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Photo acoustic study of plants exposed to varying light intensity growth conditions: Spectral and morphological changes

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Abstract. In this paper we describe results of photo acoustic (PA) measurements carried out on various plants exposed to varying light intensity conditions. Depending on the species and light intensity conditions, the PA absorption spectra show differences in peaks associated with pigments and the cuticle. These differences are related to the spatial distribution of the pigments that differs from plant to plant. We have also performed systematic study of oxygen evolution at different wavelengths. The obtained oxygen spectra are equivalent to the action spectra usually acquired by determining the CO_2 uptake and energy storage. The intensities of oxygen spectra exhibit differences depending on distinct morphology of plant.

1. INTRODUCTION

The photo acoustic (PA) signal from leaves is sensitive to molecular oxygen produced in the chloroplasts as a result of photochemical reactions induced by the absorption of light. Released O_2 generates an additional component of the PA signal that is being added to the already existing signal caused by the increase of temperature. Therefore, the resulting signal can be used to study the photosynthetic activity rate of the leaf.

Several PA studies on plants have been performed. Many of these have focused on determining the relative amount of pigments (mainly chlorophylls in chloroplasts) as well as on depth profiling of the leaf. The development of the open photo acoustic cell (OPC) concept in which the leaf itself functions as one of the walls of the PA cell, allowed for *in vivo* and *in situ* studies of leaves [1,2]. Using the OPC technique it is possible to preserve normal conditions for the plant and monitor the photosynthetic activity during growth and treatments for a long period of time [3].

In this work we have used the PA spectroscopy technique, both, with conventional as well as the OPC cell to study the absorption spectra of leaves and their photosynthetic activity through the oxygen evolution. Different species of plants, genetically adapted to shade or to sunlight conditions were investigated. The conventional PA cell was employed to obtain the absorption spectra, while the photosynthetic activity was assessed by OPC.

2. EXPERIMENTAL

The PA spectrometer comprises a Xenon arc lamp (Oriel, mod.6128, 1000 W), a mechanical chopper (PAR, model 192) and an Oriel 77250 monochromator operating in the 300-800 nm range. The measurements were carried out at 17 Hz. The continuous white light used to saturate photosynthesis

(~350 W/m^2) was provided by a tungsten lamp. A double-branched optical cable (a set of lenses and mirrors) was used to transport each light beam to the OPC (conventional cell).

Investigated plants originated from the two contrasting environments: that of tropical rain forest and of cerrado (savannah). The forest plants are adapted to shade conditions as they grow surrounded by trees that preclude them from being fully exposed to sunlight. On the other hand, cerrado plants are adapted to a full sunlight incidence. Four plant species were used: Zeyhera tuberculosa (forest), Zeyhera digitalis (cerrado), Eriotheca candolleana (forest) and Eriotheca gracilipes (cerrado). The former two species were grown under 50% sunlight intensity conditions (labelled F and C, forest and cerrado), while the other two species were grown under two different light conditions i.e. 10% (shadow, labelled FS and CS, forest-shadow and cerrado-shadow) or 60% sunlight intensity (labelled FL and CL, forest-light and cerrado-light).

The PA measurements were performed on the second pair of fully expanded leaves 100 to 120 days after the sowing. The absorption spectra were obtained by means of conventional PA cell (cut leaves) in presence of saturating white light (to avoid oxygen from contributing to the signal). For each kind of sample, measurements were repeated about 10 times (4 leaves for each plant/3 plants of each kind). The evolution of oxygen from intact leaves was measured by OPC.

3. RESULTS AND DISCUSSION

Figure 1(a) shows the PA spectra of CS, CL, FS and FL samples (average spectra). It is clear that cerrado plants (CS and CL) show lower absorption peaks in the visible range. These peaks are due to the absorption of pigments located in the chloroplasts. The forest plant grown under 60% sunlight condition (FL) displays absorption peaks slightly higher than the cerrado ones. However for the same species plants when grown in the shadow (FS) the intensity of peaks is twice that much. On the other hand, the amplitude of the PA signal (due to the cuticle absorption) in the UV range is practically the same for all the samples. Furthermore, the phase difference $\Delta\Phi$ (defined as $\Delta\Phi=\Phi_C - \Phi_P$) between the PA signals from the cuticle (at 330 nm) and chlorophyll (680 nm) is 41, 48, 20 and 44 degrees deg for CS, CL, FS and FL samples, respectively. Summarizing, the FS plant shows substantially higher signal amplitude in the visible and a significantly smaller phase difference between pigments and cuticle.

Assuming a simple model of the well-defined pigments containing layers at the distance d from the cuticle (leaf surface), one can write $\Delta\Phi = d/\mu$, where μ is the effective thermal diffusion length. For each sample it is possible to estimate d using aforementioned $\Delta\Phi$ and assuming thermal properties of water being representative for the leaf, which is quite reasonable. The results are: d_{CS} = 37 μm, d_{CL} = 43 μm, d_{FS} = 18 μm, d_{FL} = 39 μm; similar results were found for cuticle versus carotenes (470 nm). Hence, the average depth of pigments in FS plant is roughly a half of that found in other samples. Figure 1(b) shows cross sections of leaves grown in a full sunlight and in the shadow. The leaf that grew under full conditions has a thicker cuticle and longer palisade parenchyma cells (these latter contain the majority of chloroplasts), making the average depth of pigments larger than that in the "shaded" leaf.

Such a difference in spatial distribution of pigments explains higher amplitude of the PA signal observed in the spectrum (Fig. 1) of FS. Indeed, for the simple model consisting of well defined layers, the amplitude of signal in the visible range (pigment peaks) is proportional to $\exp(-d/\mu)$ and to the concentration of the pigment. Using d/μ values found above, one can verify that the ratio of chlorophyll (carotenes) peaks in different samples very well matches the ratio of corresponding $\exp(-d/\mu)$ factors. This result leads to the conclusion that, despite observed differences in absorption spectra, the concentrations of pigments in samples investigated here are not significantly different. However, in the case of a forest plant grown under shaded conditions, spatial distribution differs remarkably.

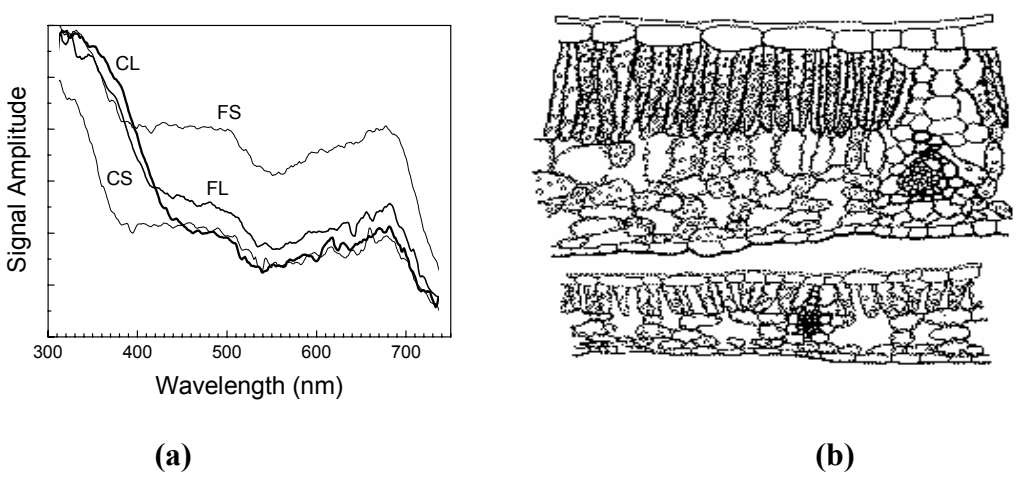


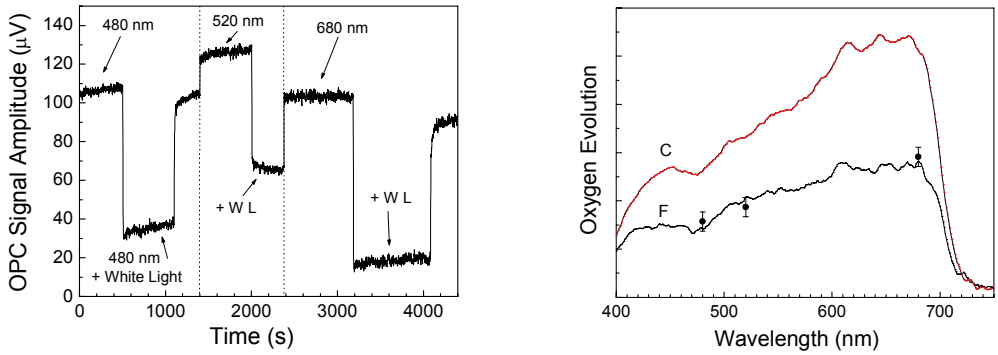
Figure 1. (a) Photo acoustic absorption spectra of (CS) cerrado plants grown in shadow, (CL) cerrado plants grown in sunlight, (FS) tropical rain forest plants grown in shadow and (FL) tropical rain forest plants grown in sunlight. Measurements were performed using a conventional photo acoustic cell. (b) The cross section of leaves grown under sun (top) and in the shadow (bottom) [4].

In an attempt to determine the photosynthetic activity in cerrado and forest plants, we have used the OPC that permits the *in vivo* and *in situ* analysis. As a first step we have carried out measurement of the so-called "negative effect" in C and F samples grown under same illuminating conditions but having distinct genetic characteristics. Figure 2(a) shows the results of such measurements (at 480, 520 and 680 nm) obtained from sample F. To obtain a negative effect, the PA signal was detected as a function of time using the modulated (monochromatic) light; to saturate photosynthesis white light was added after a certain period. The signal amplitude drops ("negative effect") due to the fact that following the incidence of white light, microphone no longer detects the oxygen component. From the difference one can then evaluate the rate of oxygen production (i.e. photosynthetic activity) that varies with the wavelength of the modulated light.

In order to investigate the rate of oxygen production as a function of wavelength in the entire visible range, we have recorded the PA spectra, using the OPC, both with and without the white saturating light. The oxygen evolution spectra were deduced from a difference between these spectra. Figure 2(b) shows such curves for F and C samples. The symbols represent the negative effect (Fig. 2(a)), normalized (at each wavelength) to intensity of the lamp emission. The agreement between symbols and continuous trace provides the evidence that the last one actually corresponds to the oxygen spectra.

The curves in Fig. 2(b) are equivalent to action spectra (that represent photosynthetic activity) usually obtained from CO_2 uptake studies [5] or PA energy storage measurements (using either inhibitor of photosynthesis [6], or by measuring the positive effect at high modulation frequencies [7]). The intensities of oxygen spectra in case of the cerrado plant (Fig. 2) are about twice of that observed for a forest plant throughout the entire wavelength range.

It is worth noticing that, unlike for conventional cell, the OPC signal originates from pressure oscillations at the rear side of the sample, i.e., the photo acoustic chamber is placed at the non-illuminated side of the leaf. Therefore, the path that oxygen and heat must travel is not the same as that traversed in the conventional PA\ cell. This explains why that curve C is double of curve F (Fig. 2(b)), while in Fig. 1(a) curves FS and FL are higher than CS and CL. Further analysis of oxygen spectra as a function of modulation frequency will allow for comparison between the mass transport parameters in different species.



(a) (b)

Figure 2. (a) The OPC measurement of the "negative effect" observed in a tropical rain forest plant (F) at three different wavelengths. (b) Continuous traces represent oxygen spectra (action spectra) of (C) cerrado and (F) tropical rain forest plants obtained via the OPC measurement. The symbols (black dots/error bars) represent the negative effect normalized to the intensity of the lamp.

4. CONCLUSIONS

In this paper we have reported the outcome of the absorption spectroscopy and oxygen evolution measurements in cerrado (adapted to sun conditions) and forest (adapted to shade) plants grown by exposing them to different level of sunlight. The PA absorption spectra show differences of pigment spatial distribution from plant to plant. Smallest average distance between cuticle and pigments was observed in forest plant grown in the shadow. This shows that next to the origin, the growth conditions also affect the morphology of the leaf.

To the best of our knowledge, the action spectrum obtained by means of OPC measurement of oxygen evolution is the first result of that kind ever reported. Two pronounced advantages of OPC method are: i) the intrinsic capability for performing *in vivo* and *in situ* (preserving the leaf) non-invasive measurements at low modulation frequencies (higher signal to noise ratio) and ii) the fact that the use of inhibitors is precluded.

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