Carbonic Anhydrase Activity Versus Acidity in Low Yielding & High Yielding Coconut Palms (*Cocos nucifera* Linn.)

G. Padmaja*, B. Sumathykutty Amma, Chacko Mathew, P. K. Ray & R. Snehi Dwivedi†

Central Plantation Crops Research Institute, Regional Station Kayangulam, Krishnapuram 690 533

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Activity of carbonic anhydrase (CA) was determined in unfertilized female flowers, one month old and two months old buttons from West Coast Tall Variety of coconut. The titratable acidity pattern was also studied in these samples. An increased CA activity was noticed in the high yielding coconut palms. A reciprocal relationship was obtained between CA activity and titratable acidity. The probable role of CA as a key enzyme regulating the *p*H of the plant cell has been envisaged.

CARBONIC anhydrase (CA) has been reported to be present in C_3 as well as C_4 plants and is believed to play a role in the fixation of carbon in plants[1,2]. Enzyme studies[3] conducted in the haustorium of germinating coconut have revealed the presence of CA in the haustorium. Carbonic anhydrase catalyses the reversible hydration of CO_2 thereby making available CO_2 for the photosynthetic activities of the plant cell[4,5].

Earlier studies in our laboratory by Dwivedi *et al*[6]. indicate a positive correlation between the enzyme activity and nut yield of the coconut palm. As a follow up of this study, CA activity was determined in 3 different stages of growth of the ovary as also their respective rachillae. An attempt has also been made to correlate the enzyme activity with the acidity of the buttons and rachillae.

The experiment was conducted in the West Coast Tall variety of coconut palms growing in two different locations, viz. farm area of CPCRI Regional Station, Kayangulam and CPCRI, Kasaragod. The former is a diseased area where as the latter is a healthy area. Ten high yielding (>80 nuts/palm/year) and 10 low yielding (<50 nuts/palm/year) coconut palms were selected from each location for the sampling purpose. Buttons (fertilized and unfertilized female flowers) at 3 different stages, viz. just after opening, 1 month after fertilization and 2 months after fertilization were collected from both sets of palms and the samples were preserved in an ice-box.

Carbonic anhydrase activity — The CA activity was determined in the buttons (both fertilized and unfertilized) by the modified procedure of Dwivedi *et al*[6]. One gram each of the tissues were chilled at 0°C for 30 min. The chilled samples were cut into 10 ml of 0.2*M* cystein-EDTA solution (*p*H 6.8-7). The samples were then blotted to remove adhering EDTA/cystein and transferred to the reaction mixture containing 2 ml of 0.2 *M* phosphate buffer, 2 ml of 0.2*M* sodium bicarbonate and 0.5 ml of 0.002% bromothymol blue indicator and contained in a wide mouthed glass vials. The mixture was incubated at 0-4°C for 20 min. The vials were then immediately exposed to room temp. to arrest the enzyme activity. Aliquot (1ml) was withdrawn from these vials and CA activity was determined by titration against N/200 HCl using methyl red indicator. The end point was marked by the sudden appearance of a pink colour. Simultaneously a control tube containing the reaction mediuma long with two drops of 0.2 *M* cystein/EDTA was also incubated and titrated against *N*/200 HCl to eliminate error due to blank.

Titratable acidity — One g each of the finely cut tissues from both set of palms was weighed into clean dry mortars, macerated well with a little acid-washed sand and extracted with 50 ml of double-distilled water at room temp. The extracts were filtered through a buchner funnel and the filtrates collected in stoppered flasks of 50 ml capacity. Five ml each of the clear filtrate was titrated against N/200 sodium bicarbonate using phenolphthalein as indicator. To eliminate error due to blank, 5 ml double-distilled water was also titrated.

The results of the study indicated a positive correlation between CA activity and yielding capacity of a palm, i.e. the high yielding palms which had a greater yielding capacity had greater CA activity. An interesting observation was that the high yielding palms which had greater CA activity had low acidity where as the low yielding palms which had lower CA activity had greater acidity (Tables 1 & 2). The activity of CA progressively increased with the maturity of the buttons (Table 1) where as in the rachillae, no steady increase could be obtained. At Kasaragod which is a healthy area, the CA activity of all the 3 stages of maturity of the buttons and

Table 1 — Carbonic Anhydrase Activity* in the bottons and Rachillae of low Yielding and high Yielding Coconut Palms at Kayangulam and Kasaragod

[Values, expressed as 1 unit = mg of CO_2/g dry wt/20 min. are, mean of 10 observations]

Description of sample	Kayangulam		Kasaragod	
	Low yielder	High yielder	Low yielder	High yielder
Unfertilized female flowers	5.96	10.56[a]	5.43	10.94[a]
Buttons (1 month after fertilization)	10.23	11.46[c]	6.68	15.49[a]
Buttons (2 months after fertilization)	11.31	13.32[c]	7.67	17.36[a]
Rachillae (from unfertilized female flowers)	5.31	8.13[b]	2.90	4.78[a]
Rachillae (from 1 month old buttons)	7.36	7.62[c]	2.80	5.86[a]
Rachillae (from 2 months old buttons)	7.03	7.48[c]	2.80	5.37[a]

*Low yielding Coconut plams were compared with high yielding plams for analysing statistical significance.

P values : [a] < 0.01; [b] < 0.05; [c] < N.S.

Present address : *Central Tuber Crops Research Institute, Sreekariyam, Trivandrum 695 017
†Central Soil and Salinity Research Institute, Karnal.

rachillae of the low yielding and high yielding palms was found to be highly significant (Table 1). However as far as the acidity is considered, a significant difference could be obtained in the unfertilized female flowers and 2 months old buttons while the 1 month old buttons showed a significance at 5% level only. However at Kayangulam which is a diseased area where most of the palms are suffering from root (wilt) disease, no significant difference could be obtained in 1 month and 2 month old buttons and their respective rachillae. But the CA activity of the unfertilized female flowers was highly significant although the rachillae showed only little significance. It can be inferred from this that at Kayangulam, as the growth of the buttons progresses some metabolic changes occur in the button and the rachillae which inhibit the CA activity thereby leading to a lowering of the active enzyme concentration. This is further supported by the Button/Rachillae graph of CA activity Fig. 1. This clearly indicates that the high yielding and the low yielding palms at Kasaragod definitely have a higher Button/Rachillae ratio when compared to those at Kayangulam. Although the exact role of CA is not known, the data show that CA significantly influences the yielding capacity of the palm. Experiments conducted in spinach chloroplasts[7] have showed that the enzyme has a catalytic role in making CO_2 available for RUDP carboxylase, a key enzyme of the photosynthetic pathway. As soon as fertilization is over and a series of metabolic reactions set in, the activity of CA also increases thereby making available CO_2 and/or HCO_3^- necessary for the reactions of the plant cell. As has been demonstrated with spinach chloroplasts[8], the permease action could not be demonstrated with coconut. However the reciprocal relationship between CA activity and acidity may be due to the fact that CA probably favours the accumulation of HCO_3^- in the high yielding palms which have a

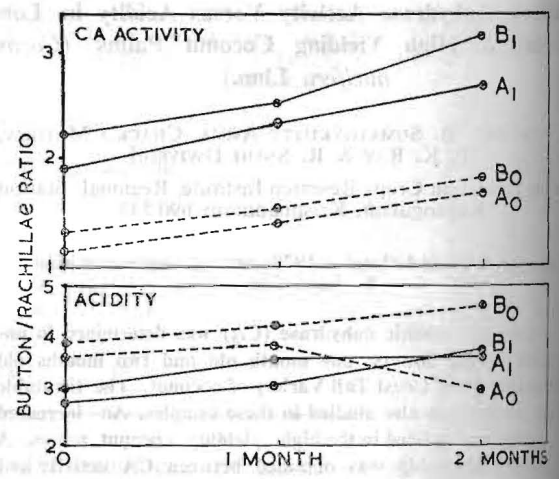

Fig. 1 — The Button/Rachillae graph of CA activity and acidity

[A_0 — Low yielder (Kayangulam); B_0 — High yielder (Kayangulam); A_1 — Low yielder (Kasaragod); B_1 — High yielder (Kasaragod)]

greater photosynthetic activity. Studies conducted in *Chlamydomonas reinhardi* by Grahman *et al.*[9] also demonstrate that cells which were grown in air where CA was present showed a much larger *p*H change when compared to those grown in 5% CO_2 where CA activity was least. The Button/Rachillae ratio of CA activity of the low yielding palms at Kasaragod and Kayangulam followed a linear pattern. However the B/R graph of the high yielding palms at Kasaraged showed a linear pattern upto one month and a steep rise was obtained in the second month. The rise was not so marked for the high yielding palms at Kayangulam. The rapid increase in CA activity (Fig. 1) with a concomitant decrease in the acidity is therefore a predisposing factor for the yield of the palm. Studies conducted in pea chloroplasts by Everson and Graham[10] have shown that the effect of CA was to counteract the light induced *p*H changes. The results of the present study indicate a similar role for CA in coconut also. As the *p*H of the plant cell is an important factor regulating the physiological and biochemical processes of the cell, the above studies lead to the conclusion that CA apart from the role in CO_2 fixation also is a key enzyme regulating the *p*H of the plant cell by favouring the reversible hydration-dehydration reaction.

TABLE 2 — TOTAL ACIDITY* IN THE BUTTONS AND RACHILLAE OF LOW YIELDING AND HIGH YIELDING COCONUT PALMS AT KAYANGULAM AND KASARAGOD

[Values, expressed as 1 unit = mg of sodium bicarbonate/100 mg dry wt, are mean of 10 observations]

Description of sample	Kayangulam		Kasaragod	
	Low yielder	High yielder	Low yielder	High yielder
Unfertilized female flowers	25.93	20.727[c]	33.718	27.566[a]
Buttons (1 month after fertilization)	28.08	22.047[c]	39.418	31.079[b]
Buttons (2 months after fertilization	22.518	19.63[c]	39.614	30.652[a]
Rachillae (from unfertilized female flowers)	7.138	5 436[b]	11.363	9.992[c]
Rachillae (from 1 month old buttons)	6.879	5.064[c]	10.583	8.992[c]
Rachillae (from 2 month old buttons)	6.99	3.988[a]	9.926	7.578b

*Low yielding Coconut palms were compared with high yielding palms for analysing statistical significance.

P Values : [a] < 0.01; [b] < 0.05; [c] < N.S.

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Testosterone & Accessory Sex Gland Lipids

E. UMAPATHY, K. S., MANIMEGALAI & P. GOVINDARAJULU
Division of Endocrinology, Department of Physiology, Postgraduate Institute of Basic Medical Sciences, Madras 600 042

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Testosterone propionate (TP) 1 mg/100 g body wt was administered to adult male rats and its effect on lipids of accessory sex glands was studied. Short and long term studies were undertaken to find out the early and late responses. TP was found to increase the total lipids even in one day treatment in all the accessory gland tissues, which was due to its preferential stimulation of phospho- and neutral lipid synthesis.

THE importance of lipids and lipid fractions in sperm maturation is well established. Recent research has implicated the epididymis as a major source of substrates- which can support the metabolic activity of maturing sperms. Glyceryl phosphoryl choline (GPC) has been suggested as the secretory product of the holocrine cells in rat epididymis[2]. Scott *et al.*[3] proposed that GPC is formed from phospholipids in epididymis. Lipids have been suggested to have a protecting effect on the spermatozoa, by split ejaculate studies[4]. Previous studies have shown a distinct lipid distributory pattern in various accessory sex glands[5] and preliminary studies have shown significant hormone-lipid inter-relationship in accessory sex glands[6,7]. The present study is a part of a series of experiments conducted to understand the lipid hormone inter-relation in accessory sex glands. Initial fluctuation of lipids has been studied in one day experiment and prolonged influence has been visualized through 7 day experiments following the administration of TP.

Thirty male albino rats (100-110 days old, 190-200 g body wt) of Wistar strain were used in the present investigations. The animals were divided into 3 groups of 10 each. The first group served as control and received only the vehicles (Pea nut oil). The second group was treated with Testosterone propionate (TP) (1 mg/100 g body wt) for a day. The third group was treated with TP (1 mg/100 g body wt) for 7 days.

The animals were sacrificed by cervical dislocation, 24 hr after the administration of TP in the case of group II animals and on the 8th day in the case of group III animals. Caput, cauda epididymides, seminal vesicle and prostate (all lobes) were removed immediately, freed from adhering tissues, rinsed, blotted and weighed accurately on a torsion balance, for further processing.

Experimental procedures regarding the extraction, identification and estimation of lipids have already been reported[9]. The results were treated for statistical significance using the students 't' test. The calculations were computerised in IBM-1620 computer and statistically evaluated.

Administration of TP resulted in changes in lipid parameters of accessory sex glands in both short and long term treatments which are depicted in Table 1. In caput epididymis an increase in triglycerides ($P < 0.001$) was seen in long term groups. TP had a profound influence on lysophosphatidyl choline and sphingomyelin ($P < 0.001$) in short term groups. However, in long term treatment phosphatidyl ethanolamine and phosphatidic acid were elevated ($P < 0.001$). In cauda epididymis, phosphatidyl choline ($P < 0.01$) and phosphatidic acid ($P < 0.001$) were decreased in long term group.

In seminal vesicle both in short, ($P < 0.01$) and long, term ($P < 0.001$) treatment, triglycerides were increased. Long term treatment led to an increase in phosphatidyl choline ($P < 0.02$) in seminal vesicles. In prostate, triglycerides were accumulated in both short ($P < 0.001$) and long term treatments ($P < 0.001$). Phosphatidyl choline ($P < 0.001$) phosphatidyl ethanolamine ($P < 0.05$) and phosphatidyl inositol ($P < 0.01$) showed an increase in long term group.

Total, free and ester cholesterol were not significantly altered in either short or long term studies in any of the tissues.

As evidenced in our earlier studies[7], testosterone had a differential effect on the two segments of epididymis. The differences in the response may be due to the differences in their structure and function[8]. The metabolic activities in the epididymal tissue as well as epididymal spermatozoa have been shown to be different in different regions of epididymis[9]. It is also suggested that caput primarily serves as a conduit in addition to its role in sperm maturation[10]. while cauda functions as a store house for sperms[11]. Much of the lipid changes (both neutral and phospho) that take place in sperm were reported to be essentially in caput[12], and it has been reported that sperms collected from caput contained more phospholipids than that of cauda[13]. Not only the tissues exhibited regional variations in their (metabolic) activities but the responsiveness to androgen as well as its threshold of requirement for the maintenance of the different cell organelles in the epididymis also seems to be different[14]. These different factors could have contributed for the differential response observed in the present study.

The phospholipids have shown characteristic alterations in the two segments of epididymis. This may be due to more GPC synthesis seen in caput than in