

## Seasonal Changes in Nitrate Reductase Activity and Other Indicators of Plant Water Stress in Field Cacao (*Theobroma cacao* L.) Plants<sup>1</sup>

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### ABSTRACT

Seasonal changes in nitrate reductase activity and other indicators of plant water stress in field cacao (*Theobroma cacao* L.) plants. Balasimha, D. (Central Plantation Crops Res. Inst., Regional Stn., Vittal 574243, India). *Plant Physiol. & Biochem.* 9(2) : 74-79, 1982. Seasonal changes in nitrate reductase (NR), proline, chlorophyll and relative water content (RWC) in leaves of field grown cacao plants were studied under irrigated and rainfed conditions. In rainfed conditions the plants showed low RWC, decreased chlorophyll content and NR activity with accumulation of proline in leaves during the dry season. In irrigated plants, there was no marked differences in these parameters. The canopy levels of chlorophylls and NR activity indicated that cacao tolerated shade.

Key words : Canopy variations|chlorophylls|Nitrate reductase|proline|relative water content|*Theobroma cacao*.

### INTRODUCTION

Crop plants experience periods of water stress during their life cycles especially in summer months, when water availability becomes limiting. Cacao is sensitive to water stress and shows diurnal fluctuation in leaf water potential (1, 2). Seasonal changes in water potential (3, 6) and NR activity (7, 11) in many annual crops has been reported. Among plantation crops there are a few reports on seasonal variations in NR activity in coffee (12) and water potential and proline contents in citrus (13).

Nitrate reductase is a rate limiting enzyme in the nitrate assimilation of plants (14) and has not been studied in plantation crops in detail. It is well known that this enzyme is extremely sensitive to water stress (14,15) and both substrate and light induce the enzyme. Cacao is grown as an inter-crop under the shade of

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either arecanut or coconut and in the latter it is mainly a rainfed crop. Under the prevailing weather conditions of coastal Kerala and Karnataka where cacao is extensively grown, period of dry spell during summer is inevitable. This paper examines the seasonal changes in some indicators of plant water stress in cacao grown under both these conditions viz, irrigated and rainfed. The canopy variations in NR activity and chlorophyll were also studied.

### MATERIALS AND METHODS

Five trees each of cacao (*Theobroma cacao* L. cv. Forester) grown on rainfed coconut and irrigated arecanut plots in laterite soil were sampled periodically. About 25 leaves (3rd-5th leaf of mature flush) from each tree were collected for determinations. For canopy variations, leaves were collected from three levels constituting first (lower) second (middle) and third (upper) "Jorquettes" of the tree.

Relative water content was determined by floating 1 cm<sup>2</sup> leaf discs on distilled water for 6 h (16). Proline was extracted in 3% sulphosalicylic acid and determined by the method of Bates *et al.* (17). Chlorophylls were extracted in 85 per cent (v/v) acetone and estimated spectrophotometrically (18). NR activity was determined *in vivo* (19, 20).

### RESULTS AND DISCUSSION

During December-April of 1981-82 no rain was received. The RWC of leaves of rainfed cacao plants was lower than those of irrigated plants. This tended to decrease in both conditions with progress of summer months (Fig. 1). It is difficult to discern why well irrigated plants also showed this decrease. Possibly these values decreased in both the cases because the progressive increase in the daily mean temperature led to high transpiration rates and hence rapid depletion of plant water levels. Both leaf water potential and RWC have been reported to decrease with progress of dry season in cereals (5), apple (4, 6) and citrus (3).

The content of proline in leaves was maintained at similar levels in irrigated plants, while there was considerable accumulation in rainfed plants during the dry period (Fig. 2). With the onset of monsoon the proline content decreased. Since the first discovery of proline accumulation due to water stress (21), this has been reported in several annuals (22, 25) and plantation crops (13, 26). There has been much speculation about role of proline in drought tolerance (24) though the view is disputed (27). In cacao, proline accumulation

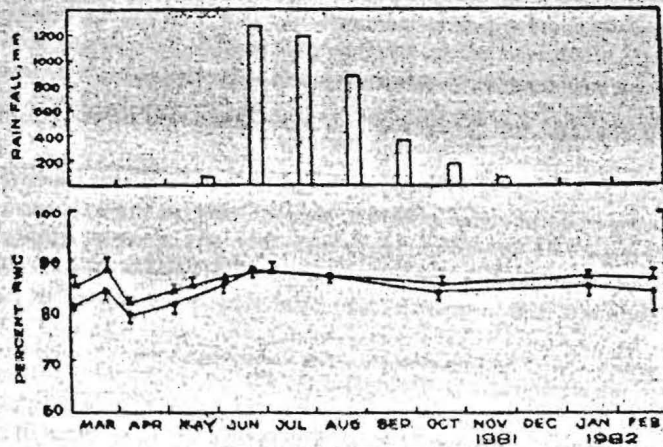


Fig. 1 : Seasonal changes in rainfall and RWC (circles-rainfed; triangles-irrigated; vertical lines represent standard error of mean values).

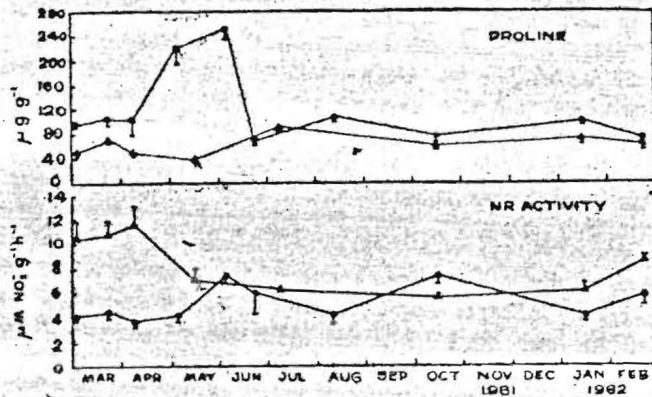


Fig. 2 : Seasonal changes in proline and NR activity (Symbols as in Fig. 1).

might at least be of some adaptive significance and efficient utilization of proline was associated with better growth and maintenance of leaf turgidity in seedlings (Balasimha and Subramonian, unpublished).

The NR activity showed interesting trends in irrigated and rainfed plants (Fig. 2). In irrigated plants NR activity was high during February-April and low during the rainy season. Whereas in unirrigated plants, the activity was low during the dry period, rose after the onset of rains when it was comparable to that recorded in the irrigated plants. Seasonal and canopy variations have been reported for several crops (7, 12). The seasonal decrease in NR activity has been mainly attributed to the physiological age (7, 9) or water stress (10).

Two important conclusions can be drawn. The decrease in NR activity in rainfed plants is an adaptation for stress conditions as has been shown for other plants (10, 14). The reported decline in respiration and photosynthesis (28) would decrease reducing power, thus affecting NR activity. With the recovery from stress, these biosynthetic processes are revived and the NR activity also recovers completely. The activity of NR is inducible by light (29) and high solar intensities during February-April adequately supplied with water might also

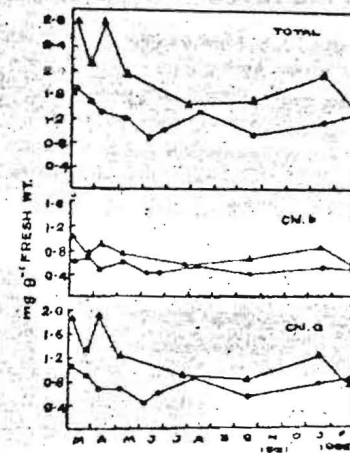


Fig. 3 : Seasonal change in chlorophyll contents (Symbols and order of months as in Fig. 1).

have resulted in higher enzyme activity in the cacao plants. Increase in NR activity by higher solar radiation has been reported in sorghum (10) and coffee (12). In coffee the NR activity varied depending on rainfall, humidity and water supply. Possibly, due to poor sunlight and persistent rains the NR activity in coffee was lower in October (12). There was also no significant variations at different canopy levels indicating relative shade tolerance of cacao trees (Table 1).

The contents of chlorophylls (a, b and total) was higher in irrigated than in rainfed plants (Fig. 3). The chlorophyll content varied among different canopy levels sampled at different seasons (Table 1). The upper canopy showed decreased

TABLE 1

Seasonal variation in chlorophyll content (mg/g fresh wt.) and NR Activity ( $\mu\text{mol-NO}_3/\text{g fresh wt/h}$ ) in cacao leaves at three canopy levels

Date	Lower*	Middle	Upper
<i>Chl a</i>			
22 January 1981	1.051	0.842	0.743
9 July 1981	1.002	1.119	1.213
22 October 1981	0.852	0.866	0.769
10 March 1982	0.744	0.647	0.589
<i>Chl b</i>			
22 January 1981	0.307	0.464	0.461
9 July 1981	0.588	0.658	0.649
22 October 1981	0.683	0.660	0.613
10 March 1982	0.496	0.539	0.415
<i>NR activity</i>			
22 January 1981	3.80	4.88	4.88
9 July 1981	6.13	5.50	6.50
22 October 1991	6.30	6.55	4.85
10 March 1982	6.66	5.90	5.00

\* The lower, middle and upper canopy levels indicate first, second and third "Jorquettes", respectively, of cacao plants.

chlorophyll contents during January, March and October when solar radiation was maximum. Cacao being shade grown, high solar radiation might result in photobleaching. Eventhough the intensity of sunlight would be rather low during the rainy season (July), chlorophyll content was not affected.

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