Similar DEM model was also developed for evaluating phosphorus requirement of oil plam (Muralidharan and Biddappa, 1992). This approach appears to be simple and less time consuming rather than conducting field experiments. which would take otherwise such a long period to arrive the potassium requirement of the crop. Apart from that the increasing cost of fertilizers warrants their judicious application and usually the application of the arbitrarily recommended dose of fertilisers would not be in accordance with the requirement of the palm. The proposed model aims at maintaining the soil potassium fertility level at 80 ppm of available potassium. Although in the oil palm, this has not been established either through field experimentation or through exhaustion, this was established for the coconut through long term field experimentation at CPCRI. ıragod (CPCRI. 1985). These results of the coconut have utilised for the oil palm, since the nutrient requirements of these two perennial oil palnts are closely comparable (Ochs, 1977).

The DEM for potassium was plotted for evaluating the buffering capacity (β^0/ψ^0). Srinivasan and Biddappa (1990) also used nutrient buffering capacity for evaluating the nutrient requirement in cardomom soils. Since the buffering capacity was closely related to the rate of potassium diffusion and other availability parameters, it could be used in the computation of potassium requirement. It may be stated that this factor would be a constant for a given soil under standard conditions. The model was based on the recovery of added potassium, which was 80 per cent in the present study. The fertilizer experiment (Nair and Sreedharan, 1982) conducted so far in the oil palm growing tracts of southern Kerala have not given any conclusive evidence so as to evolve critical limits for major nutrients. In the absence of computer models and ıate data from field experiments, this model, which has taken into account the response and reaction of the soil to the added fertilizer may be considered most suitable for potassium scheduling for oil palm on the basis of soil test data. Thus, the present arbitrary recommendations of 1200g K_2O per palm per year for the oil palm grown in south India can be modified by using this model.

4. Conclusion

The TKR model computed based on the DEM would find applications among the oil palm cultivators. Thus, depending upon the purchasing power of the oil palm cultivators the soil available potassium status can be maintained to any desired level by supplying the indicated amounts of K_2O *in the table.*

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DROUGHT INDUCED PHOTO OXIDATIVE STRESS AND INHIBITION IN PHOTOSYNTHESIS IN *HEVEA BRASILIENSIS*

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Abstract: The effects of severe drought stress on leaf photosynthesis and photosystem II (PSII) activity were studied in *Hevea brasiliensis*. Drought inhibited leaf photosynthetic rate at any given light intensity and ambient CO_2 concentration. The quantum yield of CO_2 assimilation and *in vivo* carboxylation efficiency were inhibited by drought stress. The quantum yield of PSSII activity and coefficient of photochemical quenching of chl fluorescence decreased and the coefficient of nonphotochemical quenching of chl fluorescence increased in the stressed leaves. Diversion of more photosynthetic electrons away from carbon to oxygen reduction led to oxidative damage of PSII and loss of total soluble proteins as well as proteins associated with PSII. It is concluded that drought induced oxidation strees lead to senescence as indicated by loss of chlorophyll.

1. Introduction

Natural rubber tree, *Hevea brasiliensis*, is one of the most important plantation crops of Kerala. About one quarter of the cultivable land in Kerala is occupied by this crop. The impact of natural rubber cultivation on Kerala's economy is substantial (1).

Plantation crops are mostly perennial in nature, and therefore, are exposed to seasonal unfavourable environmental extremes such as drought almost every year. The long term impact of drought stress on crop productivity can be substantial in a plantation species like *Hevea* (2). Certain rubber growing regions of Kerala are prone to moderate to severe drought stress.

Several key physiological parameters are adversely affected during environment strees. Photosynthetic carbon assimilation rate is particularly sensitive to drought stress (3). Drought stress is generaly accompanied by high temperature and high sun light intensity (2) which aggravate the drought induced damage to photosynthetic apparatus. (4,5). When the carbon assimilation capacity is decreased, the light energy absorbed by the leaf can be excessive which results in the over excitation of chlorophyll molecules. This affects the photochemical activity alters the fluorescence emission by the chlorophyll pigments (6). Excess light can become inhibitory to the photosynthesis when the plants are under severe stress (7). Drought stress is also known to affect the biochemical composition and activities of some photosynthetic enzymes in the leaf (8). Degradation of vital thylakoidal proteins such as the DI protein associated with PSII reaction centre causing inhibition of PSII activity and photosynthetic oxygen evolution (9) are also reported.

An imbalance between photosynthetic electron transport (ie PSII activity) and photosynthetic carbon assimilation can lead to the production of reactive oxygen species (10). The various species of reactive oxygen species (ROS) such as $O_2^{\cdot-}$, H_2O_2 and several free radicals (FR) resulting from the harmful reactions triggered by ROS with cellular constituents such as membranes causes photooxidative stress leading to further inhibition of photosynthesis (5), Quantum yield of PSII activity (11) and damage to PSII reaction center (12).

In the present study we report the results of the water deficit stress mediated changes in photosynthetic carbon assimilation and photooxidative damage to PSII activity in *Hevea* leaves.

2. Materials and Methods

Young poly bag grown *Hevea* plants were used in the present study except for one experiment where mature field grown *Hevea* trees were used to study the chloroplastic protein profile on an SDS-PAGE in relation to photosynthetic oxygen inhibition and chlorophyll contents. Water stress was created by withholding the irrigation to the poly bag grown plants at the Rubber Research Institute of India, Kottayam. Leaf photosynthetic rates (A) were determined using a portable photosynthesis system (Li 6400, LiCor, Lincoln, Nebraska, USA) at different photosynthetic photon flux densities (PPFD) and leaf intercellular CO_2 concentrations (Ci) which was obtained by altering the CO_2 concentration in the ambient air present in the photosynthetic measurement cuvette (13). An asymptotic function ($y=a*(1-e^{-bx})+c$) was fitted between, the dependent variable (A) and the independent variable PPFD or Ci to study the photosynthetic response to light and CO_2, respectively. This function gave excellent co-efficient of determination for both the light and CO_2, response curves in every measurement ($r > 98\%$), except for the light response curves studied with severely drought stressed leaf. The initial slopes of the A/PPFD response curve (defined as the apparent quantum yield for CO_2 assimilation ϕc) and the A/Ci response curve (defined as the carboxylation efficiency CE) were calculated fron linear regression analysis(13).

In vivo PSII activity was measured using a pulse amplitude chlorophyll fluorescence measurement system (PAM Walz, Germany) as described else where (14). Analyses of the photochemical and nonphotochemical quenching of chl fluorescence, calculation of photosynthetic electron transport rate (ETR) and estimation of electron transport rate used for processes other than carbon reduction (ETR*) are given in detail else where (15). The photosynthetic oxygen evolution was measured on excised leaves disks at approximately 500 μ mol^{-2} s^{-1} PPFD using a Clark-type oxygen electrode (Hanzatech, UK) in the gas phase.

Mature trees grown with and without irrigation during summer in the extremely drought prone region of Dapchari in the North Konkan region were used to extract intact chloroplast, thylakoids and stromal protein (16). Leaf protein and chlorophyll contents were estimated (17,18). Comparable amounts of protein were loaded on a gel and the proteins present in intact chloroplast, thylakoids and stroma were separated electrophoretically (19).

Independent t test was done to compare the statistical difference between the control and drought treatment.

3. Results and Discussion

As PPFD increased leaf photosynthetic rate (A) increased following an asymptotic function in the irrigated control plants (Fig 1A). The light saturated photosynthetic rate (A_{sat}) and apparent quantum yield for CO_2 assimilation (ϕc) were higher in the irrigated control than the stressed (Fig. 1A). In the water stressed plants A showed an initial

increase with PPFD followed by an decline in A at high PPFD which resulted in negative values of A_{sat}.

Table 1. Effects of drought stress on chl fluorescence, PSII activity and chlorophyll and soluble protein contents in *Hevea brasiliensis*. (n=6-10).

Parameter	Water stressed	Control (Irrigation)	t Test P
Fo (Relative units)	745	590	<0.05
Dark Fv/Fm	0.41	0.79	<0.01
ϕ_{ppII} (low light)	0.38	0.78	<0.01
ϕ_{ppII} (high light)	0.16	0.32	<0.01
q_p (low light)	0.65	0.74	<0.05
q_p (high light)	0.41	0.45	NS
[illegible] (low light)	0.53	0.21	<0.01
[illegible] (high light)	0.74	0.54	<0.05
ETR* (high light) (μ mol m-2 s-1)	36	12	<0.01
Photosynthetic O_2 evolution (rel. Units)	2.7	4.3	<0.05
Chl *a* (mg/g fr.wt.)	2.1	2.9	<0.01
Chl *b* (mg/g fr.wt)	0.63	1.7	<0.01
Sol. protein (mg/g fr.wt)	25.5	37.5	<0.01

The reduction in the ϕc and high light induced inhibition in A at the leaf level was reflected in the quantum yield of PSII activity in the water stressed plants. The maximum potential quantum yield of PSII activity (determined as a ratio of variable to maximum fluorescence Fv/Fm in the dark adapted state) showed a significant reduction in the water deficit leaf (Table 1). The reduction in the dark Fv/Fm was mostly due to an increase in the minimum fluorescence yield Fo in the dark adapted state which indicated a severe damages to PSII. As expected the ϕPSII decreased with increase in PPFD, but the extent of decrease was greater in the water deficit plants than the control. Inhibition in the ϕPSII activity would lead to an inhibition in the photosynthetic O_2 evolution by the water deficit leaf as compared to the irrigated one (Table 1).

Water deficit leads to a greater inhibition in the photochemical quenching (q_p) of chlorophyll fluorescence at high PPFD. This indicate that more number of PS II reaction center remained in the closed state in the water deficit leaf. The coefficient of nonphotochemical (q_N) quenching was large in the water deficit leaf at low and high PPFD, but the difference was small at high PPFD. The large q_N is a mechanism of dissipating the excess excitation energy absorbed by the chlorophyll molecules (15). However, simultaneous measurement of A and PS II activity indicates an excess of excitation energy present in the system in relation to the carbon reduction capacity of the mesophyll. This is evident from the increased diversion of photosynthetic electrons away from carbon reduction to other reductive processes (table.1).

The response of A to Ci indicate a substantial loss in the carboxylation efficiency of drought stressed leaf as indicated by the reduction in the initial slope and CO_2 saturated

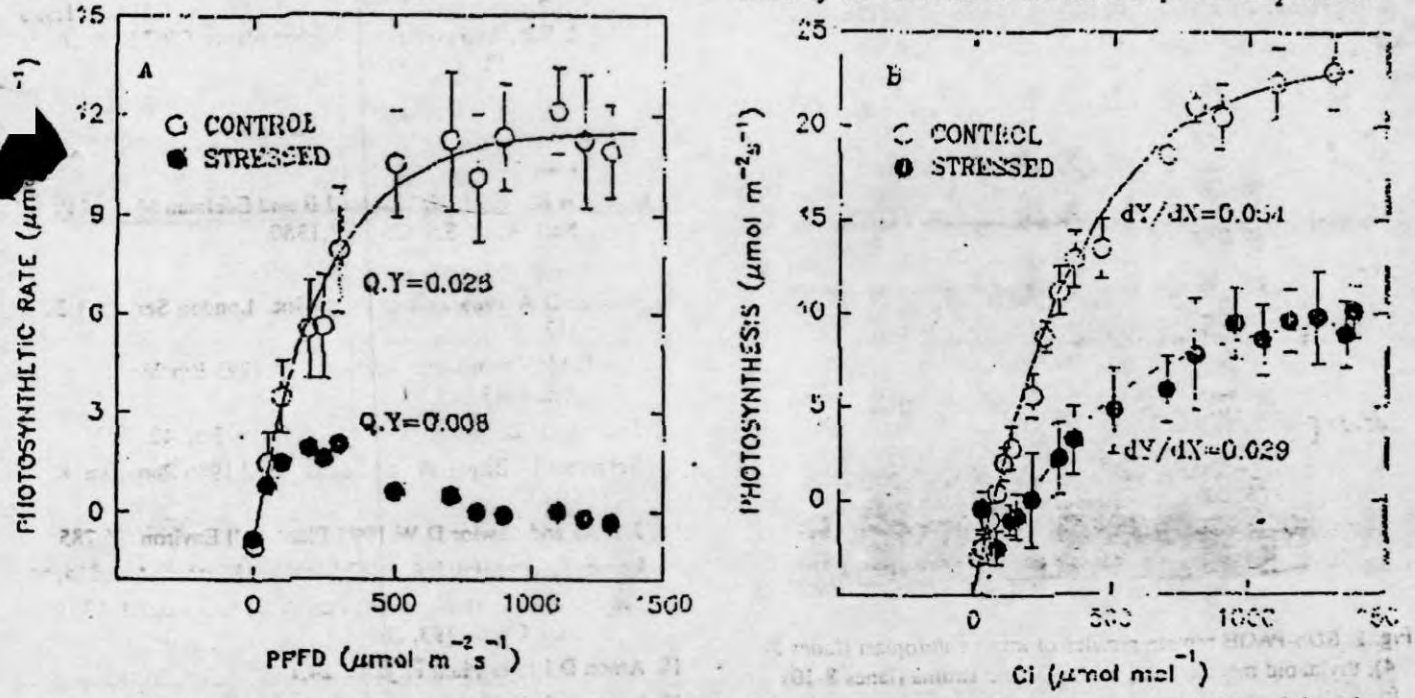

Fig. 1. Response of leaf photosynthesis to PPFD (A) and leaf cellular CO_2 concentration, Ci (B) in control (open circle) and drought stressed (closed circle) *Hevea* Plants. Each point is a mean of six to ten measurements and SE is shown.

A in the drought stessed leaf (Fig. 1B). The reduction in the carboxylation efficiency indicate an inhibition induced activity of the primary carboxylating enzyme Rubisco which also catalyses the photorespiratory pathway. Under normal conditions in a C_3 species like *Hevea* a substantial amount of excitation energy (ie photosynthetic electrons) will be utilized for the photorespiration and other process such as reduction of nitrates and sulphur etc. But under drought condition it is unlikely that the excess photosynthetic electrons would be used for these above process which are known to decrease in drought stressed plants. Hence most of the electrons which are in excess after utilization in the Calvin cycle end up in the reduction of molecular O_2 which is present in large concentrations at the vicinity of chlorophyll. This will lead to production of several species of active oxygen species such as O_2, H_2O_2 and *OH causing oxidative stress (10). If the factors causing the oxidative stress are very strong (eg severe drough) and the capacity of the cell to scavenge the free radicals and active oxygen species is impaired, the cells will start to degrade and senescence will set and hence there will be degradation of proteins and loss of chlorophyll as observed in this study in the stressed leaves (Table 1).

The PSII reaction center and antenna proteins are sensitive to extreme environmental conditions like temperature, high light and drought (4). Our results indicated a comparative loss of PSII proteins in the range of 25-27 kDa, most likely representing the light harvesting chlorophyll protein complex (LHCP) in the drought stressed plants (Fig.2) which could be attributed to the oxidative stress resulting from diversion of photosynthetic electrons to molecular O_2. The same may be responsible for the degradation in chlorophyll and soluble proteins in the drought stressed plants.

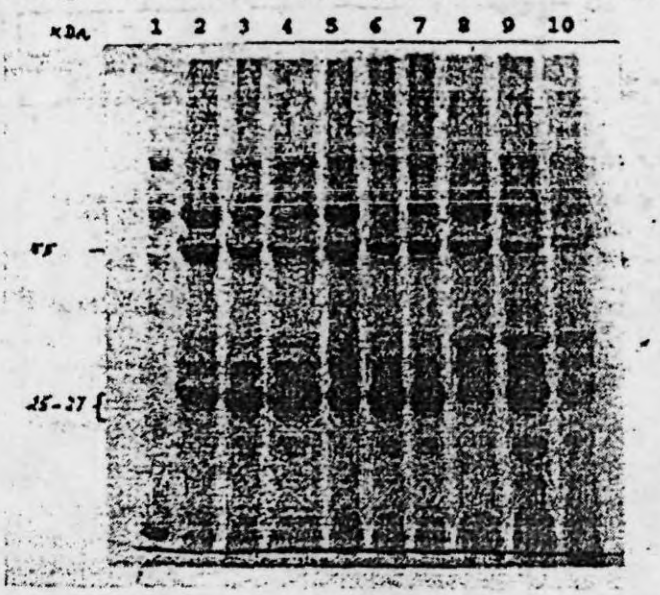

Fig. 2. SDS-PAGE protein profiles of intact chloroplast (lanes 2-4), thylakoid membranes (lanes 5-7) and stroma (lanes 8-10) from plants grown without irrigation (2,5,8). restricted irrigation (3,6,9) and full irrigation (4,7,10).

4. Conclusion

The following conclusions can be drawn from the present investigation

1. Drought strees inhibits the mesophyll capacity to fix CO_2 more than PS II activity and photosynthetic electron transport rate.
2. This imbalance results excess excitation energy in the system leading to oxidative stress.
3. Loss of PS II proteins, chlorophyll and soluble proteins in the leaf may be the result of the degradative reactio resulting from ... Such an oxi...

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