Leaf water potential, stomatal resistance and activities of enzymes during the development of moisture stress in the coconut palm

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Response of coconut palms (*Cocos nucifera* L.) to available soil moisture was studied by determination of leaf water potential, ψ, stomatal resistance and activities of three enzymes, acid phosphatase (orthophosphoryl monoester phosphohydrolase, (E.C. 3.1.3.2), L-aspartate: 2-oxoglutarate aminotransferase (E.C. 2.6.1.1) and malate dehydrogenase (E.C. 1.1.1.39). Comparison between the irrigated and rainfed palms was made during the course of a six-month period (December 1985 – May 1986) representing increasing drought conditions. A combined effect of soil and atmospheric droughts on the development of stress in terms of high stomatal resistance and low ψ was discernible in coconut. Stress-induced changes in enzyme activities was noticed. The threshold ψ for the activities of acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase was determined.

Keywords: Leaf water potential; Enzyme activity; Coconut palm; Moisture stress; Soil and atmospheric drought

The coconut is cultivated mainly as a rainfed crop in peninsular India. Although this region receives 2500–3000 mm rainfall annually, its distribution is limited to about four months in the year, June–September. Consequently, the crop is exposed to increasing soil water deficit, beginning in December and continuing until the following May, when it suffers serious damage as expressed by the drooping of leaves, button shedding and reduced fruit set. That the palm experiences water stress during the summer months is evident from the decreased yield of nuts in rainfed palms compared with those under irrigation (Nelliat and Padmaja, 1978). The effect of different frequencies of irrigation on the water relations and dry matter production of West Coast Tall coconut palms has been reported (Rajagopal *et al.*, 1989). Based on the relationship between the soil water deficit and the stomatal resistance, the critical soil water deficit for irrigation scheduling was deduced. Kasturi Bai *et al.* (1988) reported the influence of the agrometeorological parameters such as radiation, temperature and vapour pressure deficit (VPD) on the development of stress in the coconut. Response of the palm to moisture stress was shown to depend on the nature of the soil – sandy loam or laterite with different water-holding capacity (Voleti *et al.*, unpublished). Although many studies have been made on the effects of drought on enzyme activities in several annual crops (Todd, 1972; Rajagopal *et al.*, 1977), the perennial tree crops have received very little attention, presumably due to the problems encountered in stress studies from their large size, non-homogeneity in a population and long periods required to obtain increasing degrees of water stress in the field. In an earlier paper (Rajagopal *et al.*, 1988) we reported variations in the activities of acid phosphatase (orthophosphoryl monoester phosphohydrolase, E.C. 3.1.3.2), and L-aspartate: 2-oxoglutarate aminotransferase (E.C. 2.6.1.1) in detached leaves of some coconut types subjected to air dehydration for different time intervals and related to changes in leaf water potential (ψ) measured simultaneously.

In the present paper we have attempted to monitor the changes in the activities of acid phosphatase, L-aspartate: 2-oxoglutarate aminotransferase and malate dehydrogenase enzymes as a function of change in leaf water potential of coconut palms exposed to gradually increasing soil moisture stress under rainfed conditions in the field. Acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase enzymes were selected, based on our previous work in which it was shown that these enzymes are highly sensitive to water stress (Rajagopal *et al.*, 1988). Acid phosphatase, being a hydrolytic enzyme, shows rapid increase in its activity under stress; the degree of its increase has been correlated with the extent of drought tolerance in cotton (Vieira da Silva, 1970a). L-aspartate: 2-oxoglutarate aminotransferase participates in the generation of carbon skeletons required for the production of proline (Fruton and Simmonds, 1965) which accumulates in moisture-stressed plants (Singh *et al.*, 1973; Waldren *et al.*, 1974). Malate dehydrogenase was chosen to represent the enzymes of the oxidative pathway.

The objectives of the work described in the present paper were to study the changes in available soil moisture during September to May, representing the progressive development of stress, and its impact on stomatal resistance, leaf water potential and the activities of three enzymes in West Coast Tall coconut palms. The above parameters have been shown to be affected under moisture stress in

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Table 1 Agro-climatic parameters during December 1985 to May 1986. (Mean of 3 values)

	Light ($\mu E\ S^{-1}m^{-2}$)	Air temperature (°C)	Vapour pressure deficit (m bars)	Pan evaporation (mm day^{-1})
Dec. 1985	951.5	30.4	18.8	3.5
Jan. 1986	1275.3	31.1	20.2	4.1
Feb	1338.0	32.3	24.1	4.8
Mar	1410.0	33.7	23.7	5.7
Apr	1500.0	35.9	32.1	6.5
May	1520.0	33.8	21.0	6.6

other plant species also (Turner, 1979; Levitt, 1980; Balasimha and Rajagopal, 1988).

Materials and methods

Coconut palms var. West Coast Tall (WCT) were planted in 1965 in the Institute Farm in a randomized block design of three replications of six palms each. This monocrop experiment, with a spacing of 7.5 × 7.5 m, was laid out in Mapping unit 3A on a red sandy loam soil. The palms were each given N, P_2O_5 and K_2O at 500, 320 and 1200 g, respectively, in two doses – ⅓rd in May and ⅔rd in November (Anonymous, 1979). Irrigation treatment was initiated in 1984, the scheduling of which depended on the cumulative pan evaporation (CPE) (Rajagopal *et al.*, 1989). The palms were irrigated with 20 mm water at an irrigation water/cumulative pan evaporation (IW/CPE) ratio of 0.75 during the summer months, December – May. This approximated to irrigation of palms once in six days by sprinklers with perforated pipes placed horizontally between the rows of palms to wet the basins.

The present experiment on stress development was conducted during December 1985 – May 1986 at monthly intervals. The two treatments were irrigated (I) and rainfed (R). Agroclimatic conditions, light intensity, temperature and vapour pressure deficit (VPD) converted from relative humidity values, were recorded in the vicinity of the experimental palms using the portable steady-state porometer (Licor-1600 USA) as described earlier (Kasturi Bai *et al.*, 1988). Data on pan evaporation (PE) were obtained (Class A pan, USWB) from the climatological station established in the Institute farm. Monthly observations of agroclimatic conditions were recorded for three consecutive days in a month to keep the variation within the values at a minimum.

Soil moisture content was determined at three depths (0–30, 30–60 and 60–90 cm) in the basins of two palms replication^{-1} for each treatment, using the Troxler neutron moisture probe (Model 3222, USA). This was done two days after each irrigation. From the soil moisture data obtained, the available soil water content was calculated based on the soil water characteristics of the red sandy loam soil (Dastane, 1972). Water relation aspects were determined in the leaves (middle whorl) of both irrigated and rainfed palms using the steady state porometer. The stomatal resistance of the leaflets was measured between 10.00 and 11.30 a.m. twice during the season (December–May), using the method described earlier (Rajagopal *et al.*, 1986). Leaf water potentials (ψ) of the opposite leaflets were determined every month with a Scholander pressure chamber (Plant water console, 3000, Soil moisture Equipment Co., USA), following the earlier method of Rajagopal *et al.* (1988). For the above measurements, three replications each under irrigated and rainfed conditions were maintained.

The enzymes were extracted from spindle leaves by grinding leaf tissue in cold 0.1 M Na-phosphate buffer, pH 7.6, containing 0.5% β-mercaptoethanol and 10% polyvinyl polypyrrolidone. The homogenate was squeezed through four layers of muslin cloth and centrifuged at 10 000g for 20 minutes. The clear pale yellow supernatant was used for enzyme assays. All operations were carried out at 5°C.

Acid phosphatase activity was assayed using *p*-nitrophenyl phosphate as substrate. (Linhardt and Walter, 1963). L-Aspartate: 2-oxoglutarate aminotransferase was assayed by colorimetric determination with 2,4-dinitrophenylhydrazine according to Bergmeyer (1963); malate dehydrogenase activity was measured by the oxidation of NADH at 340 nm (Kitto, 1969).

All enzyme assays were performed in duplicate on three palms with four leaflets each and results are expressed as mean and SE of five values.

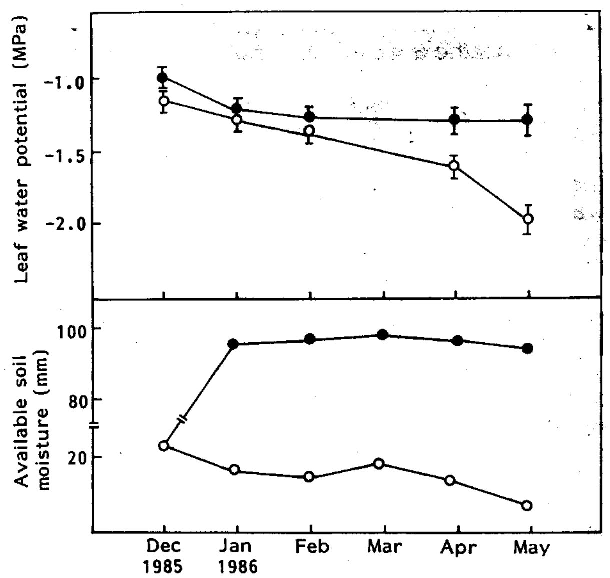

Figure 1 Changes in available soil moisture (ASM) and leaf water potential (ψ) of rainfed (○—○) and irrigated (●—●) palms. Vertical bars denote the S.E. of the mean

Results

The agro-climatic conditions that prevailed during the experimental period are shown in Table 1. It is clear that the three parameters, light, temperature and VPD, increased gradually between December and April/May. This reflected on the PE which increased from 3.5 mm day^{-1} in December to 6.6 mm day^{-1} in May.

At the start of the experiment in December, the available soil moisture was about 20 mm (Figure 1). With the imposition of irrigation treatment, it increased to about 95 mm in January and remained around that level until May, whereas under rainfed conditions, the available soil moisture fell until May, with a small increase in March due to non-seasonal rainfall compared with the initial level (December). The available soil moisture in rainfed plots during April and May was 62.5 and 35.0%, respectively, of the initial value in December. Thus, in May there was a large difference in the available soil moisture between the irrigated (94 mm) and rainfed (7 mm) plots.

There was negligible difference in the stomatal resistance initially (December) between the palms in the irrigated (1.40 sec. cm^{-1}) or rainfed plot (1.38 sec. cm^{-1}). However, by May the diffusive resistance had increased to 4.88 sec. cm^{-1} in the leaves of rainfed palms, but only to 2.99 sec. cm^{-1} in those of irrigated palms. Thus, the stomatal resistance was 63.2% higher in stressed leaves of unirrigated palms than in the turgid leaves of palms with irrigation. The ψ value ranged between −0.98 MPa in December and −1.05 MPa in May in the leaves of irrigated palms, whereas it fell from −1.10 MPa to −1.75 MPa in the rainfed palms during the same period, with a marked fall between April and May (Figure 1).

The activity of acid phosphatase in rainfed palms registered a gradual increase from December to March, followed by an increase with 52% higher activity in May than in the irrigated palms, which maintained lower activity throughout the period (Figure 2). A similar trend was observed in L-aspartate: 2-oxoglutarate aminotransferase activity which also showed a significant increase in May (89.2%). On the other hand, the rainfed palms had relatively low activity of malate dehydrogenase at all stages compared with that in irrigated palms (Figure 2).

Discussion

The increases in light intensity, air temperature, VPD and the accompanying decline in the available soil water content during December to May were reflected in the extent of soil and atmospheric droughts that prevailed during the experiment (Table 1). Consequently, the palms started to show the impact of moisture stress as revealed by changes in leaf diffusive resistance and ψ in rainfed palms (Figure 1). The changes in plant water relations in response to soil water potential are well documented in the literature (Slatyer, 1967; Turner, 1974). In coconut, a decrease in available soil water from 20 mm in December to 7 mm in May resulted in lowering of ψ from −1.0 to −1.75 MPa during the same period. This was associated with a high stomatal resistance (4.88 sec. cm^{-1}). Recently, Rajagopal *et al.* (1989) reported on the relationship between the soil water deficit and stomatal resistance in coconut which revealed the critical moisture level for the development of stress in the coconut palm. The critical soil water deficit for irrigation scheduling was deduced to be 110 mm. The influence of climatic factors, particularly the VPD, on stomatal regulation in coconut was shown by Kasturi Bai *et al.* (1988). A combined effect of soil and atmospheric droughts on the water relations of coconut is thus evident.

The impact of stress development in the coconut palm on the activity of leaf enzymes was studied by measurement of the activities of acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase enzymes which were found to increase rapidly during exposure of excised coconut leaves to air-dehydration stress (Rajagopal *et al.*, 1988). Malate dehydrogenase was also included as it serves an important role in the redox balance of the cell (Ulrich, 1941; MacDonald and Laties, 1964).

The results presented in Figure 2 show that the acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase activities of both rainfed and irrigated coconut palms remained constant or increased marginally up to March when the available soil moisture in the rainfed and irrigated conditions were about 20 mm and 95 mm, respectively. A further reduction of available soil moisture in the rainfed plots from 20 to 12 mm caused a sudden

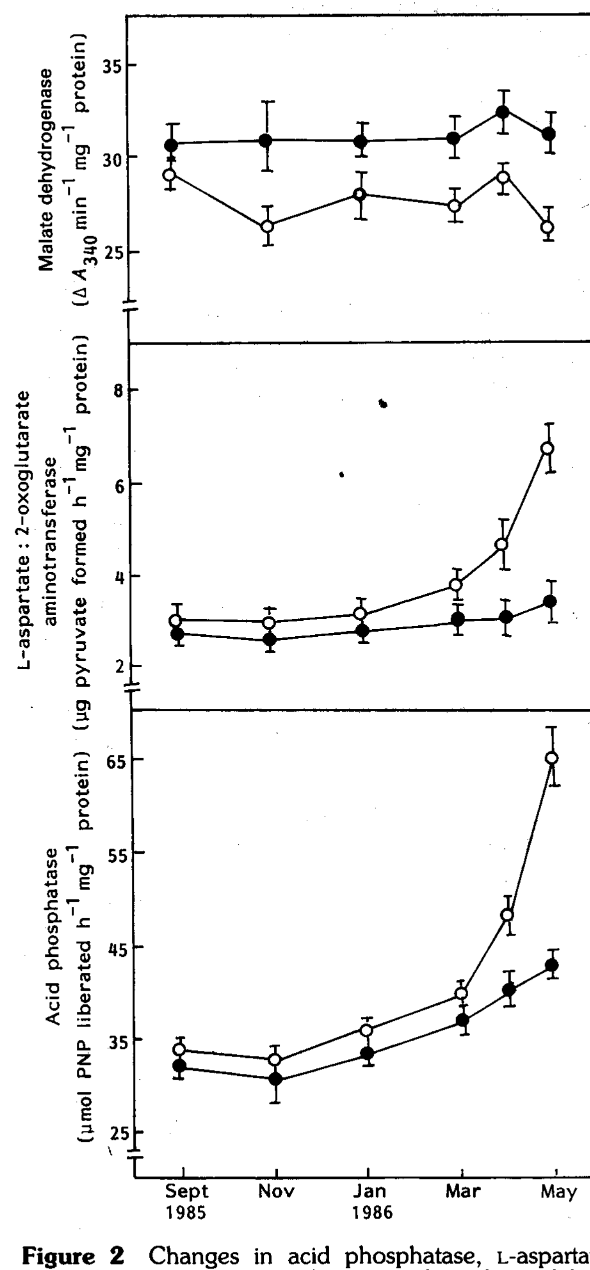

Figure 2 Changes in acid phosphatase, L-aspartate: 2-oxoglutarate aminotransferase and malate dehydrogenase activities under rainfed (○—○) and irrigated (●—●) conditions. Vertical bars indicate the S.E. of the mean

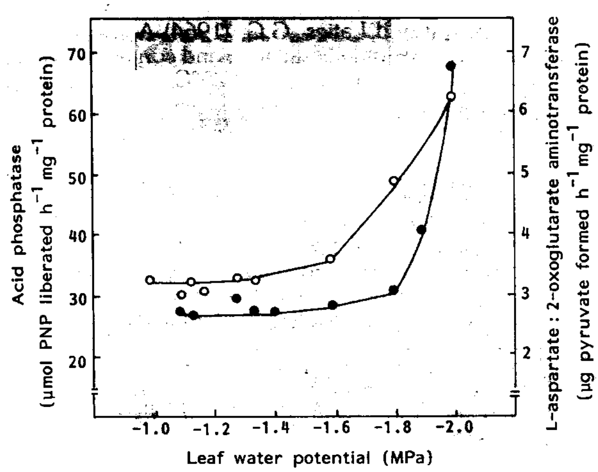

Figure 3 The relationship between leaf water potential and changes in acid phosphatase (○—○) and *L*-aspartate: 2-oxoglutarate aminotransferase (●—●) activities in coconut leaves.

increase in the activities of both acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase, reaching maximum activity during May. The irrigated palms, on the other hand, showed very little change during the same period. These results suggest that the rapid increase of acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase beyond March is mainly the effect of soil drought which could have been further accentuated by the atmospheric stress then prevailing. The slow and steady rise in the activities of these enzymes from September until May in the irrigated palms, in which the available soil moisture remained higher than 90 mm throughout, confirms the view that the atmospheric factors – including light intensity, air temperature and vapour pressure deficit – do impose a certain level of stress on the coconut palm even under non-limiting soil moisture conditions.

Increase in the acid phosphatase activity of some species of cotton under natural soil drought conditions was reported by Vieira da Silva (1970a) who found it to be inversely related to the dry matter accumulation. By further ultrastructural studies, Vieira da Silva *et al.* (1974) demonstrated that during moisture stress, some of the acid phosphatase in the chloroplast leaks into the cytosol due to injury in the chloroplast membrane. In the non-stressed tissue, the chloroplast enzyme was found to be latent (Vieira da Silva, 1970b). These reports suggest that the increased acid phosphatase activity in coconut under drought stress could be due to a possible loss of internal organization of the cell. Enhanced activity observed in L-aspartate: 2-oxoglutarate aminotransferase under stress could be explained by its role in nitrogen metabolism. It is now well established that glutamic acid and aspartic acid play important roles in nitrogen transfer through transamination reactions involving L-aspartate: 2-oxoglutarate aminotransferase, alanine aminotransferase and other enzymes (Fruton and Simmonds, 1965). Proline appears to be formed from glutamic acid *via* glutamic acid semialdehyde and pyrroline carboxylic acid (Boggess *et al.*, 1975). It would appear, therefore, that the enhanced activity of L-aspartate: 2-oxoglutarate aminotransferase in response to stress may be to supply glutamic acid for proline biosynthesis, which is known to accumulate under stress conditions in several crops (Barnett and Naylor, 1966; Singh *et al.*, 1973; Aspinall and Paleg, 1981). However, in the present study, we did not observe any marked increase in proline content (hence data not included) in the leaves of rainfed palms, indicating thereby that a ψ of -1.8 MPa was not sufficient to induce the synthesis of proline in coconut. Studies by other investigators also have shown that proline accumulates at a ψ lower than -2.0 MPa in different plant species (Waldren *et al.*, 1974; Chu *et al.*, 1976).

Malate dehydrogenase activity in the leaves of irrigated palms remained higher than that in the rainfed palms throughout the course of the experiment. A high activity of this enzyme is essential for the regeneration of NADH in the cell (Kun, 1963). The balance of malate/oxaloacetate and NAD/NADH could be greatly affected unless cells had a means of maintaining adequate malate (NAD) dehydrogenase activity (De Jong, 1973). Since L-aspartate: 2-oxoglutarate aminotransferase is associated with malate dehydrogenase activity to ensure the transfer of electrons through the mitochondrial membrane (Lehninger, 1970), the significant changes in the activities of these enzymes could upset the equilibrium, leading to slow death of the cell. Thus, the changes observed in the activities of three enzymes between irrigated and rainfed palms bring out clearly the fact that cellular metabolism is adversely affected by moisture stress.

In order to know the sequence in which the enzymes are affected by stress, the changes in activities of acid phosphatase and L-aspartate: 2-oxoglutarate aminotransferase were represented as a function of leaf water potential (Figure 3). The activities of both enzymes increased gradually with the fall in ψ up to a certain point, when any further reduction in ψ triggered their activities. Based on their responses, the threshold values of ψ for the sudden spurt in activity of these two enzymes were found to be -1.6 MPa and -1.8 MPa, respectively. This showed that during stress development, acid phosphatase enzyme was affected much earlier in the sequence than L-aspartate: 2-oxoglutarate aminotransferase. In the literature, there are reports available on the threshold ψ values for stomatal resistance, abscissic acid content or ethylene production in stressed plants (Turner, 1975; Wright, 1977); Hsiao *et al.* (1976) have documented the sequential response of different metabolic reactions to the changing water potential and suggested that the process that is affected earlier in the sequence is the more sensitive to stress. The results of the present study, when considered from that angle, show that acid phosphatase, among the enzymes investigated, is highly sensitive to stress. Therefore, it could be used as a tool for the measurement of the degree of stress in the coconut palm. The possibility of using this factor in screening for drought tolerance in coconut is being pursued.

In conclusion, it could be stated that a prolonged dry spell in which the available soil moisture falls below 20 mm could adversely affect the metabolic status of the coconut palm by its influence on leaf water potential and related components. It is evident from the data presented that the coconut palms experience a high level of stress during March to May from the combined effects of soil and atmospheric droughts.

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