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Association of chlorophyll content, phyllotaxy, photosynthesis and B group vitamins in some C_3 and C_4 plants

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Abstract. The photosynthetically efficient C_4 plants *viz Amaranthus viridis*, *Euphorbia hirta* and a C_3 plant, *Acalypha indica* with mosaic leaf pattern showed the maximum amount of *B* vitamins when compared to the other C_3 plants. It is observed that photosynthesis and vitamin synthesis go hand-in-hand showing close correlation. The results also indicate that there is a close relation between chlorophyll content and vitamin content. However, there appears to be no relation between phyllotaxy and photosynthesis. Between the two C_3 plants, *viz.*, *Acalypha* and *Carica*, the photosynthetic inefficiency of the latter might be due to more of chlorophyll *b* and less of chlorophyll *a* as seen from chlorophyll *a*/chlorophyll *b* ratios.

Keywords. Chlorophyll ; B vitamins ; phyllotaxy ; photosynthesis ; C_3 and C_4 plants.

1. Introduction

The chlorophyll content of the cell must be closely associated with photosynthetic activity because the photosynthetic rate is proportional to the chlorophyll concentration (Maksymowych 1973), but there are contrary reports also. Black (1972) concluded that the high rate of photosynthesis/mg chlorophyll is directly related to the low chlorophyll content. The photosynthetic rates of different chlorophyll mutants of pea, soybean, cotton and tobacco were studied by Benedict (1972). The photosynthetic rate/mg of chlorophyll in mutants is 2–11 times faster than in the wild type leaves. This phenomenon is compared to the photosynthetic rate of the yellow sectors of variegated leaves. These yellow sectors although containing a reduced chloropyhll content, have a much higher photosynthetic rate/mg chlorophyll than the green sectors. Bonner and Bonner (1948) found that thiamine synthesis in seedlings is often light-dependent and mature leaves of full grown tomato plants are the centre of production. Gustafson (1948) suggested that light stimulates thiamine biosynthesis. It was stated that the photosynthetic unit and not the chlorophyll content determines the rate of photosynthesis (Black 1972).

The present investigation has been designed to understand the relationship between chlorophyll content, vitamin content, phyllotaxy and photosynthesis in some C_3 and C_4 plants. Work on the relationship between phyllotaxy and photosynthesis is scanty. Earlier work (Evans 1975) indicates that canopies with more vertically inclined leaves have a higher photosynthetic rate than those with horizontal leaves.

2. Materials and methods

Young and fully expanded leaves from various plant species, *viz.*, (1) *Acalypha indica*, L., (2) *Amaranthus viridis*, L., (3) *Carica papaya*, L., (4) *Commelina benghalensis*, L., (5) *Euphorbia hirta*, L., (6) *Euphorbia pulcherrima*, Willd., (7) *Ervatamia coronaria*, Stapf., (8) *Werium odorum*, Soland., (9) *Nyctanthus arbor-tristis*, L., (10) *Petunia hybrida*, L., (11) *Sida acuta*, Burn, (12) *Tridax procumbens*, L., growing in the university Botanical Garden under natural photoperiod constitute the experimental material.

Chlorophylls were extracted with 80% acetone and estimated according to the method of Arnon (1949). The chloroplasts were isolated using N/15 phosphate buffer (pH 7·3) containing sucrose (0·33 M), disodium salt of EDTA (2×10^{-3} M) dithiothreitol (5×10^{-3} M), $MgCl_2$ (1×10^{-3} M), $MgSO_4$ ($1{\cdot}5 \times 10^{-3}$ M) and 5% (w/v) polyvinylpyrolidene as isolation medium following the procedure of James and Das (1957). The method of Jagendorf and Evans (1957) was used to assay Hill reaction activity of chloroplast preparation. $^{14}CO_2$ fixation studies were done using a technique similar to that described by Berry *et al* (1970), using ^{14}C sodium bicarbonate (specific activity, 27 mci/m mole) and the net photosynthesis was expressed as mg CO_2 fixed $dm^{-2}hr^{-1}$.

The different vitamins of the *B* group, *viz.*, thiamine (B_1), riboflavin (B_2), pyridoxin (B_6), niacin and folic acids were extracted and estimated colorimetrically following the methods given by Manzur-ul-Haque Hashmi (1973) and the results were expressed as μg/g dry wt. All the results were averages of three individual experiments.

3. Results and discussion

The results in table 1 indicate that *Amaranthus viridis*, *Euphorbia hirta* which are C_4 photosynthetic plants and *Acalypha indica*, a C_3 plant with mosaic pattern of phyllotaxy have maximum Hill activity, net photosynthesis and chlorophyll content. *Commelina benghalensis*, *Euphorbia pulcherrima*, *Ervatamia coronaria* and *Sida acuta* occupy the next position in order in this respect. *Nerium odorum* shows the minimum activity.

It is tempting to note that there is a close correlation between the photosynthetic parameters (table 1) and vitamins of the *B* group (table 2) *viz.*, thiamine (B_1), riboflavin (B_2), pyridoxin (B_6), niacin and folic acid in the plants mentioned above. *Nerium odorum* shows the minimum amount of vitamins of the *B* group. The data thus gives a circumstantial evidence to show that photosynthesis and vitamin synthesis go hand-in-hand showing a close correlation (table 3). The photosynthetically efficient C_4 plants *viz.*, *Amaranthus veridis*, *Euphorbia hirta*

Table 1. The pattern of chlorophylls and the rate of photosynthesis in different plant species.

	Plant species	Phyllotaxy	Plant type	Total chlorophylls *	Chlorophyll a/b ratio *	Hill activity +	Net photosynthesis **
1.	*Acalypha indica* L.	mosaic	C_3	4·62 ±0·84	1·16 ±0·04	163·5 + 6·21	38·4 ± 2·22
2.	*Amaranthus viridis* L.	alternate	C_4	3·58 ±0·22	1·21 ±0·12	186·2 ± 10·6	45·8 ± 5·16
3.	*Carica papaya* L.	mosaic	C_3	1·90 ±0·31	0·18 ±0·01	121·1 ± 7·81	26·5 ± 1·76
4.	*Commelina benghalensis* L.	alternate	C_3	2·43 ±0·14	1·17 ±0·06	148·4 ± 3·42	31·6 ± 4·32
5.	*Euphorbia hirta* L.	opp-superimposed	C_4	3·96 ±0·86	1·25 ±0·22	169·5 ± 12·31	42·0 ± 1·78
6.	*Euphorbia pulcherrima* Willd.	opp-decussate	C_3	3·47 ±0·86	1·28 ±0·08	142·6 ± 16·2	36·8 ± 6·52
7.	*Ervatamia coronoria* Stapf.	opp-decussate	C_3	2·02 ±0·10	1·25 ±0·12	136·0 ± 5·61	29·1 ± 1·34
8.	*Nerium odorum* Soland	Whorled	C_3	1·69 ±0·21	1·63 ±0·34	84·3 ± 7·52	17·2 ± 2·88
9.	*Nyctanthes arbortristis* L.	opp-superimposed	C_3	1·98 ±0·28	1·26 ±0·07	117·5 ± 11·6	21·6 ± 1·06
10.	*Petunia hybrida* L.	alternate	C_3	1·76 ±0·11	1·31 ±0·26	104·2 ± 18·2	19·0 ± 3·82
11.	*Sida acuta* Burm.	alternate	C_3	3·13 ±0·16	1·17 ±0·34	126·1 ± 7·01	23·2 ± 2·17
12.	*Tridax procumbens* L.	alternate	C_3	1·65 ±0·81	1·24 ±0·12	100·8 ± 9·44	21·8 ± 4·61

* mg g^{-1} fresh wt.
** mg CO_2 fixed $d^{-2}m$ hr^{-1}.
+ μ moles of DCPIP reduced mg^{-1} chl hr^{-1}.
(Values are means ± S.E. of three individual experiments).

and *Acalypha indica*, a C_3 plant with mosaic leaf pattern showed the maximum amount of vitamins. *Commelina benghalensis*, *Eurphorbia pulcherrima*, *Ervatamia coronaria* and *Sida acuta* form the second group in their vitamin contents quite parallel to their photosynthetic parameters.

The results categorically indicate that there is a close relation between chlorophyll content and vitamin content (table 3). The maximum amount of chlorophyll (total) is seen in *Acalypha indica* (4·62 mg/g fresh wt.). Nevertheless,

Table 2. Vitamin content ($\mu g\ g^{-1}$ dry wt.) in the leaves of different plant species.

Plant spp*	Thiamine (B_1)	Riboflavin (B_2)	Pyridoxin (B_6)	Niacin	Folic acid	Total
1.	369·0 ± 12·1	124·0 ± 8·9	215·0 ± 16·2	152·0 ± 20·4	134·0 ± 6·2	994
2.	280·0 ± 21·3	136·0 ± 12·4	198·0 ± 17·4	169·0 ± 11·2	149·0 ± 13·1	932
3.	114·0 ± 16·2	82·0 ± 3·2	128·0 ± 15·8	97·0 ± 8·6	78·0 ± 4·4	499
4.	232·0 ± 31·0	64·0 ± 5·7	176·0 ± 21·1	160·0 ± 11·1	52·0 ± 2·3	684
5.	291·0 ± 18·6	99·0 ± 2·8	195·0 ± 8·8	141·0 ± 6·1	130·0 ± 7·6	856
6.	207·0 ± 26·2	76·0 ± 4·9	180·0 ± 11·2	122·0 ± 10·1	104·0 ± 11·2	689
7.	197·0 ± 16·6	108·0 ± 13·1	209·0 ± 6·8	135·0 ± 14·2	126·0 ± 13·6	775
8.	74·0 ± 8·1	42·0 ± 3·6	96·0 ± 2·4	28·0 ± 3·8	49·0 ± 6·4	289
9.	139·0 ± 11·2	34·0 ± 2·7	126·0 ± 7·6	47·0 ± 1·2	61·0 ± 3·4	407
10.	98·0 ± 14·1	51·0 ± 2·6	113·0 ± 3·2	62·0 ± 5·6	93·0 ± 2·8	417
11.	156·0 ± 21·7	78·0 ± 1·7	174·0 ± 14·6	103·0 ± 2·8	82·0 ± 4·1	593
12.	124·0 ± 6·4	46·0 ± 1·1	101·0 ± 9·9	94·0 ± 2·1	76·0 ± 3·6	441

* Plant names as represented in table 1 serially.
(Values are means ± S.E. of three replications).

Table 3. Statistical analysis.

1. Correlation coefficient (for tables 1 and 2)

	between a set of parameters		correlation coefficient (r_{xy})
(a)	Total chlorophylls	× hill activity	+ 0·822 (4·564)*
(b)	,,	× photosynthesis	+ 0·826 (4·635)*
(c)	,,	× thiamine (B_1)	+ 0·895 (6·346)*
(d)	,,	× riboflavin (B_2)	+ 0·730 (3·378)*
(e)	,,	× pyridoxin (B_6)	+ 0·209 (0·676)
(f)	,,	× niacin	+ 0·676 (2·901)*
(g)	,,	× folic acid	+ 0·907 (6·809)*
(h)	,,	× total vitamins	+ 0·849 (5·083)*

2. Analysis of variance (for table 2)

		Vitamins	Plant species
F calculated	...	26·765*	10·515*
F from table at 5% level	...	2·594	2·152
C.D. at 5% level	...	15·802	8·972

* Significant at 5% level.

Values in the parantheses represent the calculated values of t-statistic for testing the significance of correlation coefficients.

chlorophyll a/b ratio is not the highest in *Acalypha indica* (table 1). Chlorophyll a/b ratio is maximum (1·63) in *Nerium odorum*, but the vitamin content is at a minimum level indicating that neither chlorophyll a nor b has anything to do with vitamin synthesis, but it is the total chlorophyll content that is associated with vitamin content, both being at a lower level (tables 1 and 3).

It is noted that there is no relationship between photosynthesis and phyllotaxy. Mosaic pattern of phyllotaxy is assumed to help light to fall directly on all the leaves without any impediment, thus making the plant photosynthetically more efficient. But, if a comparison is made between *Acalypha* and *Carica* (both show mosaic pattern) the latter is not at all efficient (table 1). Similarly *Amaranthus veridis* has alternate leaves, while *Euphorbia hirta* which is also a C_4 plant possesses opposite superimposed leaf arrangement thus indicating that there is no relation between phyllotaxy and photosynthesis. In the case of opposite decussate pattern in *Ervatamia* and *Euphorbia pulcherrima* where overshadowing is avoided, the photosynthetic efficiency is somewhat better than that of *Carica papaya* with mosaic pattern.

On the basis of computations it can be inferred that there is a highly positive correlation between total chlorophyll content and other parameters such as Hill activity, net photosynthesis, thiamine, riboflavin, niacin, folic acid and the total vitamins (table 3). The correlation coefficients of all the parameters are significant at 5% level except with pyridoxin. Hence there is an association of chlorophyll content and other parameters (except phyllotaxy).

Even an analysis of variance (for table 2) reveals a close association among the parameters. *F*-values calculated for vitamins and plant species are significant at 5% level.

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