Photochemical activities of chloroplasts from plants with and without bundle sheath in leaves*[1/]

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COMPENDIO

Se escogieron veinte especies tanto monocotiledoneas como dicotiledoneas y se investigó su anatomía foliar. Las especies estudiadas se agruparon según la presencia o ausencia de la vaina de los haces vasculares en las hojas. Se determinaron la actividad de la reacción Hill y las actividades de fosforilación cíclica y no cíclica de los cloroplastos aislados de estos dos grupos de plantas. Se observó que los cloroplastos de plantas con vainas de los haces en las hojas mostraron actividades fotoquímicas definidamente más altas que las plantas sin vaina, exhibiendo así una acentuada correlación entre la actividad de los cloroplastos y las características anatómicas. — Los autores.

Introduction

IT is fairly established that species with C_4-dicarboxylic acid pathway of photosynthesis (4) exhibit a distinctive type of leaf anatomy characterized by the presence of chlorenchymatous bundle sheath (9). Plants lacking such an anatomical characteristic possess the Calvin type C_3 photosynthesis. Of these two types of plants, the former are known to be photosynthetically more efficient than the latter. Conflicting reports, however, appeared recently on the photochemical properties of chloroplasts isolated from plants of the C_4 type. Chen *et al.* (3) showed that chloroplasts of Bermuda grass, a plant with high capacity for photophosphorylation, belongs to the high photosynthetic group of plants. Contrary to this finding, Hew and Gibbs (5) reported results with chloroplasts isolated from maize, sugarcane and sorghum and observed that their reaction were very similar to those from plants like spinach.

The present finding concerns a survey of the photochemical activities of chloroplasts from twenty species of plants with and without bundle sheath in the leaves.

Materials and methods

The plants selected for the investigation were as follows: *Elencine coracana, Hordeum vulgare, Pennisetum typhoideum, Pennisetum purpureum, Setaria italica, Oryza sativa, Sorghum bicolor, Saccharum officinarum, Cenchrus ciliaris, Musa paradisiaca, Dracaena terniflora* and *Phoenix sylvestris* of the monocotyledonous families. From the dicotyledonous families those selected were: *Polyalthia longifolia, Arachis hypogea, Helianthus annuus, Capsicum annuum, Nicotiana tabacum, Gomphrena decumbens, Chenopodium amaranticolor* and *Piper nigrum*. Leaves from all the crop plants were cultivated under field conditions in the nearby Agricultural Experiment Station. Other plant materials were collected from the garden of this department.

Fully expanded leaves were ground in a pre-cooled mortar using M/15 sucrose phosphate buffer at pH 7.3

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Table 1—Hill reaction activity*. Decrement of absorbancy/mg. chlorophyll

	Plants with bundle sheath		Plants without bundle sheath	
1	*E. coracana*	63.34 ± 6.34	*P. longifolia*	10.97 ± 0.17
2	*H. vulgare*	30.63 ± 3.28	*P. sylvestris*	10.92 ± 0.72
3	*P. typhoideum*	27.91 ± 1.71	*M. paradisiaca*	7.38 ± 0.63
4	*P. purpureum*	20.43 ± 0.43	*C. amaranticolor*	13.31 ± 0.44
5	*G. decumbens*	27.80 ± 0.60	*N. tabacum*	4.92 ± 0.15
6	*S. italica*	25.89 ± 1.22	*D. terniflora*	2.81 ± 0.08
7	*O. sativa*	13.88 ± 0.84	*H. annuus*	12.22 ± 0.31
8	*S. bicolor*	24.96 ± 0.67	*P. nigrum*	7.57 ± 0.36
9	*S. officinarum*	18.12 ± 1.62	*A. hypogea*	7.09 ± 0.50
10	*C. ciliaris*	20.02 ± 0.52	*C. annuum*	10.72 ± 0.22

* The reaction mixture consisted of the grinding medium 2.0 ml; 0.1 M KCl 1.0 ml; 0.1 per cent 2,6-dichlorophenol indophenol, 0.1 ml and chloroplast suspension equivalent to 50 μg chlorophyll. Change in absorbancy was recorded after exposing the tubes to 8,000 lux for 3 minutes

containing disodium salt of EDTA, final conc 2×10^{-3} M) and $MgSO_4$ (final conc. 1.8×10^{-3}M). Class II chloroplasts were prepared from the resultant brie (7). All manipulations during the extraction procedure were carried out in a cold room maintained at 0-2°C

Hill reaction activity was measured by following the reduction of 2,6-dichlorophenol indophenol (6). Phenazinemethosulphate (PMS) catalyzed cyclic and ferricyanide catalyzed non-cyclic photophosphorylation activities were recorded in terms of radioactivity incorporated into ATP (3). $AT^{32}P$ content was assayed according to Arney (1). Chlorophyll content was estimated by the method of Arnon (2). Light microscopic preparations were done as per the procedure outlined by Johansen (8).

Results and discussion

Study of leaf anatomy revealed the occurrence of a distinct bundle sheath separated from the mesophyll tissue in the following species: *E. coracana, H. vulgare, P. typhoideum, P. purpureum, O. sativa, S. italica, S. bicolor, S. officinarum, C. ciliaris* and *G. decumbens*.

Table 2—PMS-catalyzed cyclic photophosphorylation*. CPM in thousands/mg. chlorophyll

	Plants with bundle sheath		Plants without bundle sheath	
1	*E. coracana*	1,891 ± 222	*P. longifolia*	71 ± 3
2	*H. vulgare*	261 ± 20	*P. sylvestris*	129 ± 15
3	*P. typhoideum*	320 ± 30	*M. paradisiaca*	131 ± 3
4	*P. purpureum*	837 ± 47	*C. amaranticolor*	47 ± 3
5	*G. decumbens*	242 ± 16	*N. tabacum*	103 ± 20
6	*S. italica*	803 ± 6	*D. terniflora*	241 ± 20
7	*O. sativa*	727 ± 112	*H. annuus*	200 ± 11
8	*S. bicolor*	319 ± 20	*P. nigrum*	179 ± 38
9	*S. officinarum*	207 ± 15	*A. hypogea*	109 ± 14
10	*C. ciliaris*	1,468 ± 188	*C. annuum*	25 ± 17

* The reaction mixture in a total volume of 2.8 ml consisted of 0.01 M Tris HCl pH 8.0. 50 μM; $MgCl_2$ 2 μM; ADP 1 μM; inorganic phosphate, 1 μM; PMS. 0.02 μM and chloroplast suspension equivalent to 25 μg chlorophyll. The reaction was started by adding 5 μ Ci ^{32}P and turning on lights (8,000 lux). The reaction was terminated after 10 minutes of exposure by adding 0.1 ml of 20 per cent TCA.

Table 3.—Ferricyanide-catalyzed non-cyclic photophosphorylation* CPM in thousands/mg chlorophyll

Plants with bundle sheath			Plants without bundle sheath	
1	*E. coracana*	2,638 ± 331	*P. longifolia*	86 ± 6
2	*H. vulgare*	376 ± 51	*P. sylvestris*	156 ± 11
3	*P. typhoideum*	747 ± 13	*M. paradisiaca*	142 ± 2
4	*P. purpureum*	1,144 ± 81	*C. amaranticolor*	92 ± 4
5	*G. decumbens*	1,017 ± 6	*N. tabacum*	181 ± 21
6	*S. italica*	904 ± 6	*D. terniflora*	311 ± 3
7	*O. sativa*	924 ± 2	*H. annuus*	268 ± 11
8	*S. bicolor*	456 ± 49	*P. nigrum*	252 ± 46
9	*S. officinarum*	723 ± 44	*A. hypogea*	170 ± 10
10	*C. ciliaris*	2,194 ± 166	*C. annuum*	291 ± 3

* Additions in reaction mixture were the same as in cyclic photophosphorylation except that 0.2 μM ferricyanide was substituted for PMS

There was no differentiation into bundle sheath and mesophyll tissues in *P. longifolia, C. amaranticolor, P. nigrum, N. tabacum, C. annuum, A. hypogea, H. annuus, M. paradisiaca, P. sylvestris* and *D. terniflora*.

Hill reaction activity of chloroplasts from various species studied exhibited a characteristic trend. Chloroplasts from all those plants possessing a bundle sheath in the leaves, irrespective of belonging to monocotyledonous or dicotyledonous groups, showed distinctly higher rates when compared to those from plants without bundle sheath (Table 1). Highest rates were recorded in *E. coracana* and *H. vulgare*.

The activities of PMS catalyzed cyclic photophosphorylation and ferricyanide catalized non-cyclic photophosphorylation of chloroplasts are shown in Tables 2 and 3 respectively. Here again the activities of chloroplasts from species with bundle sheath are several times higher than those from plants without bundle sheath.

Thus, the present results show that both Hill reaction and photophosphorylation capacities of chloroplasts from bundle sheath in the leaves are much higher than those from the other group of plants. The higher photochemical activity is presumably related to the greater efficiency in carbon fixation in those plants possessing bundle sheath. These results appear somewhat in contrast to those reported by Hew and Gibbs (5) in as much as the present finding revealed distinct differences in the rates of photochemical properties of chloroplasts from the two groups of plants. Further work on the photochemistry of chloroplasts from the two types of plants is considered to be most useful in seeking an explanation for the differences in the photophysiology between the C_4 and C_3 type of plants.

Summary

Twenty species of plants belonging to both the monocotyledonous and dicotyledonous groups were chosen and their leaf anatomy was investigated. The species studied were grouped according to the presence or absence of vascular bundle sheath in the leaves. Hill reaction activity and cyclic and non-cyclic photophosphorylation activities of chloroplasts isolated from these two groups of plants were determined. It was observed that the chloroplasts from plants with bundle sheath in the leaves showed distinctly higher photochemical activities than those from plants without bundle sheath, thus exhibiting a striking correlation between the chloroplast activity and anatomical characteristics.

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