lying embedded in the kernel below the soft eye becomes active. It commences to grow in two directions, the top portion towards the soft eye and the lower one into the cavity as an absorbent spongy matter known as haustorium or the apple.

The haustorium swells and continues to grow till it completely fills the cavity and is in close contact with the endosperm or the kernel. The apple is pale yellow in colour on the outer surface and consists of loosely connected thin walled cells with large interspaces between them. This very fine corrugated surface exposes a very large absorptive surface area, and the strands of vascular tissue which branch freely run through the apple and converge to the soft eye to carry food absorbed by it initially from the coconut water and later on from the kernel to the young plant. To play this vital part it is quite certain that the apple should be the centre of intense metabolic activity in which numerous enzymes take part. This paper records the results of preliminary studies aimed at identification and characterisation of enzymes present in the haustorium.

REVIEW OF LITERATURE

The discovery of Krebs, tricarboxylic acid cycle (Krebs, 1943) as a mechanism of respiration in plants has clearly indicated and proved the presence and role of several enzyme systems in germinating seeds. Literature contains numerous references (Burris, 1953; Bonner, 1950; Vanfleet, 1952; Weier and Stocking, 1952) on the occurrence of catalase, cytochrome oxidase, peroxidase, phosphatase and polyphenolase activities in higher plants. Interesting studies on the activity of some of these important enzymes have also been reported in detail. Thus Haskins (1955), Ginter and Smith (1953),

Maxwell (1950), Fritz and Beevers (1955), Webster (1952), Goddard (1944), Bhagvat and Hill (1951) and others are among those who have studied the activity of the cytochrome oxidase during the development of corn, in the corn embryo, in the etiolated wheat and barley seedlings, pea seedlings and in tissues of higher plants. Haskins (loc. cit.) mentioned the activity of peroxidase, catalase and phosphatase in the preparation of various corn tissues. Brown and Hendricks (1952) reported studies on enzymatic activities as indications of copper and iron deficiencies in plants. The same authors as well as Eyster (1950) have examined catalase activity in the chloroplast pigment deficient types of corn, and Roca and Ondarza (1954) examined the same in different varieties of maize. Kugler and Bennet (1947) have described the histochemical localisation of acid phosphatase activity in germinating maize kernels. Price and Thimann (1954) have evolved optimum conditions for the extraction of succinic and malic dehydrogenases as well as of succinic and malic and Alpha Ketoglutaric oxidases and have carried out quantitative assays of these in plant tissues (Avena coleoptiles and pisum seedlings). The same authors (1951) have also described succinic dehydrogenases of oat and pea seedlings. Malic and citric dehydrogenases were reported in cucumber seeds by Thunberg (1929). Okumuki (1939) found succinic dehydrogenase in pollen for the first time. Stafford (1951) studied the intracellular localisation of enzymes in pea seedlings. The widespread occurrence of co-enzyme A in vascular plants has been shown by Seifter (1954). He has examined a large number of flowering plants and non-flowering vascular plants. It was found to be present in highest concentration in seeds (in peanut seed 47 units per gram dry weight). Co-enzyme A was also isolated from wheat germ. The Krebs, cycle enzyme system of pea seedlings has been described by Davies (1953). Albaum, and Eichal (1943) have studied the relationship between growth and metabolism in the oat seedling. Hayashi (1940) as well as Paleg (1960, 1961) showed that gibberellins stimulate the activity of amylase in germinating barley and wheat grains. The foregoing account clearly shows the widespread interest taken in recent years on the subject of enzyme activity during the plant metabolism particularly in the germinating seeds.

Some very preliminary studies have already been reported in this regard in the case of coconut seed also. Thus Roxas (1914) showed that the enzyme lipase is present in the germinating coconut haustorium and in lesser amounts in the coconut water. It worked best at the neutral pH and was inhibited by 0.4 per cent cyanide and arsenite. Brill (1919) has also mentioned the presence of lipase in coconut but no definite conclusions appear to have been reached. Sadasivan (1951) worked on the phosphatases and demonstrated their presence in the coconut embryo, kernel and water. The embryo also contained amylase, lipase, protease, invertase, peroxidase, catalase and dehydrogenases. The kernel and water contained peroxidase, catalase and dehydrogenases. The phosphatase had a pH optimum at 5.6 and was inhibited by 10-3 fluoride but not by cyanide or iodoacetate. Wilson and Cutter (Jr.) (1952) described the distribution of acid phosphatases during the development of fruit of *Cocos*

nucifera and found the highest activity of the acid phosphatases in the meristematic nucellus and outer layers of embryo haustorium. Throughout its development the endosperm showed relatively little acid phosphatase or inorganic phosphate although the onset of cellular differentiation was accompanied by a rise in phosphatase activity. Cutter, Wilson and Dube (1952) also described the endogenous oxygen uptake of tissues in the developing fruit of *Cocos nucifera*. They observed that the highest rates of oxygen consumption were in the youngest stages of development, in the regions of the greatest enzymic activity in the new storage tissues and in the young embryos.

While the occurrence of several enzymes is thus no longer in doubt, their characterisation, concentration and intensity of action, etc., in the germinating and growing coconut seed still remain little understood. Since it is well-known that the haustorium is particularly a site of intense metabolic activity functioning to release by enzyme activity the nutrient needs of the seedling it was thought interesting to make a careful re-examination first of the identification of the various enzymes present and their concentrations in the haustorium tissue and then the kinetics of the enzyme activity during the ontogeny of the seedling for the first twelve months after the plumule has emerged. The results of attempts made in a preliminary study to characterise the enzymes are presented in this paper

MATERIAL AND METHODS

The haustorium samples were collected in this preliminary study from six-month old seedlings, counting six months from the time seednuts sown in the nursery sprouted. The haustorium material was carefully cleaned, the required weight being taken from seedlings, which appeared for all practical purposes to be representative, good and energetic, chosen at random from the nursery. The material was then extracted with distilled water or phosphate buffer as the case may be by thoroughly mincing the soft material with the extractant. The extract was then centrifuged to sediment it for about 5 minutes at 3500 r. p. m. and the clarified supernatant liquid was used as the enzyme source.

In all cases methods of testing the enzymes were in general those described by Sumner and Somers (1953) with certain suitable modifications in certain cases. Requisite quantities of the extract were added to the appropriate substrate kept in small conical flasks followed by the necessary quantities of buffer solution to maintain the optimum pH activity range of the particular enzyme and the reaction allowed to begin. The flasks were kept in an incubator maintained at the temperature for optimum enzyme activity. Controls brought up to the same volumes as in the experimental flasks with distilled water or buffer solution as the case may be, but excluding the enzyme source were also always kept in duplicates in each trial. After known intervals the enzyme activity was tested by the appropriate method and the activity approximately assayed. The results are given in Table 1.

DISCUSSION

It is well-known that the coconut seed is self-contained as far as its nutritive requirements are concerned at least up to the first twelve to eighteen months, since the seednut is first put in the nursery bed. It would seem that even after root establishment the seedling derives some nutrition from the haustorium and surviving seednut portions. The husk, the kernel, the nut water and later the haustorium which is formed serve as the source of the nutrients. Table 2 gives the nutrient contents of some of the more important constituents of the germinating seednut. The release of the different nutrient factors to the germinating seednut and growing seedling is achieved through a variety of enzymatic reactions. When the seed is placed in an environment favourable to germination the slow metabolism of the resting seeds becomes rapid and intense, the reactions taking place include hydrolyses, oxidations, desmolyses and syntheses. Stored food is changed from insoluble immovable substances to soluble transportable compounds which get translocated to the embryo. The embryo utilizes these to manufacture the materials needed for the formation of new tissues. There is considerable increase in the enzymic activity of the seed nut during germination. Carbohydrates, proteins and fats are broken down by the appropriate enzymes and resynthesised into the requisite intermediate and final forms in which the tissues of the new growing seedling need them. Besides, hexokinase, phosphorylase, phosphatase and a number of others also appear to function due to the katabolic and anabolic processes. The tests carried out and described in this paper prove that the haustorium tissue is a site of active enzymatic activity as should be expected. There are likely to be several more enzymes active at some stage or other during the development of the seedling and a systematic study embracing the entire eighteen months' growth of the seedling will be worthwhile. These studies are now in progress.

SUMMARY

Several enzymes of the carbohydrase, proteinase and lipase group appear to be active in the haustorium tissues of the coconut seedlings. Some of these have been characterised and an approximate estimate of the intensity of their activity at one stage (during the sixth month after sprouting) of the seedling has been made. The following enzymes were found present – peroxidase, ascorbic acid oxidase, dehydrogenase, catalase, lipase, alkaline phosphatase, acid phosphatase, urease, sucrase, carbonic anhydrase, glutaminase, asparaginase, aspartase and enzyme of proteolytic activity. At the sixth month stage there was high peroxidase and lipase activity in the haustorium.

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TABLE I

Showing the enzyme activity in the haustorium of six months old seedling

Substrate	pH	Enzyme looked for	Method of testing	Intensity of activity	Reference cited for the methods followed
Pyrogallol (0.25% solution) + hydrogen peroxide (10% solution of 20 vol.)	3.5-5.5 Phosphate buffer	Peroxidase	Formation of purpurogallin; the colour of purpurogallin measured and compared	++	Method followed in general with reference to "Chemistry and Methods of Enzymes" by Sumner, J. B. and Somers, G. F. (1953), with suitable modifications.
Sodium salt of Succinic acid (0.1 N solution) + Methylene blue (0.0212%)	6.8 to 8.0 Phosphate buffer	Succinic dehydrogenase	Tested by Thunberg's technique and observed the decolourisation of methylene blue at 37°C due to the formation of leucomethylene blue	+	,, ,, and also "Practical Physiological Chemistry" by Hawk, P. B., Oser, B. L. and Summerson W. H. (1954).
Olive oil (pure)	8.8 NH_4 OH–NH_4Cl buffer	Lipase	Production of fatty acids due to hydrolysis of the substrate used, and the acidity determined by titrating against N/10KoH using thymolphthalein as indicator	++	With general reference to "Practical Physiological Chemistry" by Hawk, P. B., Oser, B. L. and Summerson, W. H. (1954).
Hydrogen peroxide (Standardised against N/20 KMnO4)	6.8 Phosphate buffer	Catalase	Decomposition of hydrogen peroxide and the residual hydrogen peroxide titrated against standard KMnO4	+	Method followed is with general reference to "Chemistry and Methods of Enzymes" by Sumner, J. B. and Somers, G. F. (1953) with suitable modifications.

1	2	3	4	5	6
Sodium-Betaglycerophosphate 0.1 M solution	9.0 NH_4OH NH_4 Cl buffer	Phosphatase (Alkaline)	Decomposition of substrate and the liberated inorganic phosphate tested using molybdenum blue method	+	Method followed is with general reference to "Chemistry and Methods of Enzymes" by Sumner, J. B. and Somers, G. F. (1953) with suitable modifications.
Sodium phenyl phosphate 0.1 M solution	5.6 NaOH CH_3 COOH buffer	Phosphatase (Acid)	" "	+	" " and "Methods in Enzymology" Vol. II by Cotowick, S. P. and Kaplan, N. O. (1960).
Urea (3% solution)	7.0 Phosphate buffer	Urease	Decomposition of urea and the ammonia evolved aerated into 2% boric acid with bromocresol green indicator and titrated against 0.002 NH_2SO_4	+	With reference to "Chemistry and Methods of Enzymes", by Sumner, J. B. and Somers, G. F. (1953) with suitable modifications and also "Practical Physiological Chemistry" by Hawk, P. B., Oser, B. L. and Summerson, W. H. (1954).
Sucrose (6.5%) solution	4.5 Acetate buffer	Sucrase	Reducing sugars formed and Fehling's solution used to determine the reducing sugars	+	" "
Sodium Bicarbonate (1.68%) solution	6.8 Phosphate buffer	Carbonic anhydrase	CO_2 formed aerated into standard Ba $(OH)_2$ solution and titrated against standard oxalic acid	—	" "
Ascorbic acid (0.5%) solution	5.6 Citrate phosphate buffer	Ascorbic acid oxidase	Formation of dehydroascorbic acid and tested with the aid of formation of Bis, 2, 4 dinitrophenyl hydrazone when reacted with 2, 4 dinitrophenyl hydrazine and after that reddish brown colour obtained by addition of 85% H_2SO_4, due to molecular rearrangement of Bis, 2, 4 dinitrophenyl hydrazone	+	" " and "Methods of Biochemical Analysis" Vol. I, Edited by David Glick (1959).

Glutamine (1.2%) solution	8.3 Phosphate buffer	Glutaminase	Ammonia formed aerated into 2% Boric acid and titrated against 0.002 NH_2SO_4	+	With reference to "Chemistry and Methods of Enzymes" by Sumner, J. B. and Somers, G. F. (1953) with suitable modifications and also "Practical Physiological Chemistry" by Hawk, P. B., Oser, B. L. and Summerson, W. H. (1954).
Asparagine (0.6%) solution	8.0 Phosphate buffer	Asparaginase	Ammonia formed aerated into 2% Boric acid and titrated against 0.002 NH_2SO_4	+	,, ,,
Aspartic acid (1.3% solution)	7.5 Phosphate buffer	Aspartase	,, ,,	—	,, ,,
Peptone (5% solution)	7.6 Phosphate buffer	Proteolytic enzymes	Amino acids formed determined by formal titration method	+	With general reference to "Chemistry and Methods of Enzymes" by Sumner, J. B. and Somers, G. F. (1953) and "Methods of Biochemical Analysis" Edited by David Glick, Vol. II, 1958 and "Practical Physiological Chemistry" by Hawk, P. B., Oser, B. L. and Summerson, W. H. (1954).

Indication: ++ Strong activity
+ Positive ,,
— Weak ,,

TABLE 2

Showing the composition of samples of nut water, kernel, husk of fully ripe coconut (12 months) seed and haustorium from six months old seedling.

Analysis for	Nut water	Kernel	Husk	Haustorium from six months old seedling
Carbohydrate				21.39%
(a) Reducing sugars expressed as glucose	0.31%	Trace	—	15.27%
(b) Non-reducing do.	1.03%		—	36.66%
(c) Total sugars do.	1.34%	3.30 % to 12.0%	—	4.170%
Protein (N% x 6.25)	0.1313%	6.563%	1.812%	17.46%
Fat or Oil	<0.1%	69.57% to 71.18%	—	0.6671%
Nitrogen	0.021%	1.050%	0.290%	0.7800%
P_2O_5	0.011%	0.500%	0.093%	1.775%
K_2O	0.259%	1.010%	1.173%	0.1150%
CaO	0.031%	0.067%	0.186%	0.2053%
MgO	0.016%	0.048%	0.038%	92.53 p. p. m.
Iron	0.1 Mg. to 0.5 Mg./100 g*	1.7 Mg./100 g*	—	115 p. p. m.
Manganese	—	—	—	1.614 p. p. m.
Copper	0.04 Mg./100 g*	0.32 Mg./100 g*	—	10.16 p. p. m.
Zinc	—	—	—	—
Sulphur	24.0 Mg./100 g*	44 Mg./100 g*	—	—
Chlorine	183.0 Mg./100 g*	—	—	

* Results from Wealth of India, C. S. I. R. Department of Scientific Research, Government of India, Delhi, 1950.