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PHOTOSYNTHETICAL PIGMENTS IN THE SPONGY AND PALISADE PARENCHYMAS AND THE ALTERNATIVE WAYS OF PHOTOSYNTHESIS

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Abstract

We have investigated the part of the spongy and palisade parenchymas played in photosynthesis. We have determined the pigment content, pigment ratios in the isolated tissues and established that:

1. More pigments are contained in the palisade parenchyma than in the spongy one.
2. Comparatively more chlorophyll b is contained in the spongy parenchyma and the chlorophyll b-/ total carotenoid ratio is higher than in the palisade.
3. The quantity of chlorophyll b is in negative correlation with the carotenoid content.
4. We suppose, on the basis of our data, there to be a ratio of photosystem I in the palisade parenchyma of the fully-developed leaves in their spongy parenchyma, but, that the ratio of photosystem II is higher. The two photosystems can function in a parallel way too, quite apart from each other. In the spongy parenchyma Hill's reaction, the non-cyclic photophosphorylation, and CO_2 fixation predominate, in the palisade one, however, the cyclic photophosphoryliation. A large part of the ATP energy produced in the palisade parenchyma can be used for forming polymers (starch, cellulose).

Introduction

Concerning the part played by the palisade and spongy parenchymas in photosynthesis, and the ratio of their participation in that, opinions differ. It may be taken as a general opinion — according to FILARSZKY (1911), HABERLAND (1914), SCHOREDER (1924), ROMELL (1927), MEYER (1962), KÁRPÁTI *et al.* (1968), as well as others — that the main assimilating tissue is the plaisade parenchyma. An opposing wiewpoint is taken by STARZECKI (1962). In his opinion, the most important tissue in the assimilation is the spongy parenchyma; the palisade parenchyma playing only the part of a light-filter in the fully-developed leaves.

The opinions are founded upon the following:

— The ratio of the palisade and spongy parenchymas depends mainly on the light conditions. STAHL (1880), TURREL (1944), STARZECKI (1962), ESAU (1969), FEKETE and SZUJKÓ—LACZA (1973).

— There is some difference between intensity and spectral composition of the light penetrating into the two tissues. The CO_2 and water supply as well as the general respiratory metabolism of the spongy parenchyma are different from those of the palisade parenchyma. EMERSON (1935), MONTFORT (1950), BRODFÜHRER (1955), RÜSCH and MÜLLER (1957), STARZECKI (1962).

— The chloroplast count per cell is generally higher in the palisade parenchyma than in the spongy one. Accodring to SCHÜRHOFF (1924), the percentage of chloroplasts in the palisade and spongy parenchymas is the following: Fragaria elatior 86 to 14, *Ricinus communis* 82 to 18, *Brassica* rapa 80 to 20, *Helianthus annuus* 73 to 27, *Phaseolus multiflorus* 69 to 31.

The researchers have so far tried to solve the above problem with leaf-histological, cytological investigations, by measuring O_2 development and CO_2 fixation.

In this paper, we draw conlusions about the activity of photosystems I and II in the spongy and palisade paranchymas from pigment content and pigment ratios known. And in our following paper (MARÓTI and GÁBOR, 1975) — we compare the electron-microscopic structure of the chloroplasts of both tissues, verifying the difference of the photosynthetic activities of mesophyll on the basis of the thylakoid aggregation, as well.

Concerning the pigment investigation of the isolated spongy and palisade parenchymas we have not found any publication. By separating the mesophyll and the bundle sheath, WOO *et al.* (1971) established that in the bundle-sheath chloroplasts of C_4-plants the photochemical system II is deficient and the chlorophyll-a/b ratio is generally higher than in the chloroplasts of the mesophyll.

Materiasl and Methods

The plants investigated: *Betula pendua* ROTH., *Hedera helix* L., *Sophora japonica L.*, *Nuphar luteum* (L) SM., *Nymphaea alba* L., *Zea mays* L. grew, under natural conditions, in the Botanical Gardens of the Attila József University. We took for our investigations fully-developed leaves. The spongy and palisade paranchymas as well as the bundle sheath were separated by means of a scalpel. The leaves were put, with lower surface upwards, on a rubber plate, and the abaxial epidermis was scratched with scalpel, with a bevelled cutting edge, the spongy parenchyma included. The other part of the leaf, the palisade parenchyma ands urface epidermis remained in goog preservation. The separation of tissues was supervised with a light microscope. We chosen only species where this was possible to do.

For our pigment investigations we took some mat ter of 0.3 to 0.5 g raw weight. The tissues separated were kept in the dark, at 1 °C during sampling (15 to 20 min), then extracted in a way already published by us (MARÓTI and GABNAI, 1971), and isolated by thin-layer chromatography. Then we measured the chlorophylls and carotenoids.

Results and their evaluation

1. Pigment content in the palisade and spongy parenchymas

It is not possible to establish in both tissues an unambiguous rule for the quantity of pigments. In the case of the plants investigated, except Sophora j., the palisade paranchyma contained many more chlorophylls and carotenoids, as referred to the weight and surface units, than the spongy parenchyma did.

In the leaves of *Hedera helix*, *Nymphaea alba*, *Nuphar luteum*, and *Betula pendula* the palisade parenchyma contains many more pigments than the spongy one. In the distribution of chlorphylls and carotenoids also, species peculiarities manifest themselves well.

Table 1. Pigment content in the palisade and spongy parenchymas as referred to a unit surface

Pigment	mg pigment (1 sq.decimetre leaf surface			
	Hedera helix		*Nymphaea alba*	
	palisade p.	spongy p.	palisade p.	spongy p.
chlorophyll a	2.35	0.86	2.57	0.21
chlorophyll b	0.52	0.21	0.52	0.05
carotene	0.24	0.04	0.21	0.01
lutein	0.31	0.14	0.44	0.06
anteraxanthine	0.04	0.02	0.04	0.01
violaxanthine	0.07	0.07	0.10	0.05
neoxanthine	0.08	0.05	0.11	0.01

Table 2. Pigment content of palisade and spongy parenchymas, as well as that of the whole leaf. Chl-a = chlorophyll a, chl-b = chlorophyll b, car. = β carotene, lut. = lutein, ant. = anteraxanthine, viol. = violaxanthine, neo. = neoxanthine

Pigments	mg pigment/100 g dry matter						
	chl-a	chl-b	car.	lut.	ant.	viol.	neo.
Hedera helix L.							
palisade p.	426	97	34	46	6	20	15
spongy p.	292	80	20	41	5	15	14
Nymphaea alba L.							
palisade p.	516	104	44	70	6	19	19
spongy p.	141	26	11	17	3	3	4
Nuphar luteum (L) SM.							
palisade p.	338	56	28	53	10	23	17
spongy p.	106	29	12	22	3	8	8
whole leaf	254	54	25	43	4	19	15
Betula pendula ROTH.							
palisade p.	444	93	40	66	5	18	18
spongy p.	284	67	24	39	3	10	13
whole leaf	409	88	34	57	4	17	17
Sophora japonica L.							
upper two cell layers of palisade p.	186	34	21	22	7	24	9
lower two cell layers of palisade p.	233	53	32	44	4	34	17
spongy p.	320	76	39	56	4	32	18

The leaves of *Hedera helix* and *Betula pendula* are hypostomatic, and those of *Nymphaea alba* and *Nuphar luteum* are epistomatic. The leaf of *Nymphaea alba* rose over the water surface, and that of *Nuphar luteum* floated on water. Between the pigment ratios of the two water-plants there is a considerable difference. The leaf of *Sophora japonica* could be separated into three tissue layers. The abaxial surface epidermis was scratched together with the spongy parenchyma. The palisade parenchyma has four cell layers. The two lower (internal) cell layers form a transition between the cells of the spongy parenchyma and the typical palisade parenchyma. The palisade parenchyma of the lower two cell layers can be separated well from the upper one that is kept together by the surface epidermis. It is a striking peculiarity of the leaf, and different from the other plants investigated, that most pigments are contained by the spongy parenchyma.

2. Pigment ratios in the various tissuse of the leaf

The chlorophyll a/b ratio is a highly characteristic value in the spongy and palisade parenchymas. In the spongy parenchyma, as a general rule, chlorophyll b is relatively accumulated. These leaves are hypostomatic and nearly of transversal position. In the spongy parenchyma, the cells are in a relatively aerobe, steam-saturated invironment and have a more hydrophilous peculiarity than the cells of the palisade parenchyma. On the basis of the low chlorophyll a/b ratio, it may be supposed that in the spongy parenchyma the ratio of the photochemical system II is higher, and its acitivity is more increased than in the palisade parenchyma.

Table 6. Chlorophyll a/b ratio in the leaves. The parenchyma of Sophora japonica with four cell layers was separated into two layers: u. = upper two, l. = lower two cell layers

Species	Whole leaf	Mesophyll	Palisade p.	Spongy p.	Bundle sheath
Hedera helix	4.2	—	4.4	3.6	—
Betula pendula	4.6	—	4.8	4.2	—
Nymphaea alba	5.1	—	4.9	5.4	—
Nuphar luteum	4.6	—	6.0	3.6	—
Sophora japonica	—	—	u. 5.5	4.2	—
			l. 4.4		
Zea mays	3.5	3.1	—	—	5.6

The epistomatic leaf of the *Nymphaea alba* investigated rose over the water surface. A higher chlorophlyl a/b ratio was found in the spongy parenchyma. In this case supposedly in the spongy parenchyma, the respiratory metebloism, and the oxygen supply are of lower degree; chlorophyll a can be accumulated just for that reason. The leaf of Nuphar luteum is epistomatic, as well, nonetheless, in the palisade parenchyma, the chlorophyll a/b ratio is higher. We are interpreting this contradiction as indicatory that, floating on the water surface, the leaf could take up water and water-solved oxygen at its lower surface.

We investigated in the spongy and palisade parenchymas the chlorophyll/carotenoid ratio, as well. For the chlorophyll a/carotenoid ratio we did not get any unambiguous connection. The chlorophyll b/carotenoid ratio was higher in the spongy parenchyma in the case of all five plants investigated. It seems to be a general rule that if the relative quantity of chlorophyll b increases, that of carotenoids decreases.

Table 7. The chlorophyll/carotenoid ratio of leaf tissues

Species	Chl.a/carotenoids		Chl.b/carotenoids	
	palisade parenchyma	spongy parenchyma	plaisade parenchyma	spongy parenchyma
Hedera helix	3.52	3.07	0.80	0.84
Betula pendula	3.02	3.19	0.63	0.75
Nymphaea alba	3.26	3.71	0.65	0.68
Nuphar luteum	2.58	2.00	0.42	0.54
Sophora japonica	2.24	2.15	0.40	0.51
	1.77			

Discussion of the results

What part may the spongy and palisade parenchymas have in photosynthesis?

With full knowledge of the pigment content of the spongy and palisade parenchymas, we may try to answer the above question but first, we have to deal with interpreting the concept of photosynthesis.

Today photosynthesis can no linger be identified only with Calvin's cycle, with a synthesis of the so-called organic compounds of primary formation. Nowadays, photosynthesis means the synthesis of any organic compounds originating from the energy of photochemical reactions in green plants [BASSHAM and CALVIN (1960), NICHIPOROVICH (1961), ARNON (1962), RUBY (1966)].

Taking into consideration the above facts, in the case of the leaves of heterogeneous mesophyll — instead of looking for the main assimiliating tissue — it is more correct to ask, what is photosynthetized by one of the tissues and what by the other The role of the palisade parenchyma may not be reduced only to light filtering. According to our supposition, the spongy and palisade parendhymas enable on alternative photosynthetetic activity. That means that the ratio of photosystems is not the same in the two tissues of mesophyll; e.g., more $NADPH_2$ comes into being in the spongy parenchyma than in the palisade one. In the spongy paranchyma the non-cyclic photophosphorylation predominates, and in the palisade parenchyma the cyclic one.

On the basis of the pigment ratios and the investigation of the electron-microscopic structure of chloroplasts we may subscribe to the opinion of STARZECKI (1962) and others that the spongy parenchyma of the hypostomatic leaves plays an outstanding part in binding CO_2. This is made possible by the structure of the spongy parenchyma, its more hydrophilous peculiarity, the presence of CO_2 and water, and the increased activity of the photochemical system II.

In the pongy parenchyma, an increased activity of photosystem II is proved by the following experimental facts:

1. The lower chlorphyll a/b ratio is showing a high photosystem II ratio. The pigment ratio is one of the most important characteristics of the two photochemical systems. According to BOARDMAN and ANDERSON (1964), GROSS *et al.* (1966), BRIANTAIS (1968), as well as others, the photochemical system I is characterized by a high, and pigment system II by a low chlorophyll a/b ratio. According to KNAFF and ARNON (1969, 1971), ARNON (1971), in photosynthesis there are three light reactions (PS—II_b, PS—II_a, and PS—I). Photosystem II is able to reduce NADP by the help of the two light reactions (II_b, II_a), independently of PS—I. The separation of the non-cyclic and cyclic photophosphorylations can be observed in the chloroplasts of the bundle sheath of C_4 plants, as well. It was demonstrated by DOWNTON *et al.* (1971), WOO *et al.* (1971) that in these chloroplasts photosystem II is deficient. According to SLACK *et al.* (1969), CO_2 fixation takes place in the mesophyll chloroplasts of C_4-plants, and the triozes formed are converted to hexose, resp. polymerized to starch in the bundle-sheath chloroplasts.
2. In the membrane of photosystem II there are more xanthophylls than in the membrane fragments containing photosystem I. LICHTENTHALER (1969), FALUDI-DÁNIEL (1973). In the spongy parenchyma, the relative quantity of the hydroxy-carotenoids (lutein, neoxanthine) is generally larger than in the palisade parenchyma.
3. According to our measuerements in the spongy parenchyma chloroplasts the length of the partitions on one square μ is much larger than in the palisade parenchyma. According to NIR adn PEASE (1973), AKOYUNOGLOU and ARGYROUDI-AKOYUNOGLOU (1974), phosystem II is in the grana, the interthylakoid place, and the partition. The spongy parenchyma chloroplasts contain many, large starch granules.
4. STARZECKI (1962) — by illuminating the leaf-discs on both sides — demonstrated in the case of several plants that the development of O_2 is much more intensive in the spongy parenchyma of the fully-developed leaves than in the palisade parenchyma.
5. We illuminated isolated leaves, after keeping them in the dark for twelve hours, in air containing 0.5 per cent CO_2 (30.000 Lux), and then separated the spongy and palisade parenchymas. After that, we measured the change in the soluble carbohydrate level in both tissues of mesophyll. In the spongy parenchyma we obtained a 5 to 15 per cent increase but in the palisade one, the quantity of soluble carbohydrate became rather smaller.

The chlorophyll a/b ratio of the palisade parenchyma chloroplasts is high. This is relates to the predominance of photosystem I. The cyclic photophosphorylation does not need water, or carbon dioxide. Light energy is pesererved — without producing oxygen — in the ATP-bondages of high energy-content. ATP can be utilized in various syntheses, and formations of carbohydrate polimers (starch, cellulose).

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