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COMPOSITION AND POSITIONAL DISTRIBUTION OF FATTY ACIDS IN LEAF PHOSPHOLIPIDS

BY

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ABSTRACT

Composition and positional distribution of fatty acids in leaf phospholipids. - The fatty acid composition and distribution in phospholipids isolated from the leaves of evergreen woody plants were investigated for seven temperate-zone and six tropical species. The relative amounts of the major phospholipids, i.e. phosphatidyl-ethanolamine (PE), phosphatidyl-choline (PC), phosphatidyl-glycerol (PG) and phosphatidyl-inositol (PI) and the fatty acid composition of each of these classes were determined. In all thirteen plants, the main fatty acid components were 16:0, 16:1(*t*), 18:0, 18:1, 18:2 and 18:3. The results showed a significant difference in the proportion of unsaturated fatty acids relative to saturated ones, especially in PG. For chilling-sensitive tropical plants, the unsaturated fatty acid content (SU= 18:1+18:2+18:3) for PG ranged from 32.4 to 44.6%, while for chilling-resistant species it was between 48.3 and 56.9%. Positional distribution of fatty acids in PG was also investigated by enzymatic hydrolysis using phospholipase A2. The results showed that 18:1, 18:2 and 18:3 are generally attached to the *sn*-1 carbon atom, whereas 16:1(*t*) is attached exclusively to the *sn*-2 carbon atom.

Key-words: Leaf Phospholipids; Phosphatidyl-glycerol; Fatty acid; Chilling-resistance; Evergreen woody plants.

Abbreviations: PE, phosphatidyl-ethanolamine; PC, phosphatidyl-choline; PG, phosphatidyl-glycerol; PI, phosphatidyl-inositol; PS, phosphatidyl-serine; TLC, thin-layer chromatography; GC, gas chromatography; MS, mass spectrum.

INTRODUCTION

The chain length, the degree of unsaturation as well as the positional distribution of fatty acids in membrane phospholipids, especially in PG, have a profound effect on the membrane fluidity (STRYER, 1988; MURATA *et al.*, 1982). Short chain lengths and a high degree of unsaturation increase the fluidity and the pliability of the membranes. On the contrary, a high content in saturated and *trans*-monounsaturated fatty acids decreases

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the fluidity of membranes and renders the plant sensitive to chilling injury (LYONS, 1973). Many studies have illustrated the relationship between the composition of the phospholipids and the plant resistance toward chilling (WATANABE *et al.*, 1981; TORIYAMA *et al.*, 1988; MURATA, 1983) and particular attention was brought on PG which is thought to play a special role in plant chilling-resistance and also in photosynthetic activity (SEKIYA *et al.*, 1990). Based on these results, altering the plant's chilling-resistance by genetic engineering has been reported (MURATA *et al.*, 1992).

In the present study, we investigated the compositions of fatty acids in four main classes of phospholipids (PE, PC, PG and PI) in the leaves of a number of chilling-sensitive and chilling-resistant evergreen woody plants from China. The positional distributions of fatty acids in PG were also determined by enzymatic hydrolysis with phospholipase A_2. The first set consisted of seven species of chilling-resistant evergreen broad-leaved trees (essentially from north to south): little-leaf box, southern magnolia, sweet osmanthus, chinese photinia, camphor tree, oleander, and oil-tea camellia which distribute in the middle region of China. The other set consisted of six species of chilling-sensitive evergreen broad-leaved trees (essentially from north to south): chu-lan tree, white michelia, ivy tree, small fruit fig, variegated india rubber fig and rubber plant, which were distributed in the south of China and Asia. All these trees are widely cultivated ornamental plants and some of them are also used for industrial purposes (camphor, camellia, rubber). It will be shown that, as expected from previous studies on herbaceous plants (MURATA *et al.*, 1982; WATANABE *et al.*, 1981; TORIYAMA *et al.*, 1988) and on poplar varieties (HU *et al.*, 1993), the chilling-resistance of evergreen woody plants is also related to the composition and positional distribution of the PG fatty acid.

EXPERIMENTAL

Plant material: Leaves of the temperate-zone trees *Buxus microphylla*, *Magnolia grandiflora*, *Osmanthus fragrans*, *Photinia serrulata*, *Cinnamomum camphora*, *Nerium indicum*, *and Camellia oleifera* were collected in the botanical garden of Nanjing Forestry University (Nanjing. China), and those of the tropical trees *Aglalia odorata*, *Michelia alba*, *Schefflera octophylla*, *Ficus microcarpa*, *Ficus elastica cv variegata* and *Ficus elastica* were taken from the greenhouse of the same garden on October 1994. All the samples were immediately inactivated and extracted as soon as possible.

Extraction and separation of phospholipids: Lipids were extracted using a chloroform/methanol/water solvent system as described in our previous studies (HU *et al.*, 1993) which was based on the procedure of BLIGH & DYER (1959). In a portion of the extract the phospholipids were purified by a silica gel TLC. When developed successively with hexane and acetone, the pigments and the glycolipids migrated while the phospholipids were retained at the origin. After having been recovered from the plate, a portion of the phospholipids was subjected to a total fatty acid analysis (see below) and another portion was used for a quantitative analysis of the major classes of phospho-

lipids (see below). The remainder of the total extract was used to isolate the individual classes of phospholipids, according to MURATA *et al.* (1982) by an ion-exchange column chromatography (DEAE-Sepharose CL-6B, Sigma) followed by a TLC (Merck silica gel plates 5724). The bands on TLC were visualized by molybdenum blue spray (Sigma) and identified by comparing with PE, PC, PG and PI phospholipid standards (Sigma). The bands corresponding to these classes were then scraped from the plates, recovered by dynamical elution with chloroform/methanol/H_2O (3:5:1 v/v) and analyzed for their fatty acid compositions (see below).

Determination of the contents of the major classes of phospholipids: The total phospholipid fraction was chromatographied on silica gel TLC using chloroform/methanol/$NH_3.H_2O$ (10:5:0.5 v/v). After visualization by molybdenum blue spray, individual phospholipid classes were identified by comparing their R_f values with those of standard samples (the R_f values for PI, PC, PE and PG were 0.12, 0.20, 0.32 and 0.46 respectively) and quantified from the peak areas of the chromatograms obtained on a Shimadzu CS-910 TLC scanner (dual-wavelength reflection mode linear scanning, λ_S= 650 nm, λ_R= 480 nm, slit 0.3mm x 8.0mm, scanning speed 40 mm/min).

Fatty acid analysis: The separated lipid classes were transesterified with 0.4 M KOH/anhydrous methanol in benzene/hexane (1:1 v/v) at 45°C for 30 min. The resulting methyl esters were analyzed by GC/MS on a VG Masslab Trio-2 mass spectrometer (70 eV, source temp. 220°C) coupled to a Hewlett-Packard 5890 Series II gas chromatograph using a DB-FFAP (30m x 0.32 mm i.d, J & W Scientific) fused silica capillary column, and identified by comparison of their mass spectra and retention times with those of authentic samples. Helium was used as the carrier gas and the temperature program was as follows: from 80°C to 170°C at 3°C /min and from 170°C to 200°C at 1°C/min. Quantifications were achieved by GC/FID from the peak areas using a CARLO ERBA Fractovap 4160 gas-chromatograph equipped with a BORWIN chromatography data system (JMBS Developments) operated under the same conditions as for the GC/MS analyses.

Enzymatic hydrolysis of phosphatidyl-glycerol with phospholipase A_2: The distributions at the *sn*-1 and *sn*-2 positions in 1,2-diacyl-PG were examined by enzymatic hydrolysis with phospholipase A_2 (EC3,1,1,4 from *Crotalus adamanteus venom*, Sigma). The selective enzymatic hydrolysis of PG was carried out essentially according to published procedures (WATANABE *et al.*, 1980; LYNCH & THOMPSON, 1986; HAVERKATE & VAN DEENEN, 1965). After hydrolysis, the lysocompounds and free fatty acids localized at the *sn*-2 position were separated by TLC using chloroform/acetic acid/methanol/H_2O (75:25:5:2.2 v/v). The lysocompounds recovered from the TLC plate were then subjected to a transmethylation and analyzed for the fatty acids at the *sn*-1 position. The fatty acids localized at the *sn*-2 position were deduced by calculation [percentage of each fatty acid in total PG - 1/2 x percentage of the same fatty acid in lysocompounds].

RESULTS

Relative contents of major classes of leaf phospholipids: In all the trees, the major phospholipids were PI, PC, PE and PG, PC being always predominant. On quantification, these classes accounted for at least 89% of the total phospholipids. Since only negligible amounts of PS were observed, we report in Table I the relative amounts of the four major phospholipid classes only. The results for PI and PE do not differ significantly in chilling-resistant and chilling-sensitive plants. On the other hand, it is obvious that the relative amount of PG increases from chilling-resistant to chilling-sensitive plants at the expense of PC.

TABLE I

Relative amounts (mol%) of major classes of leaf phospholipids in temperate-zone and tropical evergreen plants.

sample N°	plants	major classes of leaf phospholipids PI	PC	PE	PG
	Temperate-zone plants				
1	*Buxus microphylla* (box)	17.1	51.6	19.6	11.7
2	*Magnolia grandiflora* (magnolia)	16.0	50.4	22.3	11.3
3	*Osmanthus fragrans* (osmanthus)	12.1	52.3	18.5	17.1
4	*Photinia serrulata* (photinia)	17.8	45.2	22.5	14.5
5	*Cinnamomum camphora* (camphor)	15.8	49.9	21.4	12.9
6	*Nerium indicum* (oleander)	14.1	47.3	23.9	14.7
7	*Camellia oleifera* (oil-tea camellia)	10.9	52.2	22.3	14.5
	Mean (SD)	14.8 (2.6)	49.8 (2.7)	21.5 (1.9)	13.8 (2.0)
	Tropical plants				
8	*Aglalia odorata* (chu-lan)	11.5	45.3	23.4	19.8
9	*Michelia alba* (michelia)	14.3	45.4	24.3	16.1
10	*Schefflera octophylla* (ivy)	14.0	42.7	23.3	20.0
11	*Ficus microcarpa* (fig)	14.6	41.7	24.1	19.6
12	*Ficus elastica var.* (rubber fig)	14.6	43.6	20.6	21.2
13	*Ficus elastica* (rubber)	15.1	44.3	17.2	23.4
	Mean (SD)	14.0 (1.2)	43.8 (1.3)	22.2 (2.5)	20.0 (2.2)

Composition of fatty acids in temperate-zone evergreen and tropical evergreen plants: The major fatty acids in all of the four main classes of phospholipids are 16:0, 18:0, 18:1, 18:2 and 18:3. The 16:1 component was present only in PG. Besides these, there are some minor components such as 12:0, 14:0 and 17:0. The unsaturated fatty acids 16:1, 18:1, 18:2 and 18:3, were identified by GC/MS to be *trans*-3-hexadecenoic ($16{:}1^{\Delta 3t}$), oleic ($18{:}1^{\Delta 9}$), linoleic ($18{:}2^{\Delta 9,12}$) and linolenic ($18{:}3^{\Delta 9,12,15}$) acid respectively. Table II shows the distribution of the fatty acids in the total phospholipids and in each of the four classes. The degree of unsaturation ΣU (sum of 18:1, 18:2 and 18:3) and the unsaturation index UI (UI= (Σ[(number of double bonds in each fatty acid) x

TABLE II

Fatty acid distribution of leaf phospholipids of temperate-zone and tropical evergreen woody plants.

sample N°	plant	class of PL	composition (wt% of total) 16:0	16:1	18:0	18:1	18:2	18:3	ΣU	UI
	temperate-zone plants	total	33.3	0.9	1.8	11.6	15.5	36.9	64.0	153.3
	Buxus	PE	23.4	–	2.6	2.0	6.2	65.9	74.0	212.0
1	*microphylla*	PC	32.4	–	3.4	11.6	15.8	36.8	64.2	153.6
	(box)	PG	35.7	8.4	3.3	29.3	12.9	10.3	52.6	86.0
		PI	43.5	–	2.7	12.8	12.9	28.1	53.8	122.9
		total	34.9	0.5	1.4	6.3	33.0	23.9	63.2	144.0
	Magnolia	PE	22.1	–	1.9	2.9	26.8	46.3	76.0	195.4
2	*grandiflora*	PC	27.1	–	4.3	2.6	43.2	22.9	68.6	157.7
	(magnolia)	PG	33.9	7.9	1.3	35.2	11.5	10.2	56.9	88.8
		PI	43.7	–	3.6	5.6	18.5	28.6	52.7	128.4
		total	24.4	tr	2.5	14.3	30.5	28.2	73.0	159.5
	Osmanthus	PE	19.9	–	2.2	6.1	17.0	54.8	77.9	204.4
3	*fragrans*	PC	25.5	–	2.2	26.0	21.1	25.2	72.3	143.8
	(osmanthus)	PG	38.9	7.6	tr	28.2	12.3	12.9	53.5	91.5
		PI	57.5	–	tr	10.3	14.5	17.6	42.5	92.1
		total	28.4	2.3	1.6	10.7	22.0	35.1	67.7	159.9
	Photinia	PE	21.1	–	2.1	20.7	21.2	34.8	76.8	167.7
4	*serrulata*	PC	25.2	–	2.8	26.3	21.1	24.6	72.0	142.2
	(photinia)	PG	27.3	24.0	tr	11.5	20.7	16.5	48.7	101.0
		PI	44.4	–	2.2	11.1	12.8	29.5	53.4	125.1
		total	33.5	tr	1.2	6.4	19.4	39.5	65.3	162.2
	Cinnamomum	PE	23.0	–	3.8	2.7	21.0	49.6	73.2	193.3
5	*camphora*	PC	29.0	–	2.2	8.6	24.6	35.4	68.8	164.2
	(camphor)	PG	24.4	24.1	0.3	30.4	10.0	11.0	51.3	85.5
		PI	48.5	–	tr	19.4	11.6	20.5	51.5	104.1
		total	31.6	1.2	4.4	8.8	21.3	32.7	62.8	149.5
	Nerium	PE	24.1	–	6.2	3.5	17.8	48.4	69.7	183.1
6	*indicum*	PC	31.1	–	3.5	11.5	35.0	18.9	65.4	138.2
	(oleander)	PG	35.9	14.8	1.0	31.6	11.9	4.8	48.3	69.2
		PI	49.6	–	0.5	4.2	19.4	26.3	49.9	121.9
		total	29.2	0.5	0.5	21.0	13.5	35.4	69.8	154.2
	Camelia	PE	24.5	–	2.0	13.8	11.9	47.8	73.5	180.5
7	*oleifera*	PC	27.5	–	1.7	28.4	15.0	27.4	70.9	140.5
	(oil-tea camellia)	PG	22.0	29.7	tr	35.7	5.5	7.0	48.3	67.8
		PI	45.5	–	1.6	22.9	10.9	19.1	52.9	102.1
	tropical plants									
		total	36.3	tr	3.3	11.2	20.3	28.8	60.3	140.2
	Aglalia odorata	PE	22.2	–	3.3	1.4	21.8	51.3	74.5	198.9
8	(chu-lan)	PC	27.5	–	3.1	9.0	35.2	25.2	69.4	155.0
		PG	46.3	9.3	6.8	14.8	19.1	3.6	37.6	64.0
		PI	47.5	–	5.1	3.0	13.2	31.2	47.4	123.0

sample N°	plant	class of PL	composition (wt% of total) 16:0	16:1	18:0	18:1	18:2	18:3	ΣU	UI
		total	28.5	tr	tr	2.1	38.0	31.4	71.5	172.4
	Michelia	PE	25.0	–	0.5	0.8	22.6	51.1	74.5	199.2
9	*alba*	PC	21.9	–	1.9	5.9	45.8	24.5	76.2	171.1
	(michelia)	PG	38.8	18.6	tr	21.5	10.3	10.8	42.6	73.7
		PI	45.3	–	2.4	2.9	18.5	30.9	52.3	132.5
		total	36.2	0.1	2.4	7.2	38.8	15.4	61.3	130.9
	Schefflera	PE	30.1	–	3.3	11.7	19.7	35.2	66.6	156.7
10	*octophylla*	PC	41.7	–	3.9	6.6	34.4	13.4	54.4	115.5
	(ivy)	PG	54.5	10.8	2.3	13.2	15.9	3.4	32.4	55.1
		PI	65.7	–	2.4	15.1	16.8	tr	31.9	48.7
		total	36.8	0.8	5.3	9.1	21.5	26.5	57.1	131.4
	Ficus	PE	24.9	–	4.9	1.9	25.7	42.6	70.2	181.1
11	*microcarpa*	PC	34.6	–	3.3	13.9	27.0	21.3	62.1	131.8
	(fig)	PG	46.4	6.3	2.7	31.1	10.3	3.2	44.6	61.4
		PI	55.6	–	0.7	29.6	12.0	2.1	43.7	59.9
		total	42.9	tr	tr	tr	30.6	26.5	57.1	140.7
	Ficus elastica	PE	29.5	–	2.7	1.7	16.2	49.9	67.8	183.7
12	*cv. variegata*	PC	30.6	–	0.2	5.4	39.6	24.2	69.2	157.2
	(india rubber fig)	PG	45.7	10.6	0.8	17.1	18.1	7.7	42.9	76.3
		PI	54.4	–	6.6	4.0	16.9	18.1	39.0	92.1
		total	41.6	tr	2.6	2.5	31.5	21.7	55.7	130.6
	Ficus elastica	PE	55.2	–	tr	tr	20.6	24.2	44.8	113.8
13	(rubber)	PC	44.6	–	1.4	6.2	32.4	15.4	54.0	117.1
		PG	44.8	14.2	3.4	17.8	17.1	2.6	37.6	60.0
		PI	69.6	–	6.4	0.9	12.9	10.2	24.0	57.4

(wt% of each fatty acid)]) are also reported. For the calculation of these two parameters, the *trans*-3-hexadecenoic acid was not considered as an unsaturated component because of its physical properties; for example its temperature of phase transition is closer to those of the saturated fatty acids (HAVERKATE & VAN DEENEN, 1965; DUBACQ & TRÉMOLIERES, 1983).

With the exception of sample No 13, the 18:3 acid is the major component in all the PE of the thirteen plants, ranging from 35 to 66%. In all PC, the contents of 16:0, 18:1, 18:2 and 18:3 are essentially the same. PI are all characterized by a high content in 16:0, ranging from 44 to 70%. In PG, although the composition of fatty acids is very different between the samples, the major components are generally 16:0, 16:1(*t*) and 18:1. No relationship seems to exist between these distributions and the chilling-sensitivity of the plants.

Comparing ΣU however, significant differences between chilling-resistant and chilling-sensitive plants are observed. The values of ΣU for the total phospholipids and for each class of phospholipid in chilling-sensitive type appear to be generally lower

than in chilling-resistant one. For a better comparison of ΣU and UI in the two sets of plant phospholipids, Table III reports the average values of the two parameters together with the standard deviations. For all classes of phospholipids the average values of ΣU and UI are higher for the chilling-resistant set. However, taking into account the standard deviations, the most significant difference between the two sets of plants lies in the ΣU values of PG. It increases from a average value of 34.0 for chilling-sensitive plants to 51.4 for chilling-resistant plants.

TABLE III

Comparison of the 'degree of unsaturation' (ΣU) and the 'unsaturation index' (UI) of phospholipids from temperate-zone and tropical plants.

phospholipid class	chilling-resistant plants					chilling-sensitive plants			
					ΣU				
	min	max	mean	(SD)		min	max	mean	(SD)
total PL	62.8	73.0	66.5	(3.8)		55.7	71.5	51.9	(5.8)
PE	69.7	77.9	74.4	(2.7)		44.8	74.5	56.9	(11)
PC	64.2	72.3	68.9	(3.2)		54.0	76.2	55.0	(9.0)
PG	48.3	56.9	51.4	(3.2)		32.4	44.6	34.0	(4.6)
PI	42.5	53.8	51.0	(4.0)		24.0	52.3	34.0	(10)
					UI				
	min	max	mean	(SD)		min	max	mean	(SD)
total PL	144	162	155	(6.5)		131	172	141	(16)
PE	168	212	191	(15)		114	199	172	(33)
PC	138	164	149	(9.9)		116	171	141	(23)
PG	68	101	84	(12)		55	76	65	(8)
PI	92	128	114	(14)		49	133	86	(36)

Positional distribution of fatty acids in 1,2-diacyl-phosphatidyl-glycerol: Table IV reports the distribution of the fatty acids at the *sn*-1 and *sn*-2 positions of 1,2-diacyl-PG together with the values of ΣU. The component 16:1(*t*) was present in the lysocompounds of PG in none of the 13 species; it is therefore exclusively present at the *sn*-2 position. The monounsaturated 18:1 acid is principally localized at the *sn*-1 position in all species, while the saturated 16:0 and the di- and triunsaturated 18:2 and 18:3 acids are found at both positions in proportions depending on the species. There are no clear differences in positional distribution of fatty acids between the two types of plants. The ΣU values of the fatty acids at the *sn*-1 position are generally higher than those at the *sn*-2 position. At both the *sn*-1 and *sn*-2 positions the ΣU values are generally higher in chilling-resistant plants than in chilling-sensitive plants. At the *sn*-1 position they range from 27.0% (magnolia) to 44.8% (osmanthus) and from 20.6% (fig) to 35.6% (india rubber fig) respectively while at the *sn*-2 position they vary from 8.6% (osmanthus) to 29.9% (magnolia) and from 4.9% (rubber) to 24.0% (fig).

TABLE IV

Fatty acid distributions at the *sn*-1 and *sn*-2 positions of PG from leaf phospholipids of temperate-zone and tropical evergreen plants.

sample N°		fatty acid composition (wt%)						ΣU
		16:0	16:1	18:0	18:1	18:2	18:3	
Temperate-zone plants								
1	C-1	20.2		2.3	25.7	1.5	tr	27.2
	C-2	15.5	8.4	1.0	3.6	11.1	10.3	25.0
2	C-1	21.7		1.3	24.5	2.5	tr	27.0
	C-2	12.2	7.9	–	10.7	9.0	10.2	29.9
	C-1	5.2		tr	25.0	10.4	9.4	44.8
3	C-2	33.7	7.6	tr	3.2	1.9	3.5	8.6
4	C-1	20.0		tr	11.5	12.5	6.0	30.0
	C-2	7.3	24.0	–	–	8.2	10.5	18.7
5	C-1	18.5		tr	28.2	2.0	1.3	31.5
	C-2	5.9	24.1	0.3	2.2	8.0	9.7	19.9
6	C-1	9.7		1.0	28.4	7.6	3.3	39.3
	C-2	26.2	14.8	–	3.2	4.3	1.5	9.0
7	C-1	13.1		tr	33.5	2.8	0.6	36.9
	C-2	8.9	29.7	tr	2.2	2.7	6.4	11.3
tropical plants								
8	C-1	25.4		2.5	12.0	7.0	3.0	22.0
	C-2	20.9	9.3	4.3	2.8	12.1	0.6	15.5
9	C-1	17.5		tr	21.0	3.6	7.8	32.4
	C-2	21.3	18.6	tr	0.5	6.7	3.0	10.2
10	C-1	26.0		2.0	10.0	12.0	tr	22.0
	C-2	28.5	10.8	0.3	3.2	3.9	3.4	10.5
11	C-1	26.6		2.7	17.0	3.6	tr	20.6
	C-2	19.8	6.3	–	14.1	6.7	3.2	24.0
12	C-1	14.4		tr	15.8	17.4	2.4	35.6
	C-2	31.3	10.6	0.8	1.3	0.7	5.3	7.3
13	C-1	17.3		tr	16.5	16.1	tr	32.6
	C-2	27.5	14.2	3.4	1.3	1.0	2.6	4.9

DISCUSSION

Previous comparative studies on leaf phospholipids of some herbaceous (MURATA *et al.*, 1982) and woody plants (TASAKA *et al.*, 1990) have suggested a relationship between the fatty acid composition of PG and the chilling sensitivity, the PG of chilling-resistant plants being characterized by higher contents in unsaturated fatty acids. The

present studies, based on a higher number of woody plant species, confirm clearly this relationship. The unsaturated fatty acid content of the PG, expressed as the degree of unsaturation ΣU, is significantly higher in chilling-resistant evergreen woody plants than in chilling-sensitive species. The unsaturation index UI, which takes into account the number of double bonds in the unsaturated components, has a less distinctive character. On the other hand, the fatty acid compositions of the other phospholipids do not show a significant correlation with the chilling-sensitivity.

An unexpected feature is however shown by this study. The relative amounts of PG in the leaf phospholipids of chilling-resistant species are lower than those of chilling-sensitive ones. As a result, the ΣU values of total phospholipids are less related with chilling sensitivity (Table III).

It is now generally admitted that chilling injury to plants is due to the phase transition of the membrane lipids at low temperatures (TASAKA *et al.*, 1990). In PG, the combination of two saturated fatty acids would produce the molecular species having the highest transition temperature and chilling sensitivity. Thus, as the component 16:1(*t*) is only found at the *sn*-2 position, the molecular species of PG 16:0/16:0, 16:0/16:1(*t*), 16:0/18:0, 18:0/16:0 and 18:0/16:1(*t*) are those which are responsible for increasing the chilling sensitivity. In the present work we did not determine the molecular species of PG, but it is possible to estimate the maximum possible content in saturated molecular species of PG from the positional distributions at the *sn*-1 and *sn*-2 positions reported in Table IV. After transforming the wt% values into mol% values, one can calculate the maximum molar percentage of these wholly saturated species as 45, 40, 10, 40, 39, 19 and 26% for the temperate-zone plants No 1 to 7 respectively, and 56, 35, 56, 54, 29 and 34% for the tropical plants No 8 to 13 respectively. Although these theoretical values are, as expected, generally higher for the second set of plants, there is an important overlapping and they certainly do not reflect the actual combination of fatty acids in PG. Further studies on the molecular species of PG are required for a better assessment of its relationship with chilling sensitivity.

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RÉSUMÉ

Distribution des acides gras dans les phospholipides de feuilles: La composition et la distribution des acides gras dans les phospholipides extraits des feuilles d'arbres à feuilles persistantes furent étudiées pour des espèces de sept zones tempérées et six tropicales. Les quantités relatives des phospholipides majeurs, c.-à-d. phosphatidyl-

éthanolamine (PE), phosphatidyl-choline (PC), phosphatidyl-glycérol (PG) et phosphatidyl-inositol (PI) et la composition des acides gras de chacune de ces classes furent déterminées. Dans les treize plantes, les principaux acides gras sont 16:0, 16:1(*t*), 18:0, 18:1, 18:2 and 18:3. Les résultats montrent une différence significative dans la proportion d'acides gras insaturés par rapport aux saturés, spécialement dans PG. Dans les plantes tropicales sensibles au froid, la teneur en acides gras insaturés de PG (ΣU= 18:1+18:2+18:3) varie de 32.4 à 44.6%, tandis que pour les espèces résistantes au froid, elle varie de 48.3 à 56.9%. La distribution de la position des acides gras dans PG fut recherchée par hydrolyse enzymatique à l'aide de la phospholipase A_2. Les résultats montrent que les acides 18:1, 18:2 et 18:3 sont généralement liés à l'atome de carbone *sn*-1, alors que l'acide 16:1(*t*) est lié exclusivement à l'atome de carbone *sn*-2.

REFERENCES

BLIGH, E, G. & DYER, W, J. 1959. *Can. J. Biochem. Physiol.*, 37: 911-917.

DUBACQ, J,P. & TRÉMOLIERES, A. 1983. *Physiol. vég.*, 21: 293-312.

HAVERKATE, F .& VAN DEENEN, L, L, M. 1965. *Biochim. Biophys. Acta,* 106: 78-92.

HU, Z,L., BAO, H. & GUO, H,L. 1993. *Scientia Silvae Sinicae*, 29: 543-546.

LYNCH, D,V. & THOMPSON, Jr, G, A. 1986. in: *Modern Methods of Plant Analysis* (LINSKENS, H, K. & JACKSON, J, F. eds.) Vol. 3. pp. 100- 120, Springer-Verlag. Berlin, Heidelberg.

LYONS, J. M. 1973. *Annu. Rev. Plant Physiol.*, 24: 445-466.

MURATA, N., SATO, N.,TAKAHASHI, N. & HAMAZAKI, Y. 1982. *Plant Cell Physiol.*, 23: 1071-1079.

MURATA, N. 1983. *Plant Cell Physiol.*, 24: 81-86.

MURATA, N., ISHIZAKI-NISHIZAWA, O., HIGASHI, S., HAYASHI, H., TASAKA, Y. & NISHIDA, I. 1992. *Nature*, 356: 710-713.

SEKIYA, J., NAGAI, K. & SHIMOSE, N. 1990. *Agric. Biol. Chem.*, 54: 2777-2782.

STRYER, L. 1988. *Biochemistry*, 3rd edition. pp.284-287. W. H. Freeman & Company. New York.

TASAKA, Y., NISHIDA, I., HIGASHI, S., BEPPU, T. & MURATA, N. 1990. *Plant Cell Physiol.*, 31: 545-550.

TORIYAMA, S. I., HINATA, K., NISHIDA, I. & MURATA, N. 1988. *Plant Cell Physiol.*, 29: 615-621.

WATANABE, T., FUKUSHIMA, H. & NOZAWA Y. 1980. *Biochim. Biophys. Acta*, 620: 133-141.

WATANABE, T., FUKUSHIMA, H., KASAI, R. & NOZAWA, Y. 1981. *Biochim. Biophys. Acta*, 665: 66-73.