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SIMULTANEOUS MEASUREMENTS OF CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHESIS : A POWERFUL TOOL IN STRESS PHYSIOLOGY

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Most crop plants in India, including the perennial crops such as rubber, tea, coffee, etc. experience environmental extremes on an yearly basis. Combinations of high load of solar radiation with drought and high temperature and evaporative demand of the atmosphere or with low temperature are such extreme scenarios that lead to environmental stress. One of the primary effects of environmental stress is inhibition of photosynthesis at high light intensities (photoinhibition). Disturbances in normal photosynthesis due to environmental perturbations increase the production of superoxide radicals, which is harmful. Changes in the equilibrium between the photosynthetic biochemistry and photosynthetic electron transport will alter the fluorescence emission characteristics of chlorophyll that could be detected. From simultaneous measurements of photosynthesis and chlorophyll fluorescence from the same leaf, partitioning of photosynthetic electrons between carbon reduction and other processes could be estimated. Pulse amplitude modulated chlorophyll fluorescence signals could be used to calculate the coefficients of useful photochemical quenching (q_p) and wasteful nonphotochemical quenching (q_N) of excitation energy present in the chlorophyll. The relationship of q_p with the quantum yield of photosynthetic electron transport and the quantum yield of CO_2 assimilation and the relationship of q_N with the production of active oxygen species (AOS) and the resulting oxidative stress in leaves is discussed in the context of environmental stress.

INTRODUCTION

Unlike animals which are mobile, plants are unable to move away from unfavorable environmental conditions. Often such unfavorable conditions lead to environmental stress to plants. Extremes of atmospheric temperatures, soil and atmospheric drought, salinity, nutrient deficiency etc. are the common environmental stresses that affect the growth and productivity of plants. Plantation crops, being perennial species are exposed to periodic cycles of various environmental stresses during their economic life span.

Loss of photosynthetic capacity of leaves is one of the early symptoms of environmental stress. The physical diffusion of CO_2 into the leaf through the stomata, photosynthetic biochemistry and photosynthetic photochemistry are affected to varying degrees during environmnetal stress. Photosystem II (PSII) is very sensitive to the physical environment of plants (Baker, 1991). Depending upon the degree to which the photochemistry is affected during stress, the fluorescence emission by chlorophyll molecules changes. Pulse amplitude modulated chlorophyll fluorescence has emerged to be a powerful non-invasive technique for elucidating the biological status of plants experiencing different environmental stresses (Krause and Weis, 1991). The purpose of this paper is to introduce the usefulness of combined measurements of photosynthetic CO_2 assimilation and chlorophyll fluo-

rescence in stress physiology with particular reference to plantation crops.

THEORY OF FLUORESCENCE

Most of the fluorescence emitted by green leaves at room temperature originates from PSII. The antenna pigments present in the PSII absorb light and the excitation energy is transferred to the reaction center molecule of PSII, P680. Deactivation of excited pigments results in the emission of heat and fluorescence. Krause and Weis (1991) has given a detailed analysis of the theory of chlorophyll fluorescence.

The rate of fluorescence emission or the fluorescence yield 'F' is proportional to the intensity of the light absorbed by the leaf and its biological state. When all the reaction centers are in the active state Q_A is fully oxidized. When Q_A is fully oxidized the fluorescence yield is minimum (Fo) (Fig. 1). When Q_A is fully reduced, the fluorescence yield is maximum (Fm). The difference between Fm and Fo is called variable fluorescence (Fv). The ratio of Fv to Fm is an important parameter of the physiological state of the leaves. This ratio is highly conserved (0.832 ± 0.004) in the plant kingdom (Björkman and Demming, 1987). Severe environmental stresses decrease this ratio.

There exists an inverse relationship between the rate of fluorescence emission and the rate of photochemical reactions. When the fluorescence yield increases from Fo to Fm, there is increased reduction of Q_A (Duysens and Sweers, 1963). This increase and the subsequent decay in the fluorescence yield represent complex kinetics (Butler, 1977; Strasser, 1986). All processes that contribute to lowering the fluorescence yield below the maximum are collectively called quenching mechanisms (Krause and Weis, 1988). Resolution of the various mechanisms of fluorescence quenching is possible based on their relaxation kinetics which gives important information about the functional state of the photosynthetic apparatus in general and PSII in particular.

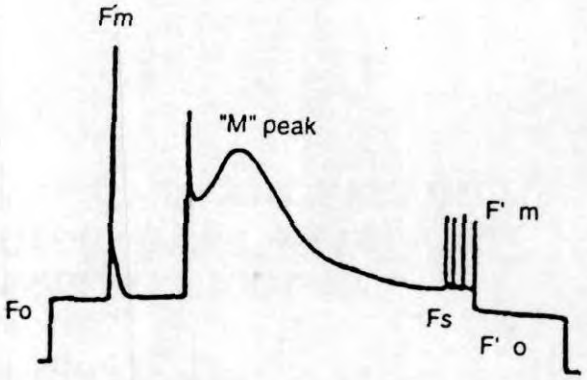

Fig. 1. A typical chlorophyll fluorescence transient obtained using pulse amplitude modulated technique

Reoxidation of Q_A^- causes fluorescence quenching which is termed "photochemical quenching" (q_p). Quenching of fluorescence that is not related to reoxidation of Q_A^- is collectively called "non photochemical" quenching (q_N). There are basically three major mechanisms that contribute to q_N. They are "energy dependent quenching (q_E) (related to the transthylakoid pH gradient), quenching related to "state 1-state 2" transition, q_T (which is dependent on the phosphorylation of LHCII) and photoin-hibitory quenching, q_I (which is related to the photoinhibition of PSII). Photochemical and nonphotochemical quenching processes are often considered as 'useful' and 'wasteful' photochemistry, respectively. In general, environmnetal stresses decrease q_p and increase q_N (for example phosphate deficiency, Fig. 2).

DEFINITIONS

A typical fluorescence transient signal generated from a leaf using pulse amplitude modulated technique is shown in Fig. 1. The following parameters can be derived from the various fluorescence yields shown in the transient (Jacob, 1995; Schreiber *et al.*, 1986).

Efficiency of excitation energy capture in the dark adapted state, ϕ_e = Fv/Fm′ where Fv = Fm–Fo

Efficiency of excitation energy capture in the light adapted state, $\phi'_e = Fv'/Fm'$, where $Fv' = Fm'-Fo'$.

Coefficient of photochemical quenching, $q_P = (Fm'-Fs)/Fv'$

Coefficient of nonphotochemical quenching, $q_N = (Fm-Fm')/Fv$.

The quantum yield of PSII photochemistry, ϕ_{PSII} is calculated after Genty *et al.* (1989) as the product of q_P and ϕ_e' :

$\phi_{PSII} = q_P \times \phi_e' = (Fm' - Fs/Fv') \times Fv'/Fm' = (Fm' - Fs)/Fm' = 1 - (Fs-Fm')$.

Hence if the steady state fluorescence yield (Fs) and the maximum fluorescence yield in the light adapted state (Fm') are known, the quantum yield of PSII photochemsirty, ϕ_{PSII}, which is the number of electrons generated per photon absorbed by PSII can be calculated. Pulse amplitude modulated chlorophyll fluorescence measurement techniques make it possible to delineate between the fluorescence signals due to the incident light and the biological state of the leaf.

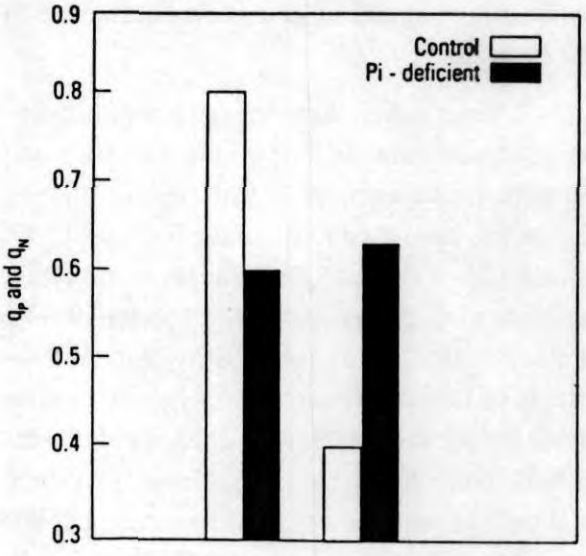

Fig. 2. Photochemical and non photochemical quenching coefficients in control and Pi deficient sunflower leaves

APPLICATION

The simultaneous measurements of chlorophyll fluorescence and CO_2 assimilation can be used to study the partitioning of photosynthetic electrons between photosynthetic carbon reduction and other processes. Recent studies show that a significant proportion of the photosynthetic electrons generated by PSII are used for various process other than photosynthetic carbon reduction (Jacob, 1995; Peterson, 1990) even in unstressed plants grown under optimum conditions (Fig. 3 and 4). These processes include photorespiration, NO_3 assimilation, Mehler reaction etc. Photorespiration and NO_3 assimilation help to drain the photosynthetic energy which is in surplus of carbon reduction and thus prevent photoinhibition of PSII. However, when the leaves are experiencing extreme abiotic stress and the carbon assimilation capacity has decreased substantially, the rates of photorespiration and NO_3 assimilation may not be large enough to take care of the excess electrons. This leads to increased diversion of excited electrons to processes such as Mehler reaction where molecular O_2 will act as the terminal electron acceptor of the photosynthetic electron transport chain instead of NADP. There may be other sites also on the electron transport chain from where molecular O_2 might accept electrons. Diversion of electrons to molecular O_2 results in the production of super oxide which is an active oxygen species. This can lead to production of very damaging other radicals if the active oxygen scavenging mechanisms are not effective. Thus, diversion of photosynthetic electrons to processes other than carbon reduction during abioic stresses will produce large amounts of free radicals in the leaf, particularly in the presence of high intensity of light. The ability of a leaf to scavenge these free radicals determines its photosynthetic response to abiotic stresses (Sengupta *et al.*, 1995).

Simultaneous measurements of photosynthetic carbon assimilation rate and ϕ_{PSII} can

be used to calculate the partitioning of photosynthetic electrons between the various processes mentioned above (Jacob and Lawlor, 1993a). If the net CO_2 assimilation rate is A, then the total number of electrons needed to sustain this rate of carbon assimilation is $4 \times A$. If the intensity of light incident on the leaf is Ii and the per cent absorptance of the leaf is a, the rate of (noncyclic) electron transport across $\phi_{PSII(ETR)}$ is $\phi_{PSII} \times I_i \times a \times 0.5$ where 0.5 corrects for the light distribution between PSI and PSII (assuming that the absorbed light is distributed equally between the two photosystems). Therefore, the ratio $(ETR-4 \times A)/ETR$ is the proportion of photosynthetic (noncyclic) electrons used for processes other than carbon assimilation. During conditions of abiotic stress an increase in this ratio is an indication of increased flow of electrons to molecular O_2 and hence, possibly production of more free radicals.

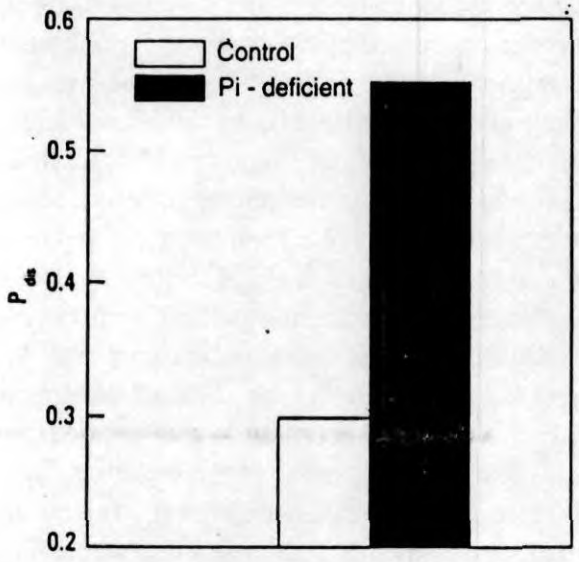

Fig. 3. Proportion of photosynthetic electrons diverted to photorespiration (P_{dis}) in control and Pi deficient sunflower leaves

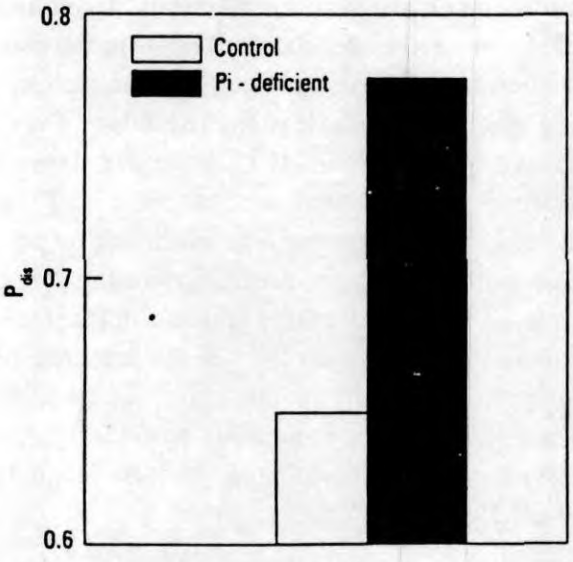

Fig. 4. Proportion of photosynthetic electrons used for all processes other than CO_2 reduction (P_{dis}) in control and Pi deficient sunflower leaves

Plantation crops remain in the field for many years as they are mostly perennial species. During their economic life span they experience various abiotic stresses. In the case of natural rubber, for instance, conditions in the non traditional areas of cultivation such as the North Konkan and the North Eastern states of India are often stressful. Other plantation crops such as cardamom, tea, coffee etc also experience abiotic stress in some areas of India and during certain periods of the year. The technique outlined here will be useful in evaluating the performance of various clones or genotypes for their relative stress tolerance, non-destructively and with relative ease. This will be particularly handy when large number of germplasm lines have to be evaluated.

Chlorophyll fluorescence is a very intensively studied area in modern plant biology and its potentials are immense. Chlorophyll fluorescence has been used to study the *in vivo* CO_2/O_2 specificity of Rubisco, the primary carboxylase enzyme in C_3 plants (Jacob and Lawlor, 1993b; Peterson, 1990) and in elucidating the rate constants of various primary photochemical events taking place in PSII (Havaux *et al.*, 1991; Jacob, 1995). While the technique has immense potential both in basic and applied research, a sound understanding of the biophysics of photochemistry and biochemistry of photosynthesis is very crucial in using this technique correctly.

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