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Photoacoustic characterisation of the *in vivo* levels of chlorophyll a in the adaxial and abaxial sides of the leaf

A S KOLASKAR, K R NAIDU, Y SEETHAMBARAM and V S RAMA DAS*

School of Life Sciences, University of Hyderabad, Hyderabad 500 134, India

*Department of Botany, Sri Venkateswara University, Tirupati 517 502, India

Abstract. Photoacoustic spectroscopy was applied to determine the distribution of chlorophyll a in the abaxial and adaxial sides of the leaf in 27 species of angiospermous plants. Two distinct patterns were observed in the ratio of the level of chlorophyll a in the abaxial to adaxial side of the leaf in the monocotyledons and in dicotyledons plants. The ratio was not correlated with the C_3 or C_4 type of photosynthetic pathways.

Keywords. Photoacoustic spectroscopy; chlorophyll a; Abaxial-adaxial ratio.

1. Introduction

The pigment chlorophyll a plays a crucial role in the photosynthetic process. It is also well known that nature provides a supra abundance of chlorophyll for photosynthesis. Though in the past the content of chlorophyll was widely estimated *in vitro* comparatively few studies were made of *in vivo* distribution (French 1960). Particularly the distribution of chlorophyll a on the two sides of a leaf in various plants was hitherto unreported, mainly because most common methods available for the determination of chlorophyll in the leaf were made in inorganic solvents (Arnon 1949; Winterman and De Mots 1965). Since the anatomical structure of the leaf near the abaxial and adaxial is quite different it is logical to assume that the chlorophyll content on the two sides might be different in most of the dicotyledonous leaves. Because of lack of suitable method for *in vivo* estimation, quantitative information on this aspect is not available. In this communication the recent technique of photoacoustic spectroscopy has been applied for the relative amount of chlorophyll a in the two sides of the leaf.

2. Materials and methods

Photoacoustic effect, though discovered in 1881 by Bell, has recently been used to study the optical properties of solids (Rosencwaig 1975). It has also been used to study the biological samples (Rosencwaig and Pines 1977). In photoacoustic spectroscopy chopped or modulated monochromatic light is allowed to fall on a sample surrounded by non-absorbing inert gas in an enclosed cell due to which periodic light absorption occurs. These excited molecules decay non-radiatively producing periodic heat flow to the surface of the sample. Heat transfer from the sample surface to the surrounding gas generates pressure fluctuations which are detected by sensitive microphones. The acoustic signal is then normalised against the signal of carbon black to compensate for the variation of power output of the light source. Plotting the normalised photoacoustic (PA) signal as a function of wavelength produces a PA spectrum which exhibits the characteristic absorption peaks and non-radiative decay processes of the sample. Since the strength of the PA signal is closely related to the amount of light absorbed and since only absorbed light can produce an acoustic signal this method has been used to study chlorophyll content under *in vivo* condition on the two sides of the plant leaf. PA spectra were recorded (using Prinston Applied Research Model 6001 instrument), corrected for blank absorption and normalized using carbon black as reference.

Leaves of plants fully grown under natural (*ca* 12 hr) photoperiod (temperature about 35°C day and 22°C night) were thoroughly washed with distilled water and dried under pressed condition for more than 24 hr. The cut sample of the leaf (6 × 4 mm), was scanned in the range of 500 to 700 nm. To reduce noise, the spectra were noted after two scans.

3. Results and discussion

Pennisetum typhoides, a C_4 monocot plant, NADP-ME type was used to study variation of chlorophyll content from leaves in different positions. PA spectra for I, II and III leaf under dry condition are given in figure 1. It is seen that the chlorophyll a content increases monotonically from I to III leaf. This phenomenon has also been observed on various other plants (Sestak 1977). This indicates that PA spectroscopy gives results

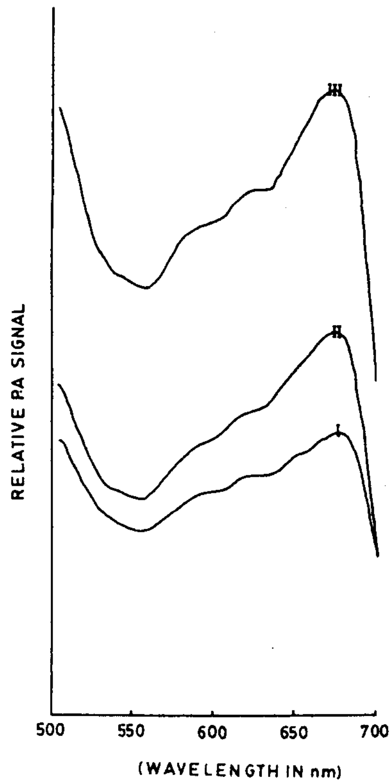

Figure 1. PA spectra of chlorophyll a on adaxial side of I, II and III leaf (from the top) of *Pennisetum typhoides*.

similar to those obtained using other conventional methods. However, PA spectra recorded for two different sides of these leaves gave the abaxial to adaxial ratio of chlorophyll a (R) as 1·13, 1·26, 1·05 and 1·05 respectively for I, II, III and matured leaf. These results suggest that chlorophyll a distribution is not symmetrical during the early development of the leaf and probably the cell distribution is isobilateral only when the leaves are matured.

The PA spectra for the different parts of same leaf, base, middle and tip are given in figure 2(a) for *Amaranthus hypochondriacus*, a C_4 plant. These spectra show that the amount of chlorophyll a increases from the tip to the bottom of the leaf and agrees with the results obtained using Arnon's method in our laboratory. Figure 2(b) gives the PA spectra using the same leaf from midrib to margin at base. These spectra also show that chlorophyll a content decreases from mid to margin. Similar results were obtained when Arnon's method was followed. It is interesting to note that the R value does not change monotonically as one goes from tip to base or midrib to margin.

Results of the two experiments discussed above show that the R value varies from species to species as well in different parts of the same leaf. In order to get some insight

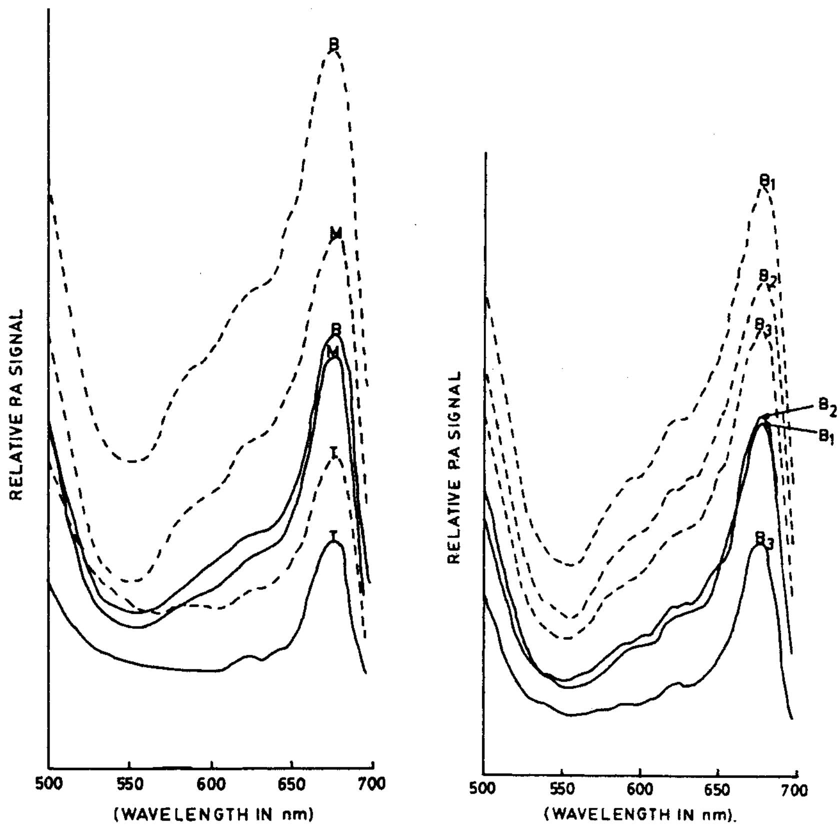

Figure 2. PA spectra of chlorophyll a of *Amaranthus hypochondriacus* from **a**. Tip to base in two sides of the leaf. **b**. Midrib to margin at the base of the leaf. (-----) abaxial side. (————) adaxial side. (T = tip; M = middle; b = base; B_1 = midrib; B_2 = middle of midrib and margin; B_3 = margin).

Table 1. Abaxial to adaxial ratio of chlorophyll a (R) for the leaves of various plants (Fully mature leaves were scanned and R value calculated)

Plant	R value	Plant	R value
Monocots		Dicots	
C_4 plants:		C_4 plants:	
NADP-ME type		NADP-ME Type	
Panicum antidotale, Retz.	1	*Alternanthra pungens*, HBK.	1·66
Pennisetum purpureum, Rich.	1	*Cleome gynandra* DC.	1·26
Pennisetum typhoides, Stampf and Hubb.	1·05	*Euphorbia hirta*, L.	0·87
		Gomphrena decumbens, Jacq.	0·83
Sorghum vulgare, Moench.	1·03	NAD-ME Type	
Zea mays, L.	0·92	*Amaranthus edulis*, L.	1·14
NAD-ME type		*Amaranthus hypochondriacus*, L.	1·24
Panicum coloratum L.	0·65	C_3 plants:	
PEP-CK type		*Arachis hypogaea*, L.	0·49
Chloris gayana	1·02	*Cassia siamea*, Lam.	0·81
Panicum maximum, Jacq.	1	*Cleome viscosa*, L.	1·53
		Corchorus olitorius, L.	0·91
C_3 plants:		*Achanthospermum hispidum* DC	1·12
Avena sativa, L.	0·9	*Tridax procumbens*, L.	1·38
Hordeum vulgare, L.	0·9	*Achyranthes aspera*, L.	1·18
Oryza sativa, L.	1·1	*Dolichos lablab*, L.	1·21
Triticum aestivum, L.	1·02	*Waltheria indica*, L.	0·85

into this problem we have scanned both monocots and dicots of C_3 and C_4 plants. In C_4 category we have studied plants which fall under NAD-ME, NADP-ME and PEP-CK type and the results are summarised in table 1. The R value for monocots studied here is fairly constant and near unity (table 1). Thus for the plants having R value different from unity the chlorophyll a distribution on the two sides of the leaf is asymmetrical.

The R value is > 1 for *Alternanthera pungens* and *Cleome gynandra* but < 1 for *Euphorbia hirta* and *Gomphrena decumbens*. Same trend was observed for C_3 dicots studied. The orientation of leaves with respect to ground and sun position was also studied and it was found that there was no correlation with the R value.

These studies suggest that the R value is not correlated with any of the taxonomic classifications of plants and depends presumably on the anatomical structure of the leaf. The present studies indicate that abaxial to adaxial ratio of chlorophyll a (R) has certain significance in photosynthetic process and requires an indepth study to understand the asymmetry of distribution of chlorophyll a in the leaf.

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