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CELLULAR AND CHLOROPLAST LIPID COMPOSITION OF THE LEAVES OF *MIMOSA PUDICA*

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Key Word index—*Mimosa pudica*; Leguminosae; cellular and chloroplast lipids; fatty acids; sterol; acylglycerols; phospholipids; glycolipids.

Abstract—Cellular and chloroplast lipids of the leaves of *Mimosa pudica* have been analysed. Qualitatively the total lipid composition of this plant is similar to that reported for the photosynthetic tissues of other plants. Chloroplast lipids show some resemblance to those of algae. The cerebroside fraction of both leaves and chloroplasts contains a polyunsaturated fatty acid (20:4ω3) and a long chain sphingosine base whose R_f value coincides with that from ox brain cerebroside and not with that of phytosphingosine from spinach.

INTRODUCTION

Mimosa pudica is a highly sensitive plant. It responds towards various stimuli, e.g. physical touch, variation in light, temperature etc., by contracting and reopening its leaves. Such extreme sensitivity is called 'nyctinasty' which may be compared with the phenomena of neurotransmission and/or muscle contraction occurring in animal systems. This suggests the presence of a unique impulse communicating system in this plant. Since both neurotransmission and muscle contraction involve participation of cell membrane, the extreme sensitivity of *M. pudica* may also be considered to be a membrane-related phenomena.

With this view in mind we have first attempted to study the cellular and chloroplast lipid composition of *M. pudica* leaves since lipids are one of the major constituents of cell membranes. The present paper describes comparative studies on the cellular and chloroplast lipids of the leaves of this plant.

RESULTS AND DISCUSSION

Lipid composition

In the present study we have analysed cellular and chloroplast acylglycerols, sterols, phospholipids and glycolipids of the leaves of *M. pudica*. We have not examined the pigments, waxes, quinones and carotenoids which are also constituents of plant lipids. The overall quantitative lipid composition is shown in Table 1 and that of phosphatides in Table 2. Generally, in higher plants, the chloroplast fraction contains a relatively high proportion of lipid. Table 1 shows that in *M. pudica* also the lipid content of the chloroplast (13%) is rather high compared to that of leaves (5%). Acylglycerols, which are present in trace amounts in spinach and other photosynthetic tissues ([1], p. 235), are present in significant amounts in both leaves (15.7%) and chloroplasts (20%) of *Mimosa*. One reason for this may be activation of some phospholipases during lipid extraction, leading to enzymatic degradation of phospholipids into glycerides.

The overall qualitative picture regarding phospholipid and glycolipid composition of *Mimosa* is similar to that reported for other plants, ([1] pp. 230, 255).

Among the phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylinositol (PI) are the major phospholipids found in most plant tissues. Here, in addition to the above 4, small amounts of lysophosphatidylcholine (LPC) are also obtained which may have been produced from PC by lipolysis or degradation. The percentage of total phospholipid (~13%) is nearly the same in leaves and chloroplasts. In leaves, the percentage of PE (32%) is greater compared to the other 4 phospholipids, though in other plants a general predominance of PC over all phosphatides is often observed ([1], p. 236). PC is the major (54%) phospholipid in chloroplasts of *Mimosa*. In higher plants, chloroplasts are rich in PG. A close structural relationship of this lipid with the photosynthetic machinery has been established [2–4]. However, there is one green alga, viz. *Euglena gracilis* ([1], p. 236) which contains small amounts (8%) of PG and large amounts (72%) of PC, so it may be said that *Mimosa* resembles algae in having a high proportion of PC in its chloroplasts. According to Kates, in leaves and green algae, glycolipids constitute *ca* 40% of the total lipid of which monogalactosyldiacylglycerols (MGDG) are the major component. In *M. pudica*, however, the percentage of glycolipids is low, *ca* 13%

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Table 1. Total lipid composition of *M. pudica*

Constituent	% of total lipid	
	Leaves	Chloroplasts
Triacylglycerols (TG)	6.5	11.7
Diacylglycerols (DG)	5.2	4.7
Monoacylglycerols (MG)	4.0	4.5
Sterols	5.6	7.4
Sterol esters (SE)	3.4	1.8
Phospholipids	13.1	12.8
Glycolipids	13.7	21.1
Monogalactosyldiacylglycerols (MGDG)	2.4	6.8
Digalactosyldiacylglycerols (DGDG)	6.4	10.2
Sulfoquinovosyldiacylyglycerols (SQDG)	2.7	2.0
Cerebroside	2.2	2.1
Total lipids (% tissue dry wt)	5.0	13.0

Table 2. Phosphatide composition of *M. pudica*

Constituent	% of total phospholipid	
	Leaves	Chloroplasts
Phosphatidyl-glycerol (PG)	24	20
Phosphatidyl-choline (PC)	27	54
Phosphatidyl-ethanolamine (PE)	32	16
Phosphatidyl-inositol (PI)	14	9
Lysophosphatidyl-choline (LPC)	3	1

in leaves and 21% in chloroplasts. Furthermore, instead of MGDG, digalactosyldiacylglycerols (DGDG) predominate over other glycolipids. From this plant, we have also isolated a considerable amount (2%) of cerebroside which is generally present in very minor amount in other plants ([1], p. 235). TLC has shown that the R_f value (0.8) of the long chain sphingosine base isolated from *Mimosa* cerebroside (both in chloroplasts and in leaves) coincides with that from ox brain cerebroside. It is definitely a unique observation because so far no plant cerebroside has been found to contain animal sphingosine.

Sugar identification

PC has shown the presence of galactose besides glycerol in the hydrolysate of the glycolipid fraction of both leaves and chloroplasts.

Fatty acid composition

Plant fatty acids are usually characterized by their high degree of unsaturation due to the presence of large amounts of α-linolenic acid (18:3ω3) which is generally concentrated in the chloroplasts. C_{16}-Saturated acid is also abundant among the fatty acids of plant leaves.

For *M. pudica*, the fatty acid composition of acylglycerols and sterol esters are given in Table 3 and those of phospholipids and glycolipids in Tables 4 and 5, respectively. In this plant, we see that triacylglycerols (TG) of both leaves and chloroplast contain higher proportions of unsaturated fatty acids whereas acylglycerols (DG) and monoacylglycerols (MG) are rich in saturated fatty acids. With sterol ester (SE), however, some contradictory results are obtained. In leaves, this fraction contains the maximum amount of α-linolenic acid (74%) while in chloroplasts it is rich in C_{16}-saturated acid (57%). In general, it has been found that MG contains the largest amount of C_{16}-saturated acid (84%) and no unsaturated fatty acid, whereas SE, TG and DG contain both C_{16}-satuated acid and α-linolenic acid in high proportion except DG of chloroplast where the α-linolenic acid content (3.4%) is low.

The distribution of fatty acids amongst phospholipids and glycolipids of both leaves and chloroplasts is not uniform. In general, all the phospholipid components contain mainly C_{16}-saturated and α-linolenic acids, the latter, of course, is present in much lower amounts in the LPC fraction of leaves. In all the phosphatides (except LPC of leaves) unsaturated fatty acids predominate over saturated. PG in photosynthetic tissues (except in blue-green algae and photosynthetic bacteria) has been found to contain high proportions of C_{16}-monoenoic acid, *trans*-3-hexadecenoic acid ([1], p. 241). In *M. pudica*, C_{16}-monoenoic acid occurs in the PG fraction of chloroplasts and leaves, but the percentage is low compared to that present in the PG of spinach ([1], p. 240). Besides PG, this acid is also present in small amounts in PE and PC of leaves and PI of both leaves and chloroplasts.

Regarding the fatty acid composition of glycolipids, some discrepancy with other plants is also observed. Galactolipids in photosynthetic tissues generally have very high contents of α-linolenic acid and a C_{16}-trienoic acid ([1], pp. 240, 242, 245); the latter is concentrated in the MGDG fraction. In *Mimosa*, no C_{16}-trienoic acid is found in any glycolipid fraction of either leaves or chloroplasts. A C_{16}-monoenoic acid is present in the DGDG and sulfolipid fractions of chloroplasts and also in the sulfolipid fraction of leaves. As compared to phospholipids, glycolipids also contain relatively high proportions of C_{16}-saturated acid and α-linolenic acid (except cerebroside of leaves). In *Mimosa*, however, the α-linolenic acid content of the galactolipids (MGDG and DGDG) in leaves is rather high compared to that in chloroplasts. In higher plants, chloroplast galactolipids generally contain large amounts of α-linolenic acid. In fact, the galactolipids with their high content of α-linolenic acid and also PG (these polar lipids appear to be concentrated in chloroplast lamellae) are considered to be involved in photosynthetic reactions occurring in chloroplasts [5, 6]. It has been suggested that these fatty acids probably form non-aqueous complexes through their double bonds with the isoprenoid methyl groups in chlorophyll, carotenoids and quinones thereby maintaining the molecular orientation and spacing required for electron transport and enzymatic reactions associated with photosynthesis [7–10]. Therefore, the percentage of α-linolenic acid in

Table 3. Fatty acid composition (%) of acylglycerols and sterol esters of *M. pudica**

Acids	Leaves				Chloroplasts			
	TG	DG	MG	SE	TG	DG	MG	SE
14:0	—	0.4	0.2	1.3	—	—	0.3	—
16:0	27.9	68.0	85.0	11.0	39.4	69.8	84.2	57.5
16:1	—	—	—	Tr	—	—	—	5.0
18:0	8.9	8.0	14.8	2.7	6.7	18.8	15.5	5.0
18:1ω9?	5.5	Tr	—	2.4	9.4	3.3	—	12.5
18:2ω6	7.4	6.6	—	8.2	12.2	4.7	—	5.0
18:3ω3	50.1	16.9		75.5	32.3	3.4		15.0
Σ SFA / Σ UFA	0.58	3.3	100 / (−)	0.17	0.85	8.09	100 / (−)	1.66

* Abbreviations are given in Table 1. Tr = trace; (−) = absent; ? = other isomers may also be present. SFA = saturated fatty acid. UFA = unsaturated fatty acid.

Table 4. Fatty acid composition (%) of different phosphatides of *M. pudica**

Acids	Leaves					Chloroplasts				
	PG	PC	PE	PI	LPC	PG	PC	PE	PI	LPC
16:0	34.8	38.4	30.6	38.9	59.0	30.5	40.2	20.8	34.5	37.6
16:1	7.5	1.8	3.5	2.0	Tr	11.8	Tr	Tr	2.3	Tr
18:0	8.3	0.7	6.1	9.3	3.15	8.8	5.3	6.4	11.0	9.6
18:1ω9?	2.7	1.3	1.1	Tr	18.9	3.9	Tr	Tr	Tr	10.7
18:2ω6	5.9	16.1	13.7	13.7	16.4	10.1	16.3	21.1	17.6	17.1
18:3ω3	38.6	39.0	41.4	32.5	2.5	34.8	38.1	51.5	34.4	24.9
20:4ω6	2.0	2.7	3.5	3.4	—	—	—	—	—	—
Σ SFA / Σ UFA	0.75	0.64	0.54	0.66	1.63	0.64	0.83	0.36	0.83	0.88

* Abbreviations are given in Tables 2 and 3.

Table 5. Fatty acid composition (%) of glycolipids of *M. pudica**

Acids	Leaves				Chloroplasts			
	MGDG	DGDG	SQDG	Cerebroside	MGDG	DGDG	SQDG	Cerebroside
14:0	—	—	—	3.3	—	—	—	0.86
16:0	9.6	11.7	28.3	28.6	31.0	32.8	10.5	7.8
16:1	Tr	Tr	4.0	Tr	Tr	14.0	19.9	—
18:0	3.6	4.3	10.4	11.3	15.1	15.9	19.3	7.7
18:1ω9?	3.5	2.0	5.6	17.4	22.8	6.9	6.6	4.3
18:2ω6	9.0	3.0	14.0	9.6	8.1	6.3	16.2	1.5
18:3ω3	74.0	79.0	37.5	Tr	22.8	23.8	27.3	47.2
20:4ω6	—	—	—	Tr	—	—	—	Tr
20:4ω3	—	—	—	29.7	—	—	—	30.4
Σ SFA / ΣUFA	0.14	0.19	0.64	0.76	0.85	0.96	0.42	0.19

* Abbreviations are given in Tables 1 and 3.

chloroplast galactolipids of *Mimosa* is not in agreement with that in other higher plants. However, blue-green algae [11] contain monoenoic acids pigments and quinonoid compounds but no polyenoic acids and they can still carry out the Hill reaction. Therefore *Mimosa* resembles blue-green algae in this respect.

The cerebroside fractions of both leaves and chloroplasts contain high proportions (*ca* 30%) of a C_{20}-unsaturated acid (20:4ω3). In all the phospholipids of leaves (except LPC), the presence of arachidonic acid (20:4ω6) is also observed. The presence of these C_{20}-unsaturated acid in *Mimosa* is interesting since so far this acid has been reported in animal, euglenoids, diatoms, algae but not in higher plants ([1], pp. 238-239).

Sterols

The MS of the sterol acetate shows a doublet at *m/e* 456 and 458 in the high mass region. Besides these, there are other peaks at *m/e* 396/398 (M^+-MeCOOH), 366/368, 311/313, 297/299, 283/285, 276, 255/257, 213/215 and 120. The genesis of these

peaks can be explained from fragmentation of the sitosterol nucleus. Therefore, the sterol appears to be a mixture of β-sitosterol and dihydro-β-sitosterol (stigmastanol), which are common constituents of plants.

The above comparative studies reveal that the chloroplast lipid profile of *Mimosa* leaves resembles more or less that of algae. This observation is of biological significance and supports the present idea of the origin of chloroplasts from algae.

EXPERIMENTAL

M. pudica leaves, freshly collected from the field station of Bose Institute, Calcutta, were used for analysis.

Isolation of chloroplasts. These were isolated according to the procedure of ref. [12]. Fresh leaves were blended for 3 min with 2 parts by wt of the isolation medium which consisted of Tris–HCl (10 mM, pH 7.9) and sucrose (0.25 M) (1:1). The homogenate was repeatedly filtered through cheesecloth. The filtrate was first centrifuged of 200 *g* (20 min) to remove unbroken cells and cell debris, and then at 1000 *g* for 10 min. The 1000 *g* pellet was resuspended in the above isolation medium and centrifuged again (1000 *g*, 10 min) to obtain the intact chloroplast pellet.

Isolation of total cellular lipid. A modified Bligh and Dyer procedure [13] was used for the extraction of total lipid. Fresh leaves (100 g) were first chopped, then blended with $CHCl_3$—MeOH (1:2, 300 ml) for 2 min at 25° in a Waring blender. The homogenate was centrifuged and the residue re-extracted with a mixture of $CHCl_3$—MeOH—H_2O (1:2:0.8, 380 ml). After centrifugation, the residue was washed with $CHCl_3$—MeOH (1:2, 150 ml). To the combined extract, $CHCl_3$ (250 ml) and H_2O (290 ml) were added and the phases allowed to separate. The organic layer was withdrawn, dried (Na_2SO_4) and evapd under red. pres. at room temp. Residual lipids were immediately dissolved in a small volume of $CHCl_3$ and preserved with antioxidant (2,6-ditertiary-butyl-4-methyl phenol) (50 mg/l.) at −20°.

Isolation of chloroplast lipids. These were extracted following the procedure of ref. [14]. Intact chloroplasts were first homogenized with $CHCl_3$—MeOH (1:2, 4.2 ml/g of dry chloroplasts) in a glass homogenizer at 25° for 2 min and the residue, after centrifugation, was re-extracted with $CHCl_3$—MeOH—H_2O (1:2:0.8, 4.2 ml). The combined extract was diluted with $CHCl_3$—H_2O (1:1, 2.8 ml), the $CHCl_3$ layer collected and treated in the same way as described in the case of isolation of total cellular lipid.

Separation and identification of individual lipid classes. Classes of neutral and polar lipids were separated by TLC. Identity and purity of each lipid were checked by comparing the R_f value with authentic compounds, re-running in multiple solvent systems and spraying with group-specific spray reagents. For sterol esters, the Liebermann reagent [15] was used as spray reagent. Dragendorff reagent [16] was used for choline group, ninhydrin [17] for free amino group, periodate—Schiff reagent [18] for vicinal hydroxyl group, orcinol reagent [19] for sugar, and molybdenum blue reagent [20] for phosphate-containing lipids in general.

The percentage of neutral lipid was determined by direct weighing and that of phospholipids [21], glycolipids [19], sterols and steryl esters [22] by colorimetric methods. For the separation of neutral lipids on TLC, Si gel H and *n*-hexane—Et_2O—HOAc (90:10:1), were used. For sterols and sterol esters, the developing solvent was *n*-hexane—EtOAc—HCO_2H (35:5:1). For the separation of phospholipids and glycolipids, Si gel G and (i) $CHCl_3$—MeOH—H_2O (65:25:4), (ii) $CHCl_3$—MeOH—28% NH_3 (13:5:1) and (iii) $CHCl_3$—MeOH—HOAc—H_2O (25:15:4:2) were used. For analytical and preparative purposes, TLC plates of 0.25 and 0.5 mm thickness were used, respectively.

Saponification of lipids. The total polar lipid mixture (20 mg) was hydrolysed [23] with methanolic NaOH (0.2 M, 1 ml) for 15 min and the product was neutralized with M HOAc. To this, 4 ml each of H_2O and $CHCl_3$—MeOH (9:1) were added. After centrifugation, the aq. and $CHCl_3$ layers were collected separately. The $CHCl_3$ layer was tested for the presence of cerebroside by TLC using ox brain cerebroside as standard. The aq. layer was concd with C_6H_6 at 37°, dissolved in a min vol. of MeOH—H_2O (10:9) and further hydrolysed by refluxing with methanolic HCl (2 M, 5 hr) to obtain any sugars. Each glycolipid, except cerebroside, was also subjected to alkaline and acid hydrolysis in the same way to obtain sugars from each fraction.

PC of sugars. Sugars obtained after alkaline and acid hydrolysis were identified by PC (descending technique) on Whatman No. 1 paper with *iso*-PrOH—Py—HOAc—H_2O (8:8:1:4, upper phase) and detected by spraying with alkaline $AgNO_3$ [24]. Galactose, glycerol and inositol were used an standards.

Preparation of fatty acid Me esters. Method 1. Total and individual lipids (few mg) were subjected to methanolysis with KOMe (0.6 M, 0.25 ml) [25]. *Method* 2. Total and individual lipids were saponified with methanolic NaOH (0.2 M, see saponification of lipids) and the liberated fatty acids in the $CHCl_3$ layer were converted to Me esters with CH_2N_2—Et_2O [26]. Me esters were separated by prep-TLC and then analysed by GLC.

Hydrolysis of cerebroside. Isolation of Me ester. The cerebroside fraction (10 mg) was refluxed with methanolic HCl (2 M, 5 ml) for 5 hr [27] and the fatty acid Me ester extracted with petrol (60–80°). *Isolation of sphingosine.* After extracting the fatty acid Me ester of the cerebroside, the MeOH—H_2O phase was made alkaline and the long chain base extracted with Et_2O. The residue after removal of solvent was compared by TLC with sphingosine prepared from ox brain cerebroside and that from spinach (Si gel H, $CHCl_3$—MeOH—2M NH_4OH (40:10:1) [28], detection with ninhydrin).

Saponification of steryl esters and acylation of free sterols. The steryl esters were saponified with methanolic KOH (6 M, 2 hr) under reflux. The non-saponifiable part containing sterol was extracted according to ref. [29]. The sterol was acetylated with Py—Ac_2O [30]. The steryl acetates were characterized by MS.

GLC analysis. An instrument fitted with dual FID was used. For fatty acid Me esters, a 15% DEGS column (2 m × 2 mm) was used. The oven temp. was 165–170°. Me esters were identified by comparing R_t with known esters, by semilogarithmic plotting according to ref. [31] and in some cases by comparing the percentage of carbon chain length with the corresponding hydrogenated product.

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