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EXCESS PHOTOSYNTHETIC ELECTRONS - A MATTER OF LIFE AND DEATH FOR GREEN LEAVES OF *HEVEA* EXPERIENCING DROUGHT STRESS

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Matured leaves of *Hevea brasiliensis* were used to examine the relationship between excess excitation energy and senescence of green leaves experiencing stress. From simultaneous measurements of chlorophyll fluorescence and gas exchange by leaves, the rate of photosynthetic carbon assimilation (A) and the rate of *in vivo* electron transport across photosystem II (J) were determined. Rate of photosynthetic electron diversion away from the carbon reduction (J*), presumably for oxygen reduction leading to the production of reactive species of oxygen (ROS) and free radicals (FR) were estimated. Both A and J increased and J* decreased when intact leaves were exposed to elevated concentration of CO_2 and all these parameters increased at high light intensity. But A and J decreased and J* increased when the leaves were excised. In the excised leaf A was more and J* was less at elevated CO_2 concentration. An increase in J* in plants with injured roots was observed which led to an inhibition in the quantum yield of PSII activity and was related to ageing as indicated by a loss of chlorophyll content of the leaf. Green leaves experiencing stress, increased diversion of electrons away from carbon to oxygen under conditions of high light intensity hastens leaf senescence, possibly through the production of destructive ROS and FR. Significance of the light environment inside a *Hevea* canopy *vis-a-vis* leaf aging is discussed in the context of unfavorable environmental conditions.

INTRODUCTION

Production of oxygen and excited electrons through hydrolysis of water by photosystem II (PSII) in the presence of light (photolysis) is the first step in the conservation and conversion of solar energy into a form which can be used for biological process. Molecular oxygen and light are essential to sustain aerobic life as we see it today. The life saving oxygen molecule is also implicated in various destructive reactions leading to degenerative process such as diseases, ageing, etc. (Wyllie, 1997; Nooden *et al.*, 1997). In the case of green plants, sun light, the ultimate source of energy for all living organisms and thus, the sustainer of life can be harmful to plants under certain situations (Barber and Anderson, 1992).

Quite a large proportion of photosynthetic electrons (often more than 40-50%) are utilized by C_3 plants for processes other than photosynthetic carbon reduction through Calvin cycle (Valentini *et al.*, 1995) such as reduction of nitrate, sulphate, etc. Molecular O_2 can also act as an alternative acceptor of photosynthetic electrons. Under normal conditions, plants have the capacity to effectively avoid the production of reactive oxygen species

(ROS) or scavenge them if they are produced in the system (Asada, 1992).

Under unfavorable conditions, for example, a deficiency in P nutrition (Jacob and Lawlor, 1993), capacity of the mesophyll cells to reduce carbon is inhibited much more than the capacity of the chlorophyll to perform photochemical reactions. This results in the availability of large number of excited electrons which are in excess of what is required to reduce carbon through Calvin cycle. Most of these "excess" electrons find their way to molecular oxygen which is present in very high concentrations in the vicinity of PSII leading to the production of ROS and free radicals (Price and Hendry, 1991). Plants experiencing abiotic stress to shade, high CO concentration, etc. will result in a decrease in the number of electrons diverted away from photosynthetic carbon reduction and thus reduce the production of ROS and FR. In the present investigation, simultaneous measurement of photosynthetic carbon assimilation and *in vivo* PS II activity were monitored to estimate the diversion of photosynthetic electron away from photosynthetic carbon assimilation in mature leaves of *Hevea* experiencing stress.

MATERIALS AND METHODS

One year old polybag grown *Hevea brasiliensis* plants were used in the first set of experiment. Fully expanded mature leaves were excised from the plants and the rate of loss of CO_2 assimilation (A) and photosynthetic electron transport across PSII (J) were simultaneously monitored exposing the leaves to normal ambient temperature, 500 $\mu mols.m^{-2}.s^{-1}$ photosynthetic flux density and three different ambient CO_2 concentrations viz. 200, 350, 700 ppm. Carbon dioxide exchange rate was determined using a portable photosynthetic system (LI 6400, LICOR, USA). Photosynthetic electron transport rate was determined based on the principle of chlorophyll fluorescence with the help of a PAM Fluorimeter (PAM 2000, WALZ, Germany) and the number of electrons diverted for oxygen reduction (J*) was calculated as described earlier (Jacob, 1996; Jacob and Lawlor, 1993).

In a second set of experiment, the roots of one year old polybag grown RRII 105 plants were carefully pruned to cause injury to the plants. A set of plants with root injury was kept at low light intensity (roughly 150 µmol m-2 s-1) and another set under full day light. Both the set of plants were irrigated to keep the leaf water status saturated. Root injured plants kept in the open sun and shade conditions were monitored for their A and J and estimated J* as mentioned above besides the leaf chlorophyll content (Arnon, 1949), periodically, as an indicator of leaf ageing.

RESULTS AND DISCUSSION

As expected, exposing intact leaves to elevated CO_2 concentration (Ca) led to an increase in the rates of photosynthetic CO_2 assimilation (A) and photosynthetic electron transport rate through PSII (J) and a decrease in the number of electrons used for reduction of molecular oxygen (J*) (Fig. 1). Rate of photosynthetic CO assimilation, J and J* were greater at high light than at shaded conditions (Fig. 2). When the leaves were excised, along with the time, there was a progressive decline in A and increase in J* (Fig 3A,B). In the excised leaf, A was more and J* was less at elevated Ca, at any given time. From these results it was evident that in a leaf which is under progressive drying, there is more diversion of electrons for reduction of molecular oxygen than for carbon assimilation. This is independent of the concentration of chlorophyll in the leaves.

From the above results a hypothesis can be drawn that the increased diversion of electrons away from carbon presumably to molecular oxygen would lead to production of ROS and FR which hastens leaf senescence. If this is true, a faster rate of ageing can be expected under conditions of a low CO_2 concentration and high

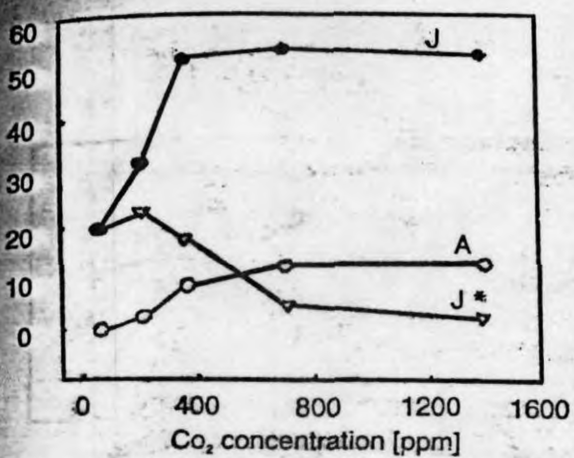

Fig.1. Rate of photosynthetic CO_2 assimilation (A), total photosynthetic electron transport across PSII (J) and the rate of electron transport used for processes other than reduction of carbon (J*) in intact leaves of *Hevea* measured at differ:nt ambient CO_2 concentrations

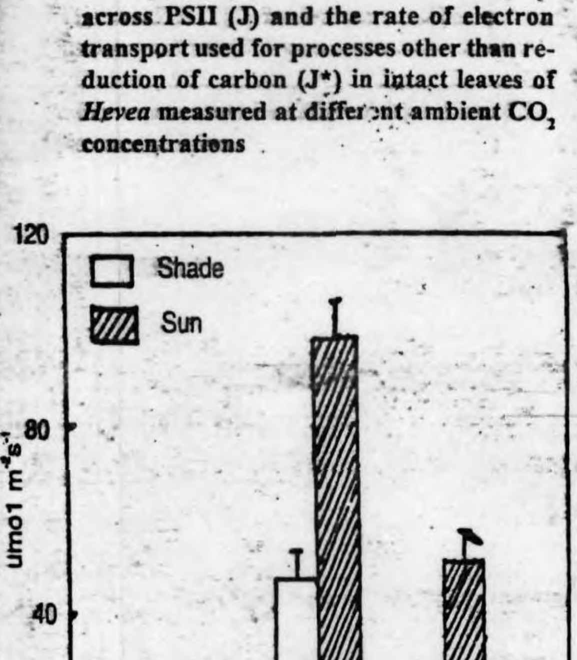

Fig.2. Rate of A, J and J* in intact leaves of *Hevea* measured at saturated light and shaded environment

light intensity which result in the production and/ or diversion of more electrons to oxygen in a stressed leaf.

Above hypothesis was tested in the second experiment where a root injury was used as an experimental tool to create stress and thus induce ageing of mature leaves in plants kept at two different light intensities. But the leaf water status did not vary between the light and shade treatments. Root pruning led to an increase in J* for the first couple of days after the initiation of the treatment (Fig. 4A). Results showed that the injured plants kept in full sun light had more number of electrons diverted away from carbon and less quantum yield of PSII activity (as determined by the ratio of maximum to variable fluorescence, Fv/Fm in dark adapted state) than their counterparts kept under shade conditions (Fig. 4A,B). An increased diversion of electrons away from carbon reduction led to an inhibition in the quantum yield of PS II activity in the injured plants kept in open sun. More diversion of electrons during the first couple of days after causing the root injury led to faster ageing of leaves as indicated by a loss of chlorophyll content in the plants kept in full sun light (Fig. 4C). Rate of photosynthetic electron transport came to a minimum as a result of the enhanced loss in chlorophyll concentration in the plants kept under sun light by the fifth day. Increased inhibition in the PS II quantum yield was strongly related with leaf aging as studied from the chlorophyll content (Fig. 5).

Thus the results of the present study proves that excess light hastens leaf aging process in *Hevea brasiliensis* plants experiencing root injury. Aging is set in by diverting more photosynthetic electrons away from carbon reduction to molecular oxygen which in turn produces ROS and FR that inhibit the activity of PSII and deplete leaf chlorophyll content. In this context, it may be observed that inhibition in the leaf photosynthetic capacity and increased rate of leaf senescence were recorded in mature *Hevea*

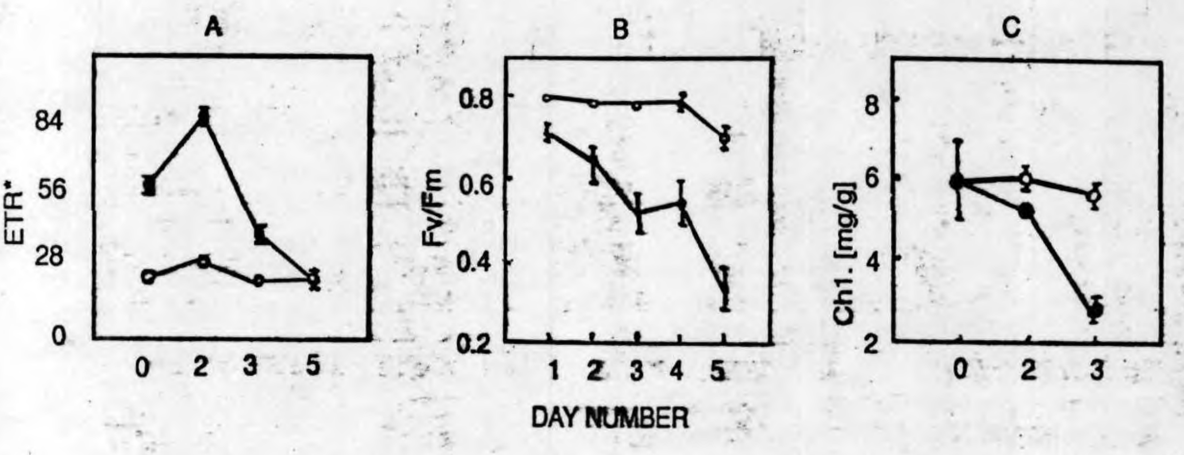

Fig.3. Leaf photosynthetic CO_2 assimilation and J* in excised leaves of *Hevea* measured at different ambient CO_2 concentrations

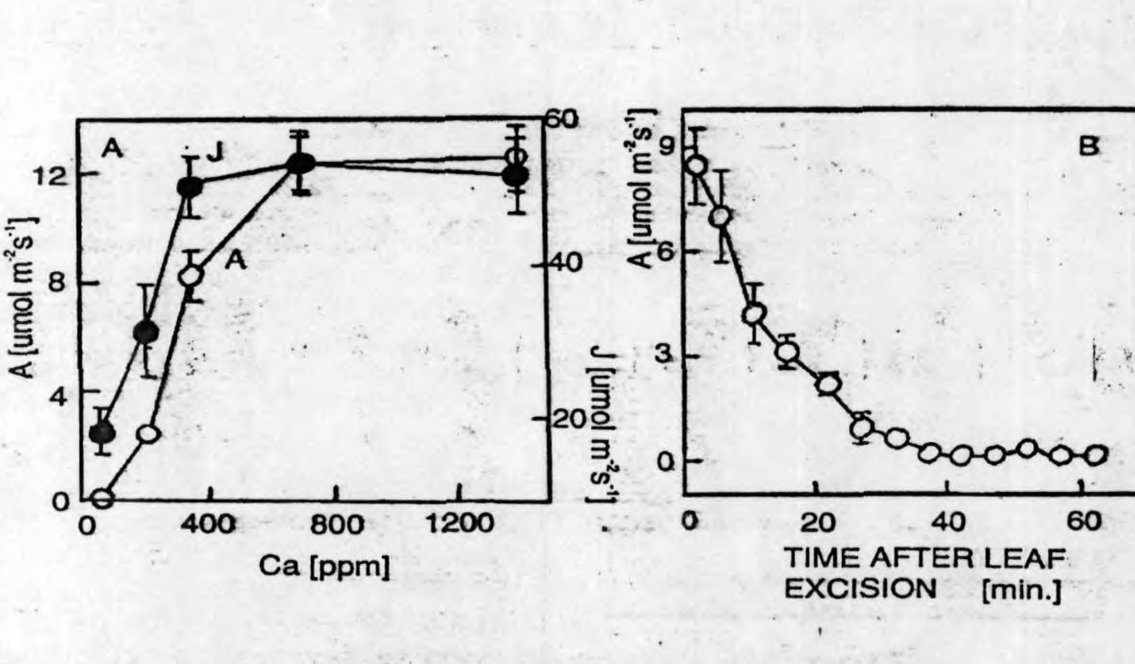

Fig. 4. Changes in the rate of electron transport used for processes other than reduction of carbon (J*), maximum potential quantum yield of PSII (Fv/Fm) and leaf chlorophyll content in *Hevea* plants kept in open sun (open circles) and shade (closed circles) after root pruning

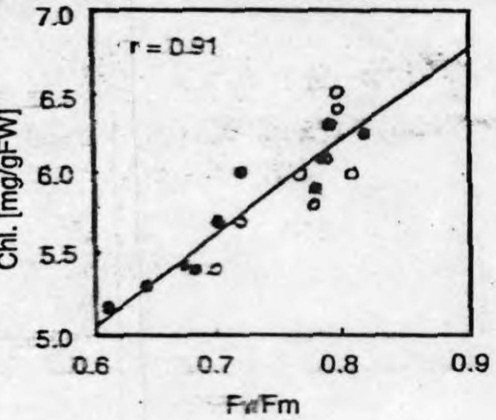

Fig. 5. Correlation between leaf chlorophyll content and the maximum potential quantum yield of PSII (Fv/Fm) in aging leaves of *Hevea* plants after root pruning

trees experiencing severe drought concomitant with high light intensity in the field (Devakumar *et al.*, 1999). Providing shade to *Hevea* plants either by growing crops such as banana in the early stages may be a useful management practice when the environmental conditions are unfavourable. In a mature plantation, mutual shading of the lower canopy leaves would take care, to some extent, the problem of excess light during summer. Avoiding interception of high amounts of light by leaf movement or reducing the light absorption by the leaf by increased reflectance will help to reduce the "excess electron load" on the top canopy leaves during adverse weather conditions. However, once the photosynthetic electrons are produced in the leaf in excess of the requirement for carbon assimilation, there will be increased production of ROS and FR which will hasten leaf aging depending up on the capacity of the plants to scavenge them.

REFERENCES

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidases in *Beta vulgaris*. *Plant Physiology* 24:1-5.

Asada, K. 1992. Ascorbate peroxidase - hydrogen peroxide -scavenging enzymes in plants. *Plant Physiology* 85: 235-241.

Barber, J. and Anderson, B. 1992. Too much of a good thing: light can be bad for photosynthesis. *Trends in Biochemical Sciences* 17: 61-66.

Devakumar, A.S., Praksh, G.P., Sathik, M.B.M. and Jacob, J. 1999. Drought alters the canopy architecture and microclimate of *Hevea brasiliensis* trees. (in press).

Jacob, J. 1996. Simultaneous measurements of photosynthetic gas exchange and *in vivo* PSII activity. pp.128-132. In: *Developments in Plantation Crops Research*. Mathew, N.M. and Kuruvilla Jacob. Eds. Allied Publishers Limited, New Delhi.

Jacob, J. and Lawlor, D.W. 1993. *In vivo* photosynthetic electron transport does not limit photosynthetic capacity in phosphate deficient sunflower and maize plants. *Plant Cell and Environment* 16:785-795.

Nooden, L.D., Guiamet, J.J. and John, I. 1997. Senescence mechanisms. *Physiologia Plantarum* 101: 746-753.

Price, A.H. and Hendry, G.A.F. 1991. Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant Cell and Environment* 14: 477-484.

Valentini, R., Epron, D., Angelis, P.D., Matteucci, G. and Dreyer, E. 1995. *In situ* estimation of net CO_2 assimilation, photosynthetic electron flow and photorespiration in *Turkey oak* (Q. cerris L.) leaves: diurnal cycles under different levels of water supply. *Plant Cell and Environment* 18: 631-640.

Wyllie, A. 1997. Clues in the p53 murder mystery. *Nature* 389: 237-238.