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Photosynthesis of Lipids from $^{14}CO_2$ in *Spinacia oleracea*[1]

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ABSTRACT

Young expanding spinach leaves exposed to $^{14}CO_2$ under physiological conditions for up to 20 minutes assimilated CO_2 into lipids at a mean rate of 7.6 micromoles per milligram chlorophyll per hour following a lag period of 5 minutes. Label entered into all parts of the lipid molecule and only 28% of the ^{14}C fixed into lipids was found in the fatty acid moieties, *i.e.* fatty acids were synthesized from CO_2 *in vivo* at a mean rate of 2.1 micromoles per milligram chlorophyll per hour. Intact spinach chloroplasts isolated from these leaves incorporated $H^{14}CO_3$ into fatty acids at a maximal rate of 0.6 micromole per milligram chlorophyll per hour, but were unable to synthesize either the polar moieties of their lipids or polyunsaturated fatty acids. Since isolated chloroplasts will only synthesize fatty acids at rates similar to the one obtained with intact leaves *in vivo* if acetate is used as a precursor, it is suggested that acetate derived from leaf mitochondria is the physiological fatty acid precursor.

Maturing spinach chloroplasts may contain >70% of the total acyl lipid of the leaf cell and their contribution to the biosynthesis of these lipids, which constitute the matrix of the photosynthetic membranes, is of great current interest. Chloroplasts have long been known to have an active fatty acid synthetase (22), but there are also conflicting reports, based on subcellular fractionation experiments, of the presence of several extrachloroplastic fatty acid synthetases in leaf cells (4, 5, 24). In a more recent study employing a sensitive radioimmunoassay using anti-ACP[3] antibodies, essentially all of the spinach leaf fatty acid synthetase was localized in the chloroplast (16).

The capacity of isolated intact spinach chloroplasts for the incorporation of acetate or photosynthetically fixed CO_2 into fatty acids has been reported from several laboratories (10, 12, 13, 18). However, the capacity of healthy expanding leaves for lipid biosynthesis from CO_2 has not been assessed to date. The rate at which well grown maturing spinach leaves could synthesize lipids from CO_2 under physiological conditions would indicate the biosynthetic capacity of the tissue against which the biosynthetic capacities of subcellular fractions could be more critically judged. We have directly compared the incorporation of CO_2 into lipids by intact spinach chloroplasts with the photosynthetic incorporation of CO_2 into lipids in rapidly expanding spinach leaves still attached to the parent plant. The results demonstrate that, whereas chloroplasts may be able to synthesize all of the saturated fatty acids required by the leaf, the biosynthesis of the fatty acid precursors and the desaturation of these fatty acids require the collaboration of other organelles in the leaf cell.

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[3] Abbreviations: ACP, acyl carrier protein; PC, phosphatidylcholine; PG, phosphatidylglycerol; MGD, monogalactosyl diacylglycerol; DGD, digalactosyl diacylglycerol; FFA, free fatty acid; MG, monoacylglycerol; DG, diacylglycerol; 3-PGA, 3-phosphoglyceric acid; PE, phosphatidylethanolamine; PA, phosphatidic acid.

MATERIALS AND METHODS

Plant Material. Seedlings of *Spinacia oleracea* L. (Yates Hybrid 102) were grown for 5 to 7 weeks in vermiculite at 25 C and 60% RH. The light intensity was 25,000 lux (0.8×10^5 erg·cm^{-2} s^{-1}) with a 12-h photoperiod and 5 C night temperature depression. Hydroponically grown plants were first germinated in vermiculite before transfer to aerated liquid culture. The nutrient solution contained 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 4 mM $MgCl_2$ plus trace elements at a final concentration of 46 μM H_3PO_3, 9 μM $MnCl_2$, 0.76 μM $ZnSO_4$, 0.32 μM $CuSO_45H_2O$, 1.2 μM $NaMoO_4$, 24 μM NaFeEDTA. Following 3 to 4 weeks growth in controlled environment cabinets, the seedlings were transferred to individual (*i.e.* one per plant) aerated jars filled with the culture solution and grown under natural light (8.5-h photoperiod) in a greenhouse for a further 2 to 3 weeks. In general, hydroponically cultured plants grew faster and gave higher rates of CO_2 fixation than vermiculite-grown plants, but the proportion of ^{14}C-fixed into lipids was independent of growth conditions. The results are reported for hydroponically cultured plants unless otherwise stated.

Exposure of Leaves to $^{14}CO_2$. Selected pots containing up to four spinach seedlings with young, still expanding leaves were placed in the exposure apparatus for equilibration 15 min before the start of each exposure. The environmental conditions in the exposure apparatus were the same as those under which the plants were grown (3). The apparatus permitted the exposure of intact plants to a constant specific radioactivity of $^{14}CO_2$ for periods from 10 s to 20 min: it contained $^{14}CO_2$ at atmospheric concentration and a specific radioactivity of 40 μCi·μmol^{-1}. An air flow of 6 liters min^{-1} ensured thorough mixing of the gases. Exposures were terminated by total immersion of the leaves in liquid N_2 and the whole leaves were used for lipid extraction.

Rapid Isolation of Chloroplasts. Chloroplast suspensions for incubation with [^{14}C]bicarbonate were prepared as previously described (12).

Lipid Extraction. Lipids were extracted from leaves and chloroplasts by homogenization in a TenBroeck ground glass homogenizer in the presence of chloroform:methanol (2:1, v/v) and the extracts dried on a rotary evaporator. The extract was redissolved in chloroform:methanol (2:1, v/v) and water-soluble contaminants removed by partitioning against 0.1 M KCl; 1% acetic acid (v/v) solution in a N_2 atmosphere at 4 C in the dark. The chloroform layer was washed three times with distilled H_2O and dried under N_2.

Lipid Analysis. Total lipid mixtures were resolved by one- and two-dimensional TLC on glass plates coated with a 250-μm layer of Silica Gel H. Galactolipids and phospholipids were separated

by development in acetone:acetic acid:water (100:2:1, v/v), chloroform:methanol (65:25:4, v/v), and chloroform:methanol:acetic acid:water (85:15:10:3.5, v/v). Neutral lipids were separated in petroleum ether:diethyl ether:acetic acid (80:20:1, v/v) and benzene:diethyl ether:ethyl acetate:acetic acid (80:10:10:0.4, v/v). Lipids were identified on the basis of co-chromatography with authentic standards and by the use of specific spray reagents.

The constituent fatty acids were analyzed as their methyl esters, which were prepared as previously described (12). Hydrolysis of the separated lipids was performed by refluxing for 45 min in 30 ml of 2.5 N KOH in 80% (v/v) methanol:water.

Galactose and glycerol were separated by ascending paper chromatography in ethyl acetate:acetic acid:formic acid:water (180:30:10:4, v/v) and visualized with $AgNO_3$ plus NaOH ethanol (23). Alternatively, the galactose/glycerol mixtures were analyzed by GLC on a Pye series 104 after silylation for 1 h in *n*-trimethylsilylimidazole (temperature 93–94 C) in pyridine. The glass columns contained OV 101 Gas-chrom Q (100–120 mesh) held at 180 C. Nitrogen carrier gas at a flow rate of 60 ml min^{-1} was used. Phospholipids and galactolipids were analyzed by the methods of Bartlett (2) and Roughan and Batt (18), respectively. Acyl-CoA and acyl-ACP were determined by the method of Mancha *et al* (9).

Radioactivity Determination. ^{14}C-labeled lipids were detected on thin layers by autoradioactivity using Kodirex X-ray film (Kodak) and by scanning on a Nuclear Chicago Actigraph III model 1006. Radioactivity was determined at 20% efficiency in solid samples using a Nuclear Chicago gas flow counter with etched aluminum planchets containing sample plated to maximum thinness. Liquid samples were counted in Bray's cocktail (12) to 85% efficiency on a Beckman LS230 liquid scintillation counter.

Chl Determinations. Total Chl was extracted in 80% acetone and determined according to the method of Arnon (1).

RESULTS

Labeling Patterns in Leaves.

Incorporation of ^{14}C into Total Lipids. Rates of light-dependent CO_2 fixation by the leaves of intact spinach plants were linear for at least 20 min and values of >100 μmol CO_2 fixed mg^{-1} Chl·h^{-1} were routinely obtained. Labeled lipids were detected after only 20 s exposure to $^{14}CO_2$ (Fig. 1) but the amount of the total fixed label found in the lipid extract exhibited a lag period of about 5 min. During this time, the precursors of lipid biosynthesis would gradually become saturated with ^{14}C up to the specific radioactivity of the $^{14}CO_2$ supplied. Once saturation of the lipid precursors had occurred then a linear rate of incorporation would be expected and this was observed over the 5- to 20-min period.

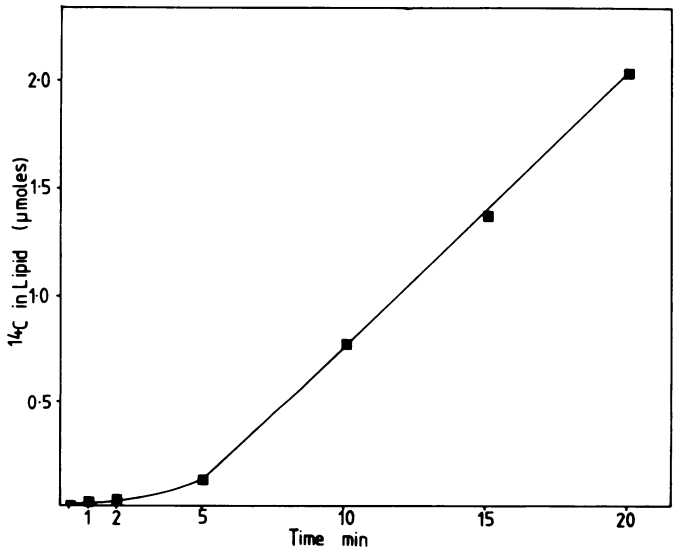

FIG. 1. Incorporation of ^{14}C into lipids from photosynthetically fixed $^{14}CO_2$ by intact spinach leaves. The values are averaged from several different experiments.

Location of ^{14}C within Lipid Molecules. The distribution of label between the aqueous and nonpolar fractions of hydrolysates of the principal labeled lipids is shown in Table I. Of the total ^{14}C incorporated, 70 to 90% was found in the acyl residues (nonpolar fraction) of PC and PG. The proportion of label in this fraction increased with time in both the phospholipids and in the galactolipids. But both galactolipids incorporated most of their ^{14}C (53–67%) into the aqueous fraction, which contained the polar moieties of the lipids, *i.e.* galactose and glycerol. Analysis of the polar moieties of MGD, where a large proportion of the newly assimilated ^{14}C accumulated (Table I), revealed that the ^{14}C was present in both the galactose and glycerol moieties. Paper chromatography and GLC showed that 72 to 78% of the ^{14}C in the polar moieties was in galactose, with the remainder found in glycerol.

Fatty Acid Labeling Patterns. The labeling patterns of the constituent fatty acids of PC, PG, MGD, and DGD in spinach leaves are given in Table II. Over 60% of the ^{14}C in PC was in 18:1,

Table I. *^{14}C Recovered in (a) Nonpolar Fractions (Containing Fatty Acids) and (b) Aqueous Fractions (Containing Galactose and Phosphate Ester Headgroups and Glycerol) from Hydrolysates of Individual Glycerolipids from Spinach Leaves Exposed to $^{14}CO_2$ for 10 Min and 20 Min in Light*

Lipid		^{14}C in Each Fraction			
		10-min exposure to $^{14}CO_2$		20-min exposure to $^{14}CO_2$	
		dpm × 10^{-3}	*% a + b*	*dpm* × 10^{-3}	*% a + b*
PC	a.	434	86	1559	89
	b.	68	14	201	11
PG	a.	97	70	3821	77
	b.	42	30	117	23
MGD	a.	191	23	737	39
	b.	396	67	1172	61
DGD	a.	37	44	141	47
	b.	48	56	158	53

Table II. *$^{14}CO_2$ Incorporation into Fatty Acids in Whole Spinach Leaves Exposed to $^{14}CO_2$ for Different Periods of Time in Light*

Fatty Acid	^{14}C in Fatty Acids							
	PC		PG		DGD		MGD	
	10 min	20 min	10 min	20 min	10 min	20 min	10 min	20 min
	% total							
12-0	tr[a]	tr	tr	tr	0.1	tr	0.2	tr
14-0	0.2	tr	0.1	tr	0.2	0.2	0.2	0.1
16-0	18.2	15.9	59.8	54.6	41.8	39.4	38.2	31.0
16-1	0.3	tr	4.6	5.7	5.2	tr	4.9	2.8
16-2	1.2	ND[b]	0.2	tr	tr	ND	4.0	7.8
16-3	tr	ND	ND	ND	ND	ND	2.0	3.7
18-0	2.7	2.4	1.9	1.7	tr	3.6	0.9	4.0
18-1	64.2	60.9	20.7	23.2	20.8	24.4	27.3	29.7
18-2	10.2	18.5	10.3	12.0	12.8	10.5	9.9	14.6
18-3	1.7	1.2	1.1	1.8	9.5	11.1	11.9	15.3

[a] Trace.

[b] None detected.

although the proportion in 18:2 increased slightly with time. Most of the label in PG was in 16:0 with a slight increase in 16:1 synthesis after 20 min. There were also small increases in the unsaturated C_{18} fatty acids. In contrast with the phospholipids, both galactolipids contained significant proportions of 18:3 after 10 min with a further rise after 20 min. These relatively short term labeling studies show the beginnings of the desaturation of PC from 18:1 to 18:2 as has been previously observed only in much longer term studies extending over 1 to 5 days (17).

Labeling Patterns of Isolated Chloroplasts—Isolated spinach chloroplasts incorporated label from [^{14}C]bicarbonate into a variety of acyl lipids in light-dependent reactions. Bicarbonate at 10 mM and pH 8.0 were found to be the optimal conditions for both total CO_2 fixation and incorporation of ^{14}C into lipids. The range of lipids synthesized *in vitro* differed considerably from those synthesized by whole leaves from $^{14}CO_2$. Table III shows a comparison of the labeling patterns of intact leaves and intact isolated chloroplasts following exposure to $^{14}CO_2$. Spinach leaves incorporated label into a wide range of glycerolipids after only 20 s. In contrast, intact spinach chloroplasts incorporated label almost exclusively into neutral lipids after a 30-min incubation with [^{14}C]bicarbonate. In addition, the isolated chloroplasts were unable to synthesize any of the polar parts of the lipid molecules, *e.g.* galactose and glycerol, and only acyl residues were found to be labeled from $^{14}CO_2$ *in vitro*.

Table IV shows the labeled fatty acids of the principal lipid products following incubation of chloroplasts with [^{14}C]bicarbonate. The pattern of fatty acid labeling in isolated chloroplasts differs considerably from that found *in vivo* (Table II) with little or no labeling of either 18:2 or 18:3 even after a 30-min incubation *in vitro*. The FFA contained mainly 18:1 but both the MG and the DG were mainly labeled in 16:0 and 14:0. There was no evidence for any significant formation of polyunsaturated fatty acids *in vitro* after 30 min, whereas they were detected *in vivo* after only 10 min.

Table III. *Comparison of Labeling Patterns of Lipids Following Exposure of Intact Spinach Leaves to $^{14}CO_2$ and Intact Spinach Chloroplasts to 10 mM [^{14}C]Bicarbonate*

Lipid	^{14}C in Lipids	
	Leaves 20 min	Chloroplasts 30 min
	% total	
PC	24.1	2
PG	7.0	ND
PE	2.4	ND
DGD	5.9	ND
PA	5.6	0.5
MGD	26.8	ND
Pigments	25.9	ND
FFA	tr[a]	37
acyl-CoA + acyl-ACP	1.2	19
MG	ND[b]	24
DG	tr	18

[a] Trace.
[b] None detected.

Table IV. *^{14}C Incorporation into Fatty Acids in Isolated Spinach Chloroplasts Incubated with 10 mM $H^{14}CO_3^-$*

Fatty Acid	^{14}C in Fatty Acids					
	FFA		MG		DG	
	20 min	30 min	20 min	30 min	20 min	30 min
	% total					
12-0	2.0	2.0	0.5	tr[a]	0.4	tr
14-0	9.8	14.3	24.0	21.3	33.5	32.9
16-0	28.6	22.2	70.7	68.7	54.6	52.9
16-1 + 16-2 + 16-3	tr	0.2	tr	0.1	0.1	tr
18-0	5.2	6.3	2.3	3.2	2.6	2.4
18-1	48.5	52.0	2.2	6.7	7.9	11.7
18-2	2.5	2.8	0.1	tr	tr	tr
18-3	tr	tr	ND[b]	ND	ND	ND

[a] Trace.
[b] None detected.

DISCUSSION

Previous studies of *in vivo* leaf lipid metabolism have involved the use of excised leaves (21), leaf discs (25, 26) or chopped leaves (6), but Macnicol (8) has reported that large differences may exist between the metabolism of leaf discs compared to intact leaves still on the plant. Murphy and Stumpf (14) recently found that polyunsaturated fatty acid biosynthesis in cucumber cotyledons was reduced by tissue damage such as excision of chopping off the cotyledons. In this study we used a $^{14}CO_2$ exposure apparatus that had been designed to take pots containing the growing spinach plants and to maintain them in environmental conditions similar to the cabinets in which the plants were grown.

Under these conditions, intact spinach leaves assimilated photosynthetically fixed CO_2 into lipid at a rate of 7.6 μmol mg^{-1} Chl·h^{-1} following a lag period of about 5 min during which the lipid precursors became saturated with ^{14}C to a constant specific radioactivity. Since the ^{14}C in fatty acids accounted for 28.4% of the ^{14}C in the whole acyl lipid fraction, the incorporation rate of CO_2 into fatty acids alone was 2.16 μmol mg^{-1} Chl·h^{-1}. This figure represents the rate of fatty acid biosynthesis from CO_2 in healthy intact spinach leaves.

In contrast to the leaves, isolated intact spinach chloroplasts were only able to utilize 0.61% of the total fixed CO_2 for lipid biosynthesis (13), *i.e.* 0.61 μmol mg^{-1} Chl·h^{-1}. In chloroplast suspensions, all of the ^{14}C was incorporated into fatty acids, whereas in the leaves ^{14}C was incorporated into all parts of the lipid molecule. Thus the rate of fatty acid biosynthesis from CO_2 by spinach chloroplasts was 0.61 μmol mg^{-1} Chl·h^{-1}, compared with 2.16 μmol mg^{-1} Chl·h^{-1} in intact leaves. Isolated spinach chloroplasts are thus able to utilize photosynthetically fixed CO_2 for lipid biosynthesis at about 28% of the rate of intact spinach leaves. Roughan *et al.* (19) have reported similar rates of fatty acid synthesis from $H^{14}CO_3^-$ by spinach chloroplasts.

Isolated chloroplasts have been shown to synthesize fatty acids at rates approaching those in whole leaves only when [1-^{14}C]-acetate is used as the substrate rather than $H^{14}CO_3^-$. Using 35 μM [1-^{14}C]acetate in the same medium as was used for the $H^{14}CO_3^-$ incubations, fatty acid biosynthesis rates in excess of 1.2 μmol mg^{-1} Chl·h^{-1} were obtained (D. J. Murphy, unpublished results). Using considerably higher concentrations of [1-^{14}C]acetate (160 μM), Roughan *et al.* (19, 20) have reported rates of over 2.0 μmol mg^{-1} Chl·h^{-1} in spinach chloroplasts, which is close to the *in vivo* rates of fatty acid biosynthesis from $^{14}CO_2$ reported here. The relatively poor capacity of isolated chloroplasts for fatty acid biosynthesis from $H^{14}CO_3^-$ is probably due to the low pyruvate dehydrogenase activity associated with the organelles (15). While some fatty acid biosynthesis may proceed directly from photosynthetically fixed CO_2 (via 3-PGA and pyruvate) it is likely that mitochondrial acetate is the normal fatty acid precursor in chloroplasts. An acetyl-CoA lyase has recently been found in spinach mitochondria (22) and a highly active acetyl-CoA synthetase capable of converting acetate to acetyl-CoA at a rate of 8 μmol mg^{-1} Chl·h^{-1} has recently been localized in spinach chloroplasts

(7).

Isolated spinach chloroplasts are able to synthesize only a very restricted range of lipids from either $H^{14}CO_3^-$ (Table II) or from [1-^{14}C]acetate (12, 13) unless exogenous UDP-galactose and glycerol 3-P are supplied (10, 11) and the newly synthesized lipids contain almost exclusively saturated or monoenoic fatty acids. Because spinach chloroplasts are capable of rates of fatty acid biosynthesis which are similar to or exceed the rates found in intact healthy leaves, it seems likely that they are the major or even the only sites of *de novo* fatty acid biosynthesis in leaves (16). However, chloroplasts are dependent upon external acetate for this fatty acid production and the subsequent desaturation steps to α-linolenic acid probably also occur elsewhere in the leaf cell.

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LITERATURE CITED

1. ARNON I 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–5
2. BARTLETT GR 1959 Phosphorus assay in column chromatography. J Biol Chem 234: 446
3. CHAPMAN DJ 1976 Amino acid formation during photosynthetic carbon assimilation in developing leaf tissue. PhD thesis. University of York, United Kingdom
4. HARWOOD JL 1975 Fatty acid biosynthesis by avocado pear. Lipids 9: 850–854
5. HARWOOD JL, PK STUMPF 1972 Fatty acid biosynthesis by subcellular fractions of higher plants. Lipids 7: 8–19
6. KANNANGARA CG, BS JACOBSON, PK STUMPF 1973 *In vivo* biosynthesis of α-linolenic acid in plants. Biochem Biophys Res Commun 52: 648–655
7. KUHN DN, PK STUMPF 1980 Subcellular localization of acetyl CoA synthetase in protoplasts of *Spinacia oleracea*. Plant Physiol 65: S-801
8. MACNICOL PK 1976 Rapid metabolic changes in the wounding response of leaf discs following excision. Plant Physiol 57: 80–84
9. MANCHA M, GB STOKES, PK STUMPF 1975 The determination of acyl ACP and acyl CoA in a complex lipid mixture. Anal Biochem 68: 600–608
10. MCKEE JWA, JC HAWKE 1978 The incorporation of [^{14}C]bicarbonate and [^{14}C]acetate into the constituent fatty acid of monogalactosyldiacylglycerol of spinach chloroplast and leaves. FEBS Lett 94: 273–276
11. MCKEE JWA, JC HAWKE 1979 The incorporation of [^{14}C]acetate into the constituent fatty acids of monogalactosyldiglyceride by isolated spinach chloroplasts. Arch Biochem Biophys 197: 322–332
12. MURPHY DJ, RM LEECH 1977 Lipid biosynthesis from [^{14}C]bicarbonate, [2-^{14}C]pyruvate and [1-^{14}C]acetate during photosynthesis by isolated spinach chloroplasts. FEBS Lett 77: 164–168
13. MURPHY DJ, RM LEECH 1978 The pathway of [^{14}C]bicarbonate incorporation into lipids in isolated photosynthesising spinach chloroplasts. FEBS Lett 88: 192–196
14. MURPHY DJ, PK STUMPF 1980 Polyunsaturated fatty acid biosynthesis in cotyledons from germinating and developing *Cucumis sativus* L. seedlings. Plant Physiol 66: 660–665
15. MURPHY JD, PK STUMPF 1980 The origin of chloroplastic acetyl CoA. Arch Biochem Biophys. In press
16. OHLROGGE JB, DN KUHN, PK STUMPF 1979 Subcellular localization of acyl carrier protein in leaf protoplasts of *Spinacia oleracea*. Proc Natl Acad Sci USA 76: 1194–1198
17. ROUGHAN PG 1975 PC: donor of 18-carbon unsaturated fatty acids for glycerolipid biosynthesis. Lipids 10: 609–614
18. ROUGHAN PG, RD BATT 1969 The glycerolipid composition of leaves. Phytochemistry 8: 363–369
19. ROUGHAN PG, R HOLLAND, CR SLACK 1979 On the control of long-chain-fatty acid synthesis in isolated intact spinach (*Spinacea oleracea*) chloroplasts. Biochem J 184: 193–202
20. ROUGHAN PG, R HOLLAND, CR SLACK, JB MUDD 1979 Acetate is the preferred substrate for long-chain fatty acid synthesis in isolated spinach chloroplasts. Biochem J 184: 565–569
21. SLACK CR, PG ROUGHAN 1975 The kinetics of incorporation *in vivo* of [^{14}C]-acetate and $^{14}CO_2$ into the fatty acids of glycerolipids in developing leaves. Biochem J 152: 217–228
22. STUMPF PK 1972 Fatty acid synthesis in spinach chloroplasts. Methods Enzymol 24: 394–397
23. TREVELYAN WE, DP PROCTOR, JS HARRISON 1950 Detection of sugars on paper chromatograms. Nature 166: 444–445
24. WEAIRE PJ, RGO KECKWICK 1975 The synthesis of fatty acids in avocado mesocarp and cauliflower bud tissue. Biochem J 146: 425–437
25. WILLIAMS JP, GR WATSON, K MOBASHSHER-UDDIN, SPK LEUNG 1975 Galactolipid synthesis in *Vicia faba* leaves. I. Galactose, glycerol and fatty acid labeling after $^{14}CO_2$ feeding. Plant Physiol 55: 1038–1042
26. WILLIAMS JP, GR WATSON, SPK LEUNG 1976b Galactolipid synthesis in *Vicia faba* leaves. II. The formation and desaturation of long-chain fatty acids in phosphatidyl choline, phosphatidyl glycerol and the galactolipids. Plant Physiol 57: 179–184