Journal of Plantation Crops 21 (Supplement) : 313-321 (1993)

# SOLUBILIZATION AND UTILIZATION OF SEED RESERVES DURING THE GERMINATION OF COCONUT (*COCOS NUCIFERA* L. )

C. MANJULA, B. CHEMPAKAM and V. RAJAGOPAL
*Central Plantation Crops Research Institute Kasaragod 671 124, Kerala*

ABSTRACT

**The time taken for germination differs in coconut, depending on the cultivars. In this paper, the solubilization and utilization of seed reserves are monitored during the early stages of germination in two late germinators namely, West Coast Tall(WCT) and the hybrid WCT x Chowghat Orange Dwarf (COD) and in two early germinatiors namely, COD and Malayan Yellow Dwarf (MYD). As germination proceeded, marked changes occurred in the seed reserves like total lipids, proteins, amino acids and carbohydrates. There was a rapid increase in the total sugars, reducing sugars and starch in the haustorium, which revealed the assimilation of sugars during the development of embryo. Similar changes were noticed in the contents of lipids and protein in both the kernel and haustorium. The role of hydrolytic enzymes like amylase, invertase, lipase, esterase and protease in the solubilization and utilization of seed reserves is also clearly brought out.**

## INTRODUCTION

Cultivars of coconut differ in the pattern of seed germination as reported by many workers (Davis and Anandan, 1956; Menon and Pandalai, 1958; Foale, 1968; Thampan, 1975). The first morphological sign of germination is the enlargement of embryo and protrusion of the apical mass outside the shell, which progressively differentiates into shoot and root (Kartha, 1981).

Germination as well as the vigour of the seedlings depends very much on the quantity and quality of the nut. The biochemical changes which are mostly concerned with the breakdown and synthesis of storage products viz. carbohydrates, lipids and proteins influence the same. Several enzyme systems present in the seed also regulate the process of germination. The changes taking place during germination had been reported in several crops like rice (Palmiano and Juliano, 1973; Brown, 1970), cowpea (Harris *et al* 1975) and wheat rye, oat and maize (Okamoto *et al* 1980). But so far only very few studies were carried out in coconut (Nagarajan and Pandalai, 1963; Balasubramanian *et al* 1973; Carpio, 1982 and Balachandran, 1985). In the present paper, the solubilization and utilization of seed reserves in the early phase of germination as well as involvement of hydrolytic enzymes during the process are dealt with in detail.

## MATERIALS AND METHODS

Four coconut cultivars viz. COD (Chowghat Orange Dwarf), MYD (Malayan Yellow Dwarf), WCT (West Coast Tall) and WCT x COD were selected for the experiment. Fifty nuts of uniform maturity from each cultivar/ hybrid were collected and stored under laboratory conditions. The nuts were sown in four beds at random during June 1990. MYD and COD were sampled at weekly intervals, whereas WCT and WCT x COD were sampled at fortnightly intervals. For each sampling, five nuts were collected at random. There was a total of 10 stages, the first stage being taken as prior to sowing. Sampling was recorded in terms of days after sowing (DAS). The experiment was terminated 77 DAS for COD and MYD and 115 DAS for WCT and WCT xCOD. Seednut was taken at every stage, dehusked and split open into halves. The kernel was separated from the shell. At later stages the haustorium, shoot and root were also taken separately for analysis.

### Biochemical constituents
### Preparation of extracts

a) Kernel : Water extracts were prepared from the kernel (1 gm/20 ml w/v). The extract was shaken with a mixture of methanol : petroleum ether : chloroform (2:2:1) twice to remove lipids. The lower aqueous layer was filtered and the clear filtrate was used for the

estimation of total sugars, reducing sugars and amino acids.

b) Haustorium : 0.5 gm tissue was extracted with 15 ml water and squeezed through a cheese cloth. After centrifugation for 5 minutes at 1000g, the clear supernatant was used for analysis.

c) Shoot and root : 0.5 gm tissue each was extracted twice with 10 ml of 80% alcohol. The pooled extract was evaporated to remove alcohol, the aqueous extract made up to 10 ml with water, filtered and used for analysis.

**Analysis**

Total sugars were estimated in the extracts using phenol sulphuric acid method (Dubois *et al*, 1951) and reducing sugars by Nelson's method (Nelson, 1944). The pellet obtained after the centrifugation of the haustorium was washed twice with alcohol, centrifuged and weighed to constant weight, for the determination by the method of Yapinlee and Takahashi (1966) using ninhydrin reagent. The oil content of all the genotypes were determined in the dried kernel (copra) using soxhlet extraction method (AOAC, 1984).

**Enzyme assay**
**Preparation of extracts**

In the case of kernel, 5 gm tissue was extracted under ice cold conditions in 20 ml distilled water and squeezed well through cheese cloth. The final volume made upto 25 ml was centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was used for enzyme assay.

Haustorium (0.5 g) also was treated as above and the supernatant used for enzyme assay.

**Assay**

i) **Amylase and invertase :** The activities were determined by measuring the amount of reducing sugars released by enzymes from their respective substrates (Colowick and Kaplan, 1955), using DNS reagent (Miller, 1972). Specific activities of amylase and invertase were expressed in terms of mg glucose liberated at 30°C per hour per mg protein.

ii) **Protease :** The proteolytic activity was measured by the method of Robinson, E (1956), in which the peptide fragments were estimated by the method of Lowry *et al* (1951). One unit of enzyme activity is defined as the amount that will liberate 1 mg equivalent of peptide fragments under the assay conditions.

iii) **Lipase :** Lipase was assayed in terms of free fatty acids (FFA) using a mixture of equal parts of pure olive oil and 5% gum accacia as substrate (Crandall and Cherry, 1931).

## RESULTS AND DISCUSSION

Since coconut is an oilseed crop, lipids are the prodominant seed reserve, followed by carbohydrates and proteins. It is the solubilization of these seed reserves by hydrolytic enzymes that enhances the rate of assimilation by the embryo in the early stages of germination.

Fig. 1 shows the oil percentage in WCT and WCT x COD, having higher content than the dwarfs COD and MYD. Oil percentage decreased more in the case of COD and MYD (4.8% and 4.1%) over the initial value than in WCT and WCT x COD (3.2% and 2.3%). This could be attributed to the lipid-solubilizing enzyme i.e., lipase. The relationship between oil content and lipase activity is shown in Fig. 2. The fact that higher lipase activity was seen in the dwarfs initially (14-28 DAS) as compared to talls clearly reveals the earlier advantage in the availability of reserve material for germination of the dwarfs. Earlier workers also reported the activity of lipase in seed crops like oil palm (Stumpf, 1983), groundnut (Aruna Sharma and Sen Gupta, 1987) and also in coconut (Sadasivan, 1951; Nagarajan and Pandalai, 1963).

Fig. 3 and 4 show the levels of total and reducing sugars in the kernel, haustorium, root and shoot. Both total and reducing sugars in the kernel showed a more or less decreasing trend as germination proceeded. In haustorium, root and shoot, a steady increase was observed upto the last stage for all the genotypes. The

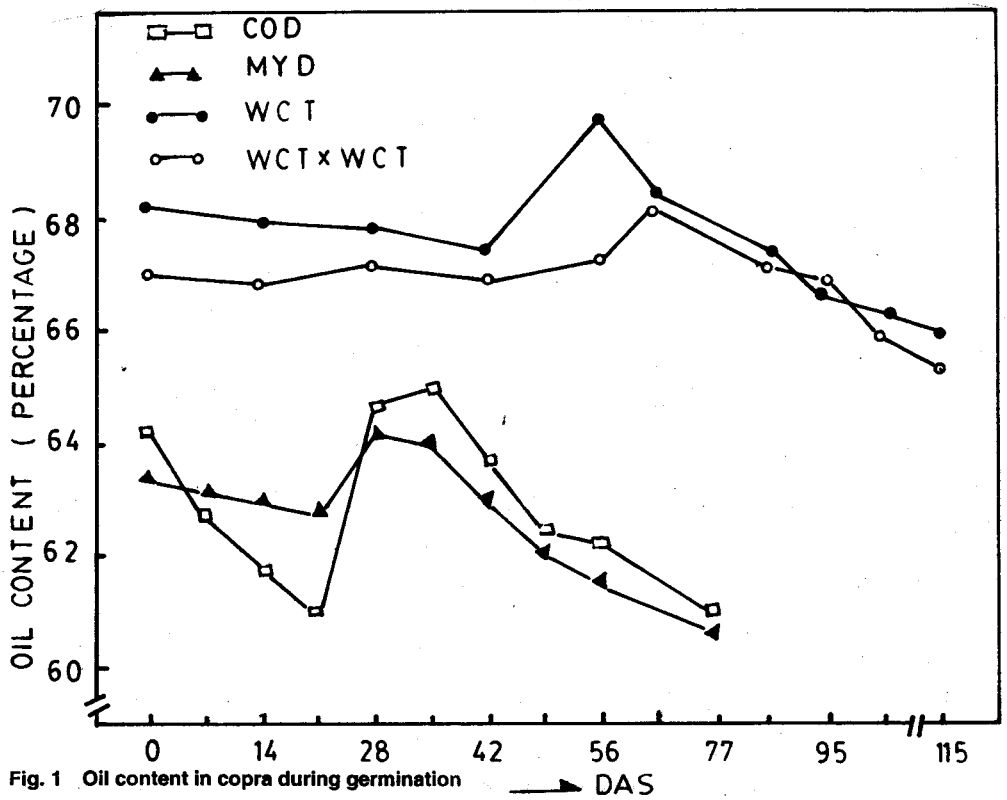


**Fig. 1 Oil content in copra during germination**

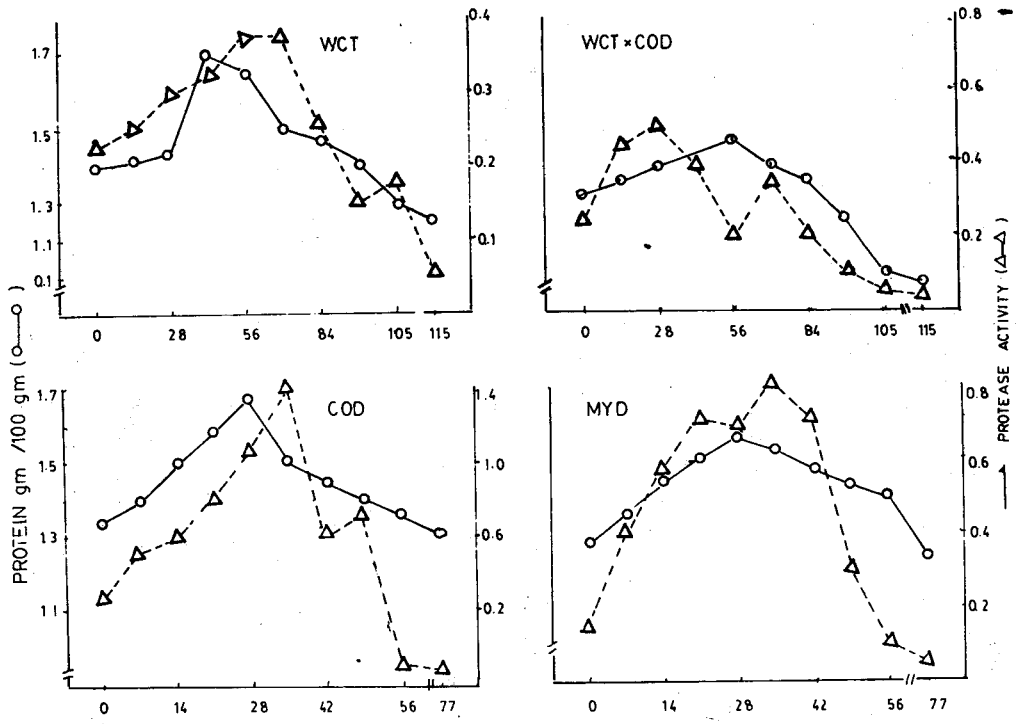


**Fig. 2 Relationship between oil percentage and lipase activity in kernel.**

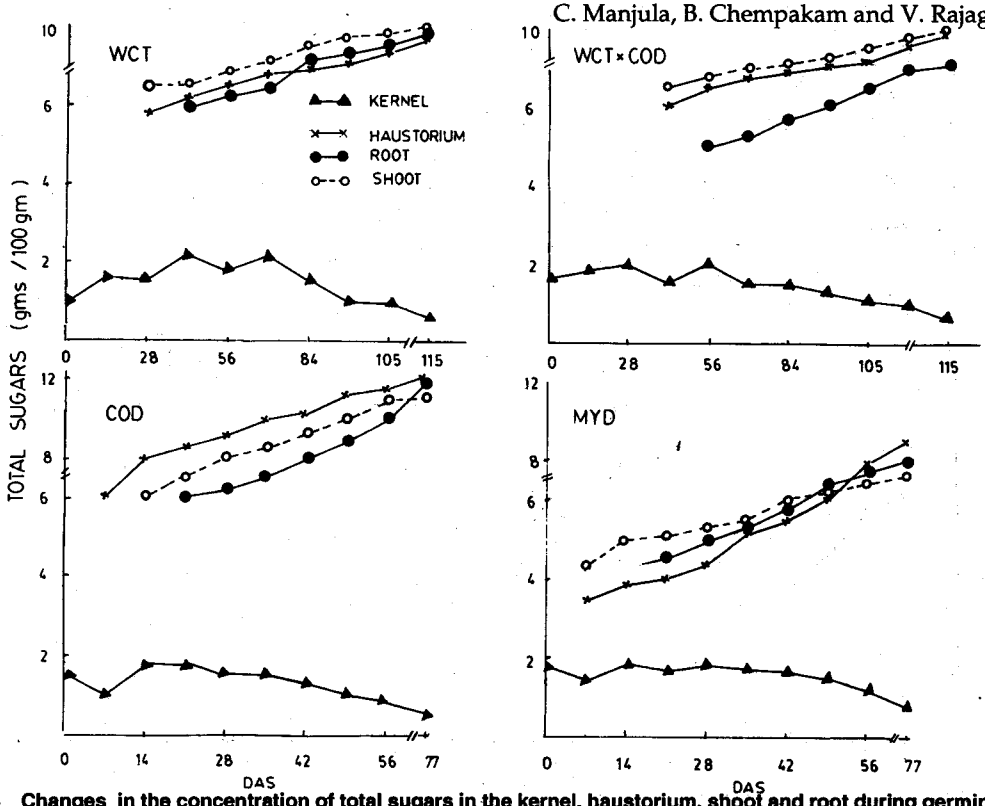


**Fig. 3 Changes in the concentration of total sugars in the kernel, haustorium, shoot and root during germination**

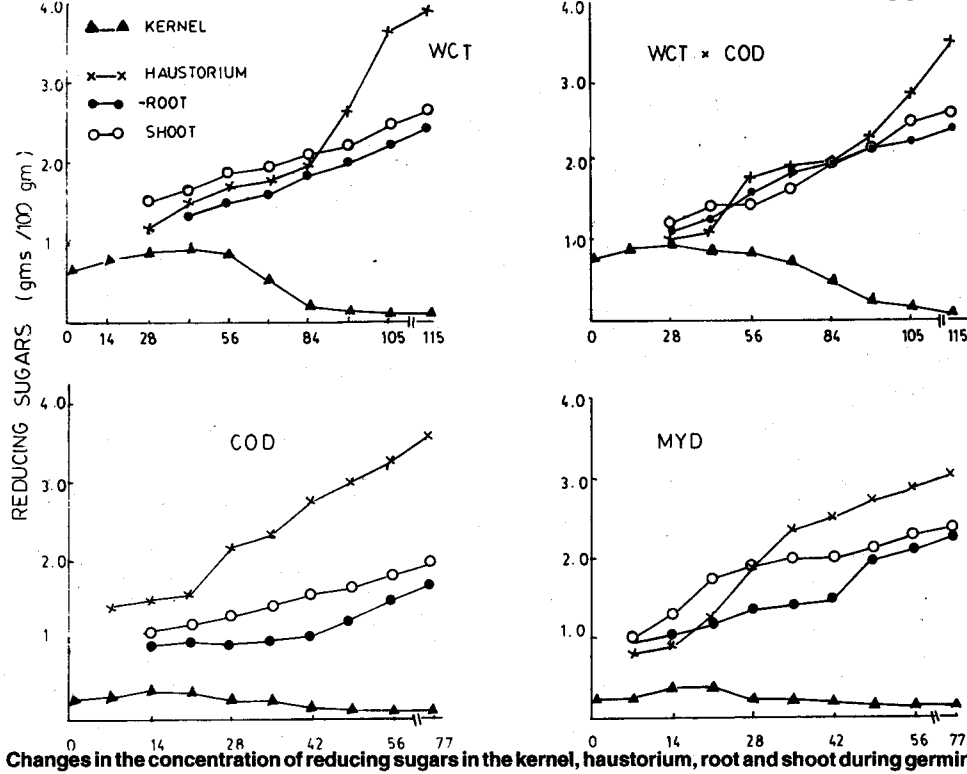


**Fig. 4 Changes in the concentration of reducing sugars in the kernel, haustorium, root and shoot during germination**

changes in the total sugars showed a direct correlation with that of amylase activity (Fig. 5). A similar relationship existed between reducing sugars and the activity of invertase (Fig. 5).

The amount of starch present in the developing haustorium had shown a uniformly increasing trend as germination proceeded. This increase in the haustorium corresponded with a decrease in sugars in the kernel, which is in agreement with earlier observations by Balasubramanian *et al* (1973). The rapid increase in the starch content beyond 58 DAS in WCT and WCT x COD and 42 DAS in dwarfs coincided with lower activity of amylase, as is evident from the correlation graph (Fig. 6). This probably indicates that the soluble sugars serve as food for the growing embryo and the excess is stored as starch in the haustorium to be used in times of need. A similar relationship between the starch and amylase activity during germination has been reported in different plant species like groundnut (Aruna Sharma and Sen Gupta, 1987), rice (Palmiano and Juliano, 1973), barley (Aref and Abdul Bakshi, 1969) and also in coconut (Nagarajan and Pandalai, 1963). The decline in the activity of amylase during the later stages of germination may be attributed to the feedback inhibition by the presence of relatively high content of reducing sugars, which resulted in the accumulation of starch. This is in support of the explanation offered by Eugeniesz *et al*, (1973) on the regulation of enzyme activity.

The content of total free amino acids in the kernel, haustorium, shoot and root is represented in Fig. 7. Amino acids in the kernel showed the maximum content at the time of formation of the haustorium, which then steadily decreased in all the four cultivars. An increasing trend is seen in other tissues as germination proceeded, with the haustorium having the highest content.

With regard to the kernel protein which also serves as a source of reserve food material

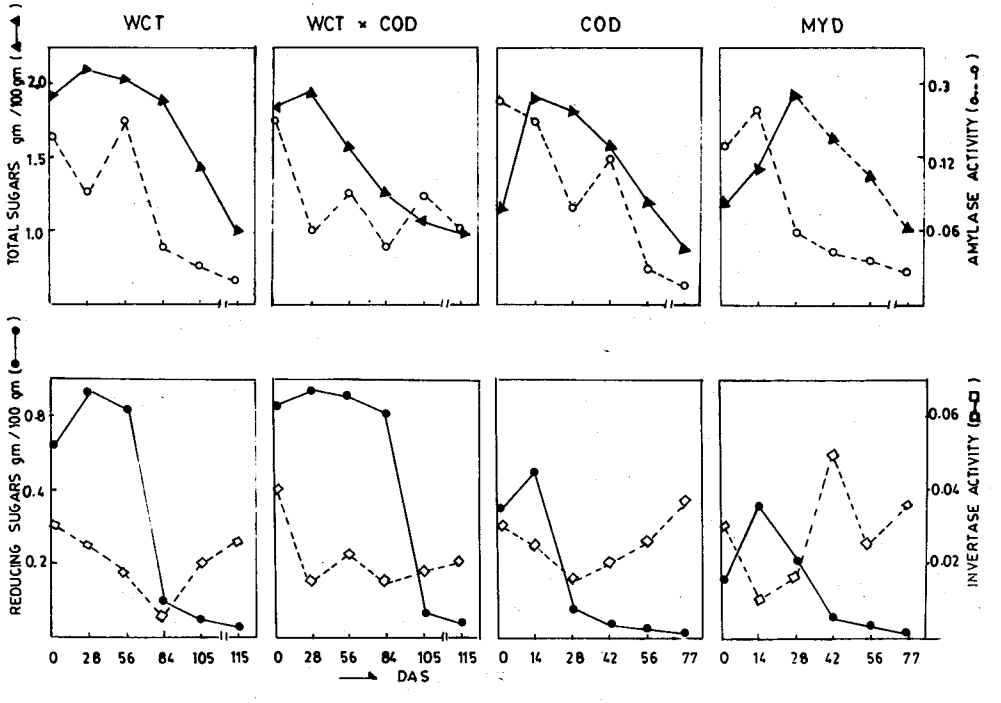


**Fig. 5 Relationship between the amylase activity and total sugars and also invertase activity and reducing sugars in the kernel**

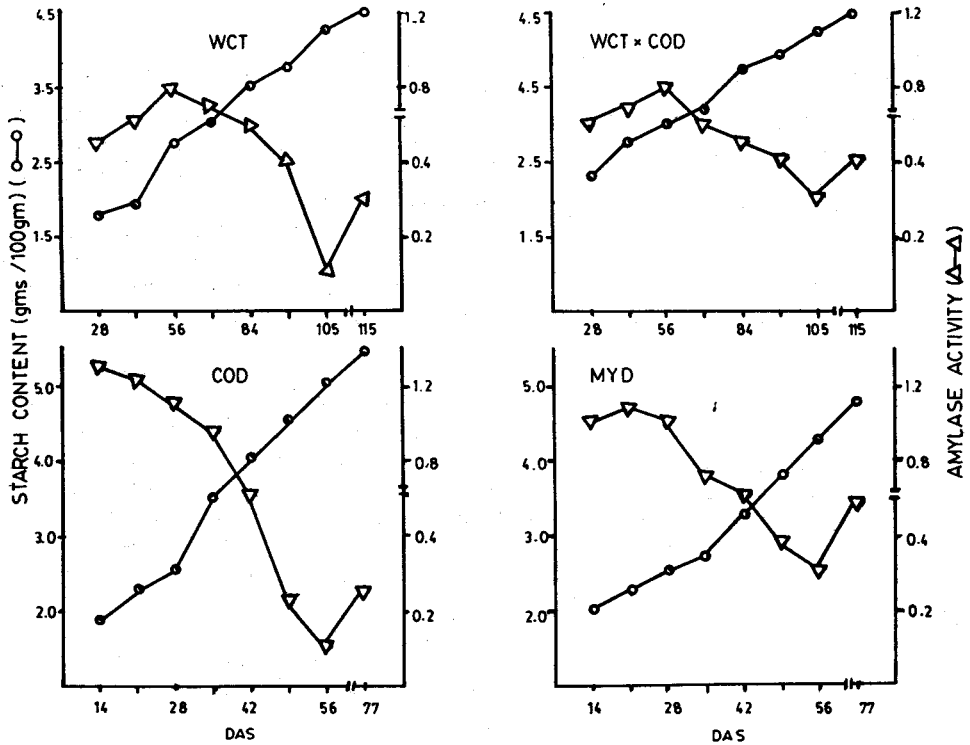


**Fig. 6 Relationship between amylase activity and starch content in haustorium**

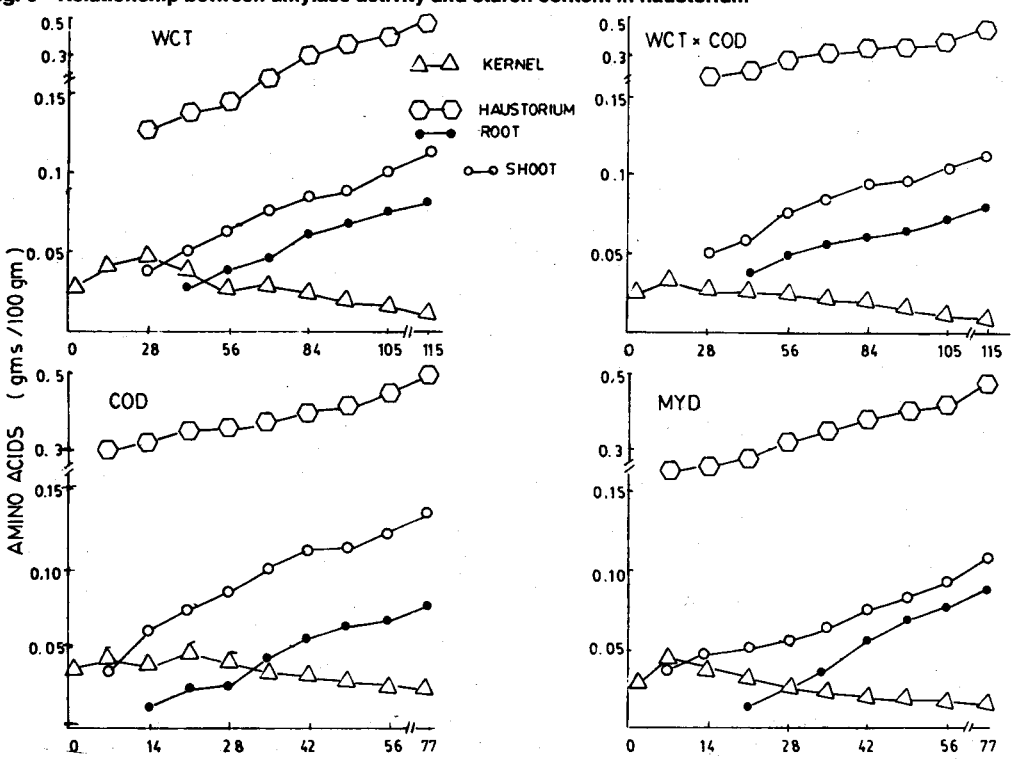


**Fig. 7 Amino acids in the kernel, haustorium, root and shoot during germination**

for the developing embryo, a gradual increase until the formation of the haustorium is seen, followed by a rapid decline throughout the experimental period. The protease activity is almost running parellel to the protein content (Fig. 8) indicating thereby that the function of protease might be different from that directly involved in the breakdown of storage protein (Shoutov and Vaintraub, 1987).

That the embryo continuously draws all the nutrients from the kernel is again indicated by the rapid increase in free aminoacids in the haustorium (Fig. 7) with a cncommitant decline in the kernel. An initial increase in the sugars and aminoacids in the nut water has also been observed (Manjula, 1990), which further supports these observations. Thus it is apparent that the growth of embryo is influenced by the level of reserve food materials in the endosperm. The activities of hydrolytic enzymes affect the solubilisation of these seed reserves accounting for the variability in germination. Early germinating nature of the dwarfs could possibly be due to the faster solubilization of the main reserve food material i.e., lipids followed by carbohydrates and proteins which is reflected in an increase in the activity of the concerned hydrolytic enzymes.

## ACKNOWLEDGEMENTS

The authors are grateful to Sri R. V. Pillai for helpful suggestions and to Sri M. V. George for the statistical analysis of the data.

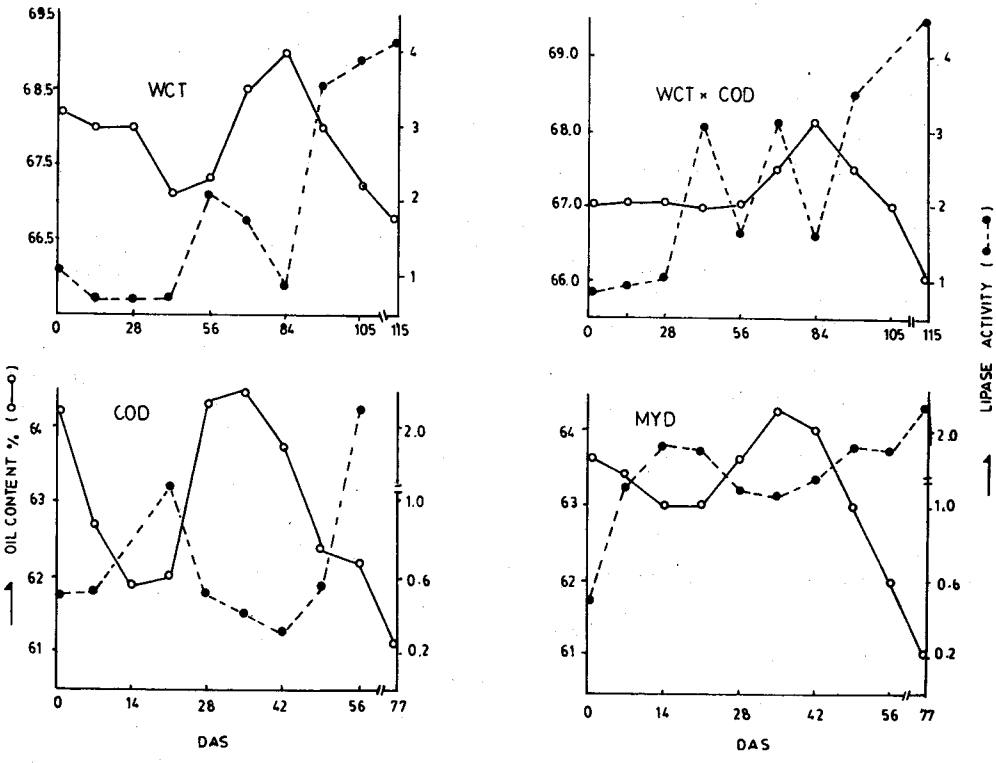


**Fig. 8 Relationship between the activity of protease and total protein in the kernel**

## REFERENCES


AOAC 1984. Edited by Sidney Williams. 14th Edition, Arlington, Virgenia, USA P. 503

AREF, A. and ABDUL BAKSHI 1969. Metabolism of barley seed during early hours of germination. *Plant Physiol* **44** : 733-738.

ARUNA SHARMA and SEN GUPTA 1987. Changes in protease and α-amylase activity in germinating seeds of groundnuts. *Ind. J. Plant Physiol.* **30** : 176-182.

BALACHANDRAN, C. 1985. Distribution of major chemical constituents and fatty acids in different regions of coconut endosperm. *JAOCS* **62** (11) : 1583-1586.

BALASUBRAMANIAN, K., ATUKORALA, T. M. S., WIJESUNDARA, S and HOOVER.A.A. 1973. Biochemical changes during germination of coconut. *Ann. Bot.* **37** : 439-445.

BROWN, R. 1970. Studies on germination and seedling growth, water content, gaseous exchange and dry weights of attached and isolated embryo of barley. *Ann. Bot.* **7** : 93-113.

CARPIO, C. B. 1982. Biochemical studies of *Cocos nucifera* L. *Kalikasan* **11** (2-3) : 319-338.

COLOWICK, S. P. and KAPLAN, NO. 1955. *Methods in Enzymology* Vol. I Academic Press Inc. New York, pp. 657-659.

CRANDALL, L.A., and CHERRY, I.S. 1931. Lipases *In* : Methods in Enzymology Ed. by Colowick. S.P. and Kaplan, N.O. Vol. I. p. 629-630.

DAVIS, T. A. and ANANDAN, A. P. 1956. The first root of coconut. *Indian Cocon.* J. **10** : 9-14.

DUBOIS, M.K., GILLES, K., HAMILTON, J.K., REBERS, D.A. and SMITH 1951. A colorimetric method for the determination of sugars. *Nature* **168** : 174.

EUGENIESZ, PARYS., ELZBIETA ROMANOWSKA and JERSEY POSKUTA. 1973. Amylase activities in etivities in attached and excised cotyledons and in embryonic axis of cotyledons and in embryonic axis of *Pisum sativum. Plant and Cell Physiol.* **24** (2) : 181-188.

FOALE, M.A. 1968. The growth of coconut seedlings, the relationship of endosperm to growth and the effect of nut size and variety on seedling growth. *Oleagineux* **23** : 651-645.

HARRIS, N. M., CHRISPEELS, J. and BOULTER, D. 1975. Biochemical and histochemical studies on protease activity and reserve protein metabolism in cotyledons of germinating cow peas. *J. Exp. Bot.* **26**(5) : 544-554.

KARTHA, S. 1981. Embryo of coconut and its germination - A short scientific note. *J. Plantn. Crops* **9** (2) : 125-127.

LOWRY, O.H., ROSEBROUGH, N.J., FARB, A.L. and RANDALL, R.J. 1951. Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **193** : 265-275.

MANJULA, C. 1990. Physiological and biochemical changes during the germination of coconut seeds, M.Phil. Dissertation, Mangalore University. pp.1-79.

MENON, K.P.V. and PANDALAI, K.M. 1958. The *Coconut Palm - A Monograph.* Indian Central Coconut Committee, Ernakulam, South India. pp. 384.

MILLER, G. L 1972. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31** : 426-428

NAGARAJAN, M. and PANDALAI, K.M. 1963. Studies on the enzyme activity in the haustorium of the germinating coconut. *Indian Cocon.* J. **17** : 25-34.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153** : 375-380.

OKAMOTO, K. , HEDEMI KITANO and TAKASHI AKAZAWA 1980. Biosynthesis and excretion of hydrolases in germinating cereal seeds. *Plant* and *Cell Physiol.* **21** : (1-4).

PALMIANO, E.P. and JULIANO, B.O. 1973. Changes in the activity of some hydrolases - Peroxidases and Catalases in the rice seeds during germination. *Plant Physiol.* **52** : 274-277.

ROBINSON, E. 1956. Proteolytic enzyme in growing root cells. *J. Exp. Bot.* **7** : 296.

SADASIVAN, V. 1951. The phosphatases in coconut. *Arch. Biochem.* **30** : 159-164.

SHOUTOV A. D. and VAINTRAUB, I. A. 1987. Degradation of storage proteins in germinating seeds. *Phytochemistry,* **26** : 1557-1566.

STUMPF, P.K. OOKE 1983. Some enzymic activities in germinating oil palm seedlings. *Plant. Physiol.* 73 : 1028-1038.

THAMPAN, P.K. 1975. *The Coconut palm and its products.* Green Villa Public house, Cochin. pp. 302.

YAPINLEE and TUNEKAZU TAKAHASHI. 1966. An improved colorimetric determination of aminoacids with the use of ninhydrin. *Analytical Biochemistry,* **14** : 71-77.

## DISCUSSION

P.R.V. SUBRAMANIA IYER : Did you estimate RNA activity?

B. CHEMPAKAM : No.

M.K. MISHRA : Whether you have done any quantitative analysis of seed reserves during successive stages of seed germination? Whether you attempted for RNA, DNA and enzymatic analysis associated with it?

B. CHEMPAKAM : Quantitative studies have not been taken up in the present context. Our main objective was to analyse the seed reserves during germination. Hence the analysis of RNA and DNA was not carried out.