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PHOSPHATIDYLGLYCEROL AND SULPHOQUINOVOSYLDIACYLGLYCEROL IN LEAVES AND FRUITS OF CHILLING-SENSITIVE PLANTS

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Abstract—The fatty acid composition of phosphatidylglycerol and sulphoquinovosyldiacylglycerol from the leaves and fruits of five chilling-sensitive plants has been analysed. The sum of the contents of hexadecanoic acid, octadecanoic acid and *trans*-3-hexadecenoic acid in the phosphatidylglycerols from the leaves and fruit tissue of each plant is very similar. The sum of the contents of hexadecanoic and octadecanoic acids in sulphoquinovosyldiacylglycerol also appears to be closely related in leaves and fruits from the same plant.

INTRODUCTION

The phenomenon of chilling sensitivity in higher plants, that is the development of physiological disorders at temperatures above 0°, occurs in both tropical and temperate crops and can be a major factor in marketing losses and production costs [1]. The concept that physical changes in membrane lipids are the critical event in chilling injury has not been universally accepted [1–3] but recently Murata has suggested [4–6] that in chloroplasts of higher plant leaves, the fatty acid composition of phosphatidyl glycerol (PG) may be the determining factor in chilling sensitivity. This hypothesis predicts that PG containing a high level of fatty acid molecular species with two high melting point fatty acids will induce phase transitions in bulk chloroplast membrane lipids above 0° due to the formation of monotectic mixtures of PG with other lipids, and that the fatty acid content of PG can be related to the chilling susceptibility of a plant [5, 7]. Murata and Kurisu [8] have also reported that PG fatty acid composition of non-green plastids (etioplasts, amyloplasts) is also correlated with the chilling sensitivity of non-green tissues. The question arises, however, whether the fruits of chilling-sensitive plants contain significant levels of high melting point fatty acids in PG, in a manner analogous to leaves. We have compared the fatty acid composition of the lipids of leaves and fruit from five chilling sensitive plants and find a close similarity between the content of high melting point fatty acids in PG from both tissues, in each case.

RESULTS AND DISCUSSION

The fatty acid composition of four plastid lipids from each tissue were analysed. The fatty acids of monogalactosyldiacylglycerols (MGDG) and digalactosyldiacylglycerols (DGDG) were highly unsaturated and a phase transition above 0° in those lipids was considered unlikely. Attention was focussed on the fatty acids of PG and sulphoquinovosyldiacylglycerol (SQDG) since it is PG [5, 7] or bulk anionic lipids [9] which have been implicated in phase transitions. In leaves, PG is largely confined to the chloroplasts and analysis of leaf PG gives similar results to those of isolated chloroplast PG [6, 7].

The postulated relationship between the chilling-sensitivity of plant tissues and the fatty acid composition of PG is based on its content of three fatty acids, 16:0, 18:0 and *trans*-3,16:1 [5, 7]. The latter has been assumed to have physical properties similar to a saturated fatty acid [4, 6]. The data in Table 1 shows that there is good agreement between the sum of the contents of 16:0, 18:0 and *trans*-3,16:1 in the PG of leaf and fruit tissue in all five examples, except for pulp tissue of cucumber and pawpaw, where the values are significantly lower. These lower values in pulp tissue may, however, simply reflect a higher content of extraplastidic PG since Murata and Kurisu [8] have isolated etioplasts from squash and amyloplasts from sweet potato and found good agreement between the contents of saturated fatty acids in PG from those plastids and chloroplasts from the corresponding leaf tissue. However, the PG of all the fruit tissues have a low content of *trans*-3,16:1, compared to that of leaves even though large amounts of chlorophyll were present (with the exception of banana pulp and ripe peel). The desaturation reaction which converts 16:0 to *trans*-3,16:1 is thought to take place after the assembly of PG molecules [10] and the lowered levels of *trans*-3,16:1 in fruits may be due to a deficiency of the relevant enzyme. The total content of the three fatty acids in PG of banana peel or pulp does not change markedly during ripening.

The content of high melting point fatty acids (16:0 + 18:0) in SQDG is in all cases lower than that of PG (Table 1) but there appears to be a similar amount of these acids in SQDG of leaves and fruit from the same plant. In all cases, however, the content of 18:2 in SQDG is higher in fruits than in leaves, and this increase is at the expense of 18:3. Such changes in the content of polyunsaturated fatty acids would be unlikely to influence the occurrence of phase changes in SQDG above 0°.

There is evidence that lipid synthesis in higher plants is under strict genetic control and that the synthesis of plastid PG is confined to the plastid [10, 11]. The observation that the *sn*-2 position of plastid PG is

Table 1. Fatty acid composition of phosphatidylglycerols and sulphoquinovosyldiacylglycerols in five chilling-sensitive plants

		Major fatty acids %							
		16:0	*cis*-16:1	*trans*-3,16:1	18:0	18:1	18:2	18:3	Σ 16:0+ *trans*-3,16:1 +18:0
Phosphatidylglycerol									
Cucumber	Leaf	35	—	30	13	9	3	8	78
	Peel	61	—	—	10	12	11	5	71
	Pulp	51	—	—	6	6	8	12	57
Tomato	Leaf	15	—	37	1	5	23	18	53
	Fruit	53	—	2	2	6	24	12	57
Bell pepper	Leaf	17	—	37	3	5	24	12	57
	Fruit	55	—	—	6	8	28	3	61
Paw paw	Leaf	28	—	36	5	14	9	8	69
	Peel	59	—	7	5	13	13	3	71
	Pulp	48	—	—	3	9	28	3	51
Banana	Leaf	34	—	38	3	8	8	11	75
	Green peel	71	—	4	2	3	12	7	75
	Ripe peel	77	—	—	—	4	6	12	77
	Green pulp	78	—	+	+	4	12	5	78
	Ripe pulp	83	—	—	—	+	8	9	83
Sulphoquinovosyldiacylglycerol									
Cucumber	Leaf	35	2	—	8	2	3	48	43
	Peel	36	—	—	4	12	21	26	36
Tomato	Leaf	46	1	—	3	1	12	35	48
	Fruit	40	—	—	5	5	32	17	45
Bell pepper	Leaf	53	—	—	3	3	10	31	56
	Fruit	36	—	—	11	2	36	14	47
Paw paw	Leaf	52	1	—	4	6	8	27	56
	Peel	40	—	—	5	11	24	12	45
	Pulp	46	—	—	5	13	31	6	51
Banana	Leaf	55	—	—	2	4	9	27	57
	Green peel	55	—	—	3	5	17	21	58
	Ripe peel	50	—	—	4	3	14	26	54
	Green pulp	59	—	—	+	4	23	14	59
	Ripe pulp	52	—	—	6	+	19	23	58

occupied almost exclusively by C_{16} acids [6, 7, 10, 12] makes it possible to calculate the content of molecular species of PG containing two high melting point fatty acids, simply from fatty acid analyses. The same calculation cannot be applied to SQDG because the diacylglycerol moiety of SQDG is largely derived from the cytoplasm, except in the 16:3 plants (e.g. tomato and bell pepper) where it can be derived from both cytoplasm and plastid [2, 10]. The content of molecular species of PG or SQDG containing two high melting point fatty acids would be the determinating factor in producing a phase transition in bulk membrane lipids above 0°, because of the relatively high transition temperature of such compounds.

The results presented in Table 1 show that if the physical properties of *trans*-3,16:1 are similar to those of its saturated analogue, 16:0, then the content of high melting point fatty acids is very similar in leaf and fruit tissues. A lesser correlation is evident for SQDG. If PG is the molecule responsible for inducing phase transitions in plastid membranes then experiments with leaves should serve as a useful model for studies with fruits. The varying responses of different cultivars of the same crop to chilling [13] may indicate however that it is the rate of response to a critical event such as a phase transition induced in bulk membrane lipids by PG, rather than the event itself which is of practical significance.

EXPERIMENTAL

Cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.) and bell pepper (*Capsicum annuum* L.) were grown from seed to mature fruit-bearing plants under natural light in a glasshouse (25° day: 15° night) at North Ryde, NSW. Pawpaw (*Carica papaya* L.) and banana (*Musa domestica* L.) were field-grown at Hardy's Bay, NSW. Leaves and green fruit were harvested from the same plant in each case. Throughout the text the words peel, pulp and fruit are used to describe parts of the fruits. In the case of cucumber and pawpaw, peel refers to thin slices of epidermis, while pulp refers to parenchymatous tissue. For bell pepper and tomato, fruit refers to segments containing both epidermis and pericarp. Green bananas were allowed to ripen in the laboratory for 10 days after harvesting, without application of ethylene.

Lipids were extracted from leaves by the technique of ref. [14] and from fruit tissues as previously described [15]. The total

extracts were fractionated on DEAE-Sepharose and individual lipids isolated by prep. TLC [6]. After detection with primulin, lipids were scraped off and methylated with BF_3–MeOH [16]. Fatty acid Me esters were analysed by GC using a 25 m CPSil 58 WCOT capillary column and FID detector. Column temp. was 190°, and injector and detector temps were 250°. The split ratio was ~ 40:1 and the sample size was 0.2–0.5 μl.

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