

CARBONIC ANHYDRASE ACTIVITY IN RELATION TO NUT YIELD OF COCONUT PALM

R. SMEHI DWIVEDI, CHACKO MATHEW, P. K. RAY,
B. SUMATHY KUTTY AMMA & SUNNY NINAN

Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala, India.

The activity of carbonic anhydrase (CA) was determined in the ovaries and male flowers of West Coast Tall variety of coconut palms bearing green nuts. The enzyme was found to be absent (not detectable) in male flowers but present in the ovaries. With increasing nut yield the level of enzyme increased in the ovaries and the activity was found to be 606, 820 and 1090 $\mu\text{g CO}_2$ released/g dry Wt/20 minutes in the palm yielding <50, 50 to 80 and > 80 nuts/year respectively. A positive correlation of 0.84 between enzyme activity and nut yield was obtained. The use of CA activity as one of the parameters for selecting stable high yielding variety of coconut has been suggested.

CARBONIC anhydrase has been reported to catalyse the reversible hydration of CO_2 and thereby facilitate the diffusion of CO_2 through the liquid phase of the cell to the chloroplast, in the process of photosynthesis¹⁻². In algae grown at high CO_2 level, the photosynthetic rate was first negligible but later increased in step with increasing carbonic anhydrase activity³. The enzyme was found to be absent in C_4 plants⁴. But recent studies have indicated that it is present in the leaves of both C_3 and C_4 plants and implicate the process of carbon fixation⁵⁻⁶. Although the reproductive organs of wheat, rice, barley, etc, trap solar energy and contribute significantly in grain production⁷⁻⁸, very little is known about the presence of carbonic anhydrase in them and the relation of this enzyme with the economic yield of plants. In the present study an attempt has been made to find out the presence of carbonic anhydrase in the reproductive organs of coconut palms and its relation with nut productivity.

Material and Methods

West Coast Tall variety of coconut palms bearing green nuts were selected at two locations, viz. sandy loam and red sandy loam soil regions. Ten palms of equal age and almost similar height and vigour were marked randomly at each location and three ovaries and male flowers (Perianth lobes and stamens) were taken from each palm for carbonic anhydrase enzyme determination. The activity of carbonic anhydrase was determined by the method of Dwivedi and Randhawa⁹ with some modifications.

One gram fresh ovary/male flowers were chilled at zero degree centigrade for 30 minutes. Chilled samples were cut at 0-4°C in 10 ml of neutralised (pH 6.8-7.0) 0.2M Cystein/EDTA solution. These cut samples were blotted to remove adhered EDTA/Cystein at the same temperature and transferred

to reaction mixture (2 ml 0.2 M phosphate buffer pH 6.8, 2 ml 0.2 M Sodium bicarbonate and 0.5 ml 0.002% Bromothymal blue indicator) contained in a wide mouthed 10 ml glass vial and incubated at 0–4°C for 20 minutes. In control reaction mixture, to maintain the uniformity 0.2 to 0.5 ml Cystein/EDTA of aforesaid concentration and pH was added while incubating it along with treated samples. This was done because while cutting the plant samples, the Cystein/EDTA which is adhered to them is not removed completely by blotting and as such is added in reaction mixture automatically while transferring plant samples to it. After completion of incubation period the vials were immediately exposed to room temperature (30–40°C) or transferred to hot water for two minutes to check the enzyme activity. One ml aliquot from control and treated reaction mixture were pipetted separately and titrated against N/200 HCl using methyl red indicator till the appearance of pink colour as end point. The amount of CO₂ released in the catalytic action of enzyme was calculated.

After confirming the presence of carbonic anhydrase in the ovary (Table-I), three yield groups of coconut palms viz: 20-50, 51-80, and 81-110 nuts/year were selected in sandy loam soil region. In each yield group 10 palms marked and three ovaries from each palm were subjected for carbonic anhydrase analysis. Based on the results of this study another experiment in detail was started to find out the relation of CA with the nut productivity of coconut. In this experiment WCT variety of coconut bearing green nut and yielding 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 and 110 nuts/year were marked. From each group ten palms were selected and from each palm three ovaries were subjected for CA determination.

Results and Discussion

From the results reported in Table I, it is evident that carbonic anhydrase was present in the ovaries and absent (not detectable) in the male flowers of all the coconut palms growing in sandy loam and red sandy loam soil regions. The enzyme activity in the ovary of palms growing in the region of red sandy

TABLE—1 Carbonic anhydrase activity in the reproductive Parts of coconut growing in two soil types with special reference to their Physico-chemical properties.

Soil type	Carbonic anhydrase activity ($\mu\text{g CO}_2$ released/g drymatter/20 minutes).		*Physico-chemical properties of soils			*Zinc Content in the leaves of palms (ppm)
	Ovary	Male flowers	Organic carbon (%)	pH	Zinc content (ppm)	
Red sandy- Loam	680	Not detectable	0.36	6.1	0.88	24.6
Sandy Loam	510	Not detectable	0.31	5.8	0.78	20.6

*(Pillai *et al.* 1975 and Sankaranarayanan *et al.* 1966),

loam was higher than that of sandy loam soils. This may be due to higher concentration of zinc in the soils and palms of former region as compared to latter one (Table-I). Dwivedi and Randhawa⁹ and Randall and Bouma¹⁰ also reported that the activity of carbonic anhydrase increases with increasing zinc content in plants since the enzyme is zinc-constituted protein¹¹.

The enzyme activity in the ovary of palms belonging to high yielders (>80 nuts/palm/year) was significantly higher than that of medium yielders 50-80 nuts/palm/year), while the activity in the medium yielders was higher than that of low yielders (<50 nuts/palm/year) (Table-2A). Based on this observation, the enzyme activity was measured in the ovary of various yielding groups viz: 10, 20, 30, 40, 59, 60, 70, 80, 90, 100 and 110 nuts/palm/year and it was found to increase significantly with increasing nut yield except in palms yielding 50 and 70 nuts/year (Table-B2). A positive significant correlation of 0.84 and regression equation $Y = -27.78 + 0.11X$ was recorded between nut yield and enzyme activity. (Fig. 1). The cause for higher nut yield due to the presence of high carbonic anhydrase activity in the ovary has not yet been explored. It could be presumed that carbonic anhydrase helps in CO_2 fixation and dry matter production^{8,12} which in turn may be mobilised to nuts as a result of which competition among nuts for photosynthates supplied through

TABLE—2 Carbonic anhydrase activity in the ovary of various yield groups of West Coast Tall variety of Coconut palms

(A)

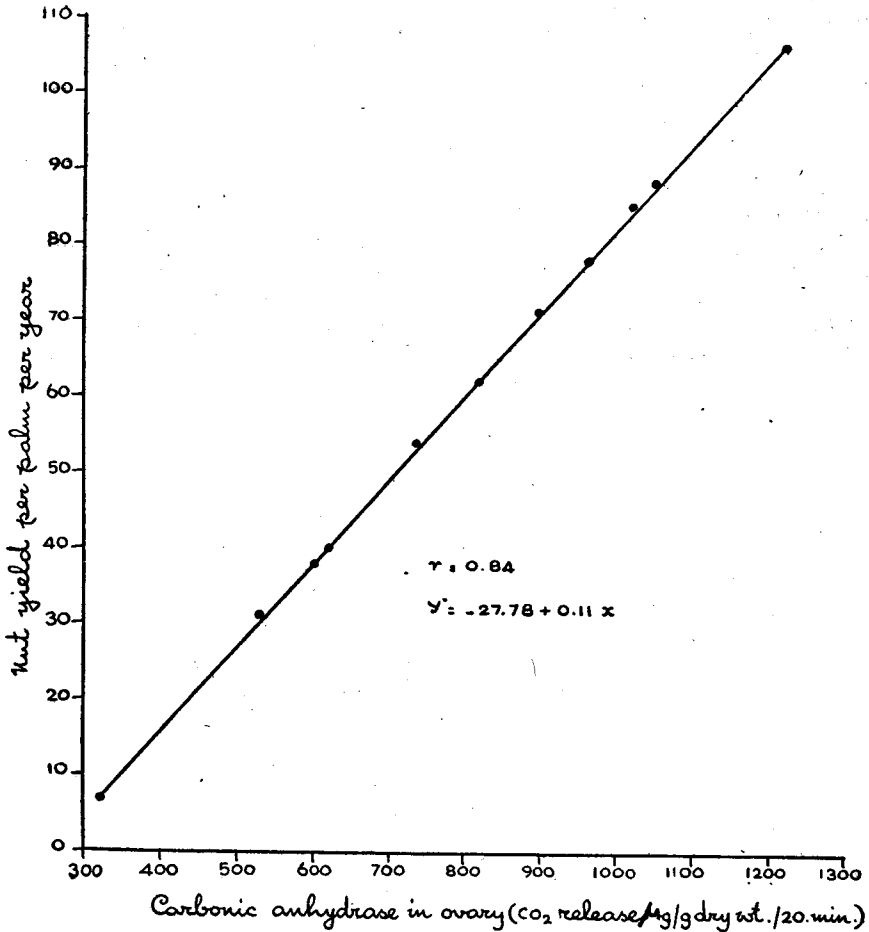
Yield group (Nuts/palm/year)	<50	50 to 80	>80
Enzyme activity ($\mu\text{g Co}_2$ released/g dry weight/20 minutes)	606	820	1097

(B)

Yield group (Nut/palm/year)	10	20	30	40	50	60	70	80	90	100	110
Enzym activity ($\mu\text{g Co}_2$ released/g dry weight/ 20 minutes)	320	620	737	820	533	897	600	963	1020	1050	1220

leaf source is reduced and good growth of individual nut occurs. The higher activity of carbonic anhydrase in the ovaries may be genetic character of high yielders. In such palms the physiological and biochemical processes related to the supply of zinc and its utilization in the synthesis of enzyme protein may be efficient and this may be perhaps one of the main reason for high carbonic anhydrase activity in their ovaries. This needs further investigation.

The coconut is an open pollinated palm and the variation among the palms for nut yield is so much that it occurs at highly significant level. Therefore on the basis of the results and correlation value of +0.84 between CA activity

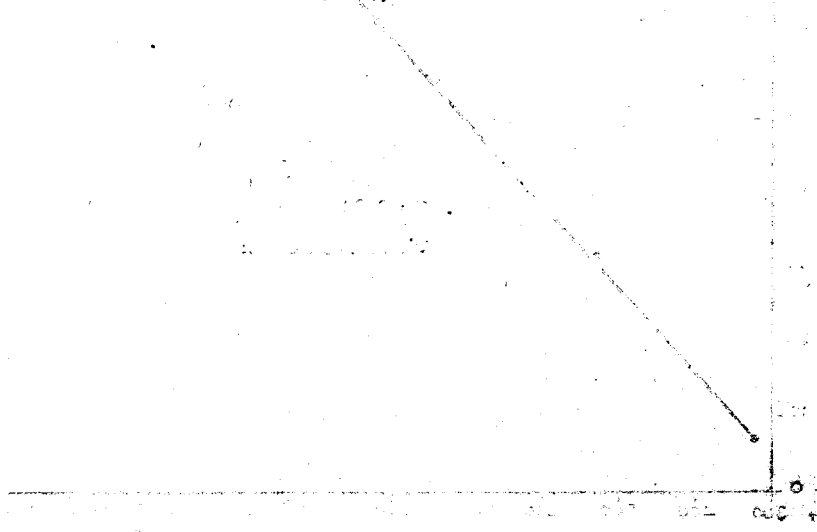


and nut yield and regression equation $Y = -27.78 + 0.11 \times$ of present investigation it could be suggested that carbonic anhydrase activity may be used as an important parameter for selection of stable high yielding varieties of coconut.

References

1. HATCH, M.D. & SLACK, C.R., *Ann. Rev. Plant Physiol.*, 21 (1970), 141.
2. ZELITCH, I., *Photosynthesis, Photorespiration and Plant Productivity*, Academic Press New York (1971), 107.
3. GRAHAM, D. & REED, N.L., *Nature. New. Biol.*, 231 (1971) 81.
4. HESKETH, J., MURANYOTS, H. & SHARKAWY, M., ET., Report No. 2 on Photosynthesis. Arizona, Agric. Res. Stn. USA. (1965).
5. POINCELOT, R. P., *Plant Physiol.*, 50 (1972), 336.
6. RATHNAM, C.K.M. & DAS, V.S.R., *Z. Pflanzenphysiol. Bd.* 75.5 (1975), 360.
7. YOSHIDA, S., *Ann. Rev. Plant Physiol.*, 23 (1972), 437.

8. DWIVEDI R. SNEHI., *Ann. Bot.*, **39** (1972), 1077.
9. DWIVEDI R. SNEHI & RANDHAWA, N.S., *Plant and Soil.*, **40** (1974), 253. †
10. RADALL, P.J. & BOUMA, D., *Plant Physiol.*, **52** (1973), 229.
11. LINDSKOG. & MALSTTRON, B.G., *Biochem. Biophys. Res. Communes.*, **2** (1960), 213.
12. GRAHAM, D., ATKIN, C.A., REED, N.L., PETERSON, B.D. & SMILLIE, R.M., in "Photosynthesis and Photorespiration" edited by Ratch *et-al.*, John Wiley & Sons Inc. New York. (1971) 267.
13. PILLAI, N.G., WAHID, P.A. KAMALADEVI, C.B. RAMANANDAN, P.L. ROBERT CECIL, P.G., KAMALAKSHI AMMA, MATHEW A.S. & BALAKRISHNAN NAMBIAR, C.K. In Fourth Session. FAO Tech. Working party on Coconut Production, Protection and Processing, (Kingston, Jamaica.) (1975), 1.1.
14. SANKARAYANAN M.P., BALAKRISHNAN NAMBIAR, C.K. RAJASULOCHANA V. & PANDALAI, K.M. *Coco. Bull.* **19** (1976), 341.



The following text is extremely faint and largely illegible. It appears to be a continuation of a report or a list of references, possibly including names of authors and institutions. Some words like 'COC' and 'COC' are visible, which may correspond to the labels on the graph above.