Rapid Papers

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Rapid Temperature-Induced Changes in the Fatty Acid Composition of Certain Lipids in Developing Linseed and Soya-Bean Cotyledons

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A change in ambient temperature caused marked alterations, over a 24 h period, in the proportions of the unsaturated C₁₈ fatty acids in 3-*sn*-phosphatidylcholine and 1,2-diacylglycerols during the development of soya-bean and linseed cotyledons. The molar proportion of oleate increased when the temperature was increased whereas that of linoleate or linolenate, depending on the species, increased when the temperature was lowered. Concomitant changes in the composition of 3-*sn*-phosphatidylethanolamine and triacylglycerols were small.

Labelling studies with detached cotyledons (Dybing & Craig, 1970; Slack *et al.*, 1978) indicated that 3-*sn*-phosphatidylcholine is probably involved in oleate desaturation and that this phospholipid may serve as a donor of polyunsaturated diacylglycerols for triacylglycerol synthesis (Slack *et al.*, 1978). There is a pronounced and rapid decline in the amount of oleate esterified to 3-*sn*-phosphatidylcholine and to 1,2-diacylglycerol and a concomitant increase in polyunsaturated C₁₈ fatty acids during the incubation of detached cotyledons (Slack *et al.*, 1978). This suggested that a large proportion of the tissue complement of these lipids had high rates of turnover. If this hypothesis is correct, it might be expected that similar changes in the fatty acid composition of these lipids would occur *in vivo* under certain conditions. It is well documented that the fatty acid composition of the triacylglycerols in several oilseed crops is influenced by the temperature prevailing during seed maturation (Hilditch & Williams, 1964; Canvin, 1965); the extent of desaturation is inversely related to temperature. Consequently, we have examined the effects of changes in ambient temperature during seed maturation in linseed and soya-bean plants on the fatty acid composition of individual lipids.

Materials and Methods

Plants of soya bean (*Glycine max* cultivar Amsoy) were grown in a controlled-environment cabinet at a constant temperature of 22.5°C, a light-intensity of 150 W/m² and a photoperiod of 12 h. Linseed plants (*Linum usitatissimum* cultivar Punjab) were grown in a heated glasshouse with the temperature maintained above 20°C. All plants were grown in pumice/peat

supplied with nutrient solution as previously described (Slack *et al.*, 1978) and the time of senescence of individual flowers noted.

Two groups of the soya-bean plants that had flowered 20–30 days previously were selected, and in each group four batches of six pods of comparable age were identified. At the start of the experiment two batches of pods were harvested from each group of plants at mid-photoperiod. One group of plants was then transferred to a controlled-environment cabinet at a temperature of 13°C and the other group to a cabinet at 32°C. Environmental conditions, other than temperature, were similar to those previously experienced by the plants. Other batches of pods were harvested at 6 h and 24 h after transfer.

Six linseed plants with developing seed capsules (20–25 days old) were selected. Three plants were transferred from the heated glasshouse to a controlled environment similar to that described above, but at a temperature of 11°C. The remaining three plants were kept in the glasshouse. At intervals over a 48 h period after transfer, capsules were harvested from the plants in each environment. Plants were then returned from the cool environment to the glasshouse and capsules harvested 24 h later. At each harvest, 21 capsules were taken from plants in each environment.

Harvested soya-bean pods and linseed capsules were placed immediately on ice. The cotyledons were removed, weighed and steamed, and the lipids extracted and concentrated as described by Slack *et al.* (1978). 3-*sn*-Phosphatidylcholine and 3-*sn*-phosphatidylethanolamine plus diacylgalabiosylglycerol were isolated from the lipid extracts by t.l.c. on silica gel G in chloroform/methanol/15 M-NH₃

(65:25:2, by vol.), triacylglycerols by t.l.c. on silica gel G in light petroleum (b.p. 60–80°C)/diethyl ether/acetic acid (75:25:1, by vol.) and 1,2-diacylglycerols by t.l.c. on silica gel G containing H_3BO_4 (5%, w/w) in chloroform/acetone (47:3, v/v) (Thomas *et al.*, 1965). 3-*sn*-Phosphatidylcholine and 3-*sn*-phosphatidylethanolamine were also purified as above from phospholipid fractions isolated from the total lipid extracts by column chromatography (Rouser *et al.*, 1967). Lipids were stained lightly with I_2 vapour and, except for diacylglycerols, were transmethylated directly on silica gel (Slack *et al.*, 1978). The diacylglycerols were eluted from the silica gel with diethyl ether and dried under N_2 . Methanol (1 ml) was added and the volatile methyl borate removed by evaporation under N_2 . The remaining diacylglycerols were transmethylated as above. The amounts of the individual fatty acid methyl esters derived from each lipid were measured by g.l.c. on columns (200 cm $\times$ 0.2 cm) of 15% (w/w) ethylene glycol succinate on Chromazorb W (80–100 mesh; Applied Science Laboratories, State College, PA, U.S.A.).

The amounts of individual phospholipids in the lipid extracts, after purification by t.l.c., were determined by the method of Rouser *et al.* (1966).

Results

The transfer of soya-bean plants from 22.5°C to either 13°C or 32°C caused rapid and pronounced changes in the fatty acid compositions of 3-*sn*-phosphatidylcholine and 1,2-diacylglycerols (Table 1). At 13°C there was an increase in the proportion of polyunsaturated acids, mainly linoleate, whereas

at 32°C there was an increase in oleate, largely at the expense of linoleate. At a particular temperature the fatty acid compositions of 3-*sn*-phosphatidylcholine and 1,2-diacylglycerols were similar. 3-*sn*-Phosphatidylethanolamine, on the other hand, contained at 22.5°C a smaller proportion of oleate than the above lipids. Although a temperature change induced alterations in the fatty acid composition of this lipid that were qualitatively similar to those shown in Table 1, the magnitude of the changes was small. The composition of triacylglycerols remained unchanged during the period of the experiment.

A decline in the amount of oleate in the 3-*sn*-phosphatidylcholine of developing cotyledons of linseed plants also occurred when these were transferred from a heated glasshouse above 20°C to 11°C. On returning plants to the glasshouse the amount of oleate in the lipid increased again (Table 2). In the linseed cotyledons, as in those of soya bean, the magnitudes of the changes in the fatty acid composition of the diacylglycerols were similar to those shown for 3-*sn*-phosphatidylcholine. By contrast, changes in the composition of 3-*sn*-phosphatidylethanolamine plus diacylgalabiosylglycerol were small. A small decrease in the amount of oleate in triacylglycerols occurred over the 48 h period at 11°C. It is noteworthy that in the linseed cotyledons the loss of oleate was compensated for mainly by an increase in linolenate, not by linoleate as in soya beans.

In both experiments the amount of 3-*sn*-phosphatidylcholine per unit fresh weight of tissue and the relative proportions of the major phospholipids, 3-*sn*-phosphatidylcholine, 3-*sn*-phospha-

Table 1. *Changes in the fatty acid composition of 3-sn-phosphatidylcholine and 1,2-diacylglycerols of developing seed cotyledons of soya-bean plants after changes in ambient temperature*

Uniform batches of developing soya-bean pods were selected on four soya-bean plants growing at a temperature of 22.5°C. Each batch contained six pods. At mid-photoperiod four batches of pods were harvested, then two plants transferred to 13°C and the others to 32°C, and single batches harvested at 6 h and 24 h thereafter. The fatty acid compositions of the lipids from individual batches were analysed as described in the Materials and Methods section.

Lipid	Ambient temperature (°C)	Time after transfer from 22.5°C (h)	Fatty acid composition (mol/100 mol)				
			C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}
Phosphatidylcholine	22.5	0	14.3 $\pm$ 0.1*	4.8 $\pm$ 0.2*	31.7 $\pm$ 1.8*	39.9 $\pm$ 1.9*	9.3 $\pm$ 0.4*
	13	6	14.3	4.6	14.2	56.1	10.8
	13	24	14.8	4.1	10.0	60.0	10.6
	32	6	15.5	6.2	44.0	27.8	6.4
	32	24	13.2	5.4	55.3	22.0	4.2
Diacylglycerols	22.5	0	12.1 $\pm$ 0.2*	5.1 $\pm$ 0.4*	35.4 $\pm$ 2.0*	37.5 $\pm$ 1.8*	10.2 $\pm$ 0.5*
	13	6	11.1	5.2	20.2	52.2	11.3
	13	24	13.6	4.3	16.5	55.0	10.5
	32	6	12.4	6.5	46.1	28.5	6.5
	32	24	11.3	5.0	53.5	24.2	5.9

* Mean $\pm$ S.E.M. for four batches of pods.

Table 2. *Changes in the fatty acid composition of 3-sn-phosphatidylcholine of developing cotyledons on transferring linseed plants to low temperature*

Six uniform linseed plants grown in a heated glasshouse at above 20°C were selected and from these three were transferred to a controlled environment at 11°C; the others remained in the glasshouse. At intervals over the following 48 h developing capsules were harvested from the plants in each environment; plants were then returned from 11°C to the glasshouse and capsules harvested after a further 24 h. At each harvest 21 capsules were taken from each treatment. The cotyledons were removed and the lipid was analysed as described in the Materials and Methods section.

Temperature (°C)	Time after transfer (h)	Fatty acid composition of 3-sn-phosphatidylcholine (mol/100mol)				
		C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}
>20		8.25±0.5*	7.6±0.4*	31.7±1.9*	16.6±0.5*	35.9±1.3*
>20 to 11	1.5	9.4	7.0	26.6	19.4	37.5
	6.0	9.2	6.0	13.8	21.2	49.8
	23.0	10.3	5.4	9.3	21.7	53.2
	28.0	14.3	5.6	8.5	18.3	53.4
	48.0	11.6	5.4	8.1	20.5	54.3
10 to >20	24.0	9.6	8.9	19.3	18.3	43.8

* Mean ± S.E.M. for five harvests.

tidylethanolamine and 3-sn-phosphatidylinositol, remained unaltered by the temperature change. In soya-bean cotyledons the average amount of phosphatidylcholine as a percentage of the combined amounts of the three lipids was 54.9±0.6% for all harvests. The comparable value for the linseed cotyledons was 58±0.7%.

Discussion

The rapid effects of an alteration in temperature on the fatty acid composition of 3-sn-phosphatidylcholine and diacylglycerols in the developing linseed and soya-bean cotyledons closely resemble the much longer-term effects of temperature on the composition of the seed triacylglycerols of these species (Hilditch & Williams, 1964; Canvin, 1965). In both cases temperature modifies the relative proportions of the unsaturated C₁₈ fatty acids, but has little effect on the ratio of saturated to unsaturated fatty acids. These observations *in vivo* support the suggestion, based on labelling studies *in vitro* (Slack *et al.*, 1978), that 3-sn-phosphatidylcholine serves as a donor of diacylglycerols for triacylglycerol synthesis. According to this hypothesis the fatty acid composition of the triacylglycerols of mature seed should reflect the average fatty acid composition of the 3-sn-phosphatidylcholine during the period of seed maturation. Furthermore the speed with which a change in the

ambient temperature modified the composition of the 3-sn-phosphatidylcholine and diacylglycerol *in vivo* supports the view (Slack *et al.*, 1978) that these lipids have a high rate of turnover.

It appears that temperature must affect differentially the relative activities of the enzymes involved in the incorporation of oleate into 3-sn-phosphatidylcholine and its desaturation. Harris & James (1969) suggested that temperature could influence the rate of fatty acid desaturation through an effect on O₂ solubility. Whether or not such an explanation is applicable in developing seed cotyledons must await further studies.

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