BIOSYNTHESIS OF ∝-LINOLENIC ACID IN LEAVES AND SEEDS OF RAPE (BRASSICA NAPUS L.)

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The biosynthesis of ∝-linolenate, the major fatty acid of green photosynthetic tissues in plants, is still unknown. Two different pathways, (i) the direct desaturation of oleate to linoleate and then to linolenic acid (Harris and James, 1965) and (ii) desaturation of twelve-carbon chain fatty acid followed by chain elongation to linolenate (Kannangara et.al., 1973), have been proposed.

In rapeseed, leaves contain about 10 % hexadecatrienoate, therefore, it was considered an ideal material to find whether both the above mentioned pathways were operative in same plant species. The seeds of rapeseed also possess 6-12% linolenate; its biosynthesis was therefore studied in seeds as well.

MATERIALS AND METHODS

Plants: Canadian cultivar "Oro" was grown in the greenhouse and growth chambers of the Institute as previously described (Brar and Thies, 1977).
Leaf and Seed embryo incubations: Radioactive substrates, at least 0.5 uCi in each case, were solubalized in 0.1 ml of 0.1% aqueous serum albumin solution and were fed to young leaves and 20-24-days old seed embryos by immersing leaf pertiole or seed embryos in the solution. Light intensity was 20-21 Klux and the temperature 21 ± 2°C.
Lipid extraction: Lipids from leaves and seeds were extracted by the method of Bligh and Dyer (1959). Affluents from GLC columns were split 1/10 through FID and 9/10 through the splitter. The radioactivity was assayed by trapping fatty-acid methyl ester (FAMES) in glass cartridges filled with silicone-coated anthracene as described by Karmen et.al. (1962).
Isolation of 16:3: Silver argentation TLC and GLC were used to isolate 16:3 from 18:3. TLC plates were developed in hexane : diethyl ether : glacial acetic acid (85:15:2), twice. The purity and identity of FAMES was checked thoroughly by several methods.
Degradation studies: FAMES were hydrolysed using KOH in methanol and reduced with PtO_2/H_2. Radioactivity at the 1-C position was determined by Schmidt decarboxy reaction (Goldfine and Block, 1961). To determine distribution of activity isolated fatty acids were subjected to chemical ∝-oxidation (Harris et.al., 1965). Reaction products were separated on GLC, identified and quantified, and the activity in each fraction (collected using Packard fraction collector) was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

Young rapeseed leaves incubated with [1-14C] or [U-14C] substrates (stearate, oleate, linoleate or acetate) incorporated a substantial part of the label into linolenic acid (Tables 1 and 2). When the leaves were fed oleic of linoleic acid, breakdown and re-synthesis occurred only to

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a small extent suggesting that these acids were desaturated directly to linolenate. Similar and low levels of the label at the 1-C position of linolenic and linoleic acids (Table 1) confirmed that [U-14C] linoleate was directly desaturated to linolenic acid. Further evidence for this conslusion was obtained by chemical α-oxidation of linolenate and linoleate isolated from leaves fed [U-14C] 18:2 (Table 3). The uniform distribution of the label reconfirmed that linoleate was directly desaturated to linoleate.

TABLE 1

THE INCORPORATION OF [1-14C] 12:0, 18:0 OR 18:1 or [U-14C] 16:3 OR 18:2 INTO FATTY ACIDS OF SEEDS AND LEAVES OF RAPESEED*

Substrate	Organ	% label unincorporated	% radioactivity in 16:0	16:3	18:0	18:1	18:2	18:3
[1-14C] 12:0	L	11	36.4	4.9	4.5	7.7	21.2	9.0
[1-14C] 18:0	L	78	5.2	-	-	20.2	37.2	5.8
[1-14C] 18:1	L	30	0.6	-	1.4	-	80.8	17.1
[U-14C] 16:3	L	53	5.4	-	3.6	4.2	5.1	81.8
	S	84	11.9	-	22.9	19.2	11.5	34.6
Label in 1-C position+	L		-	4.7	-	-	0.0	1.7
[U-14C] 18:2	L	84	6.4	4.1	0.8	1.1	-	87.0
	S	93	9.8	18.1	2.0	6.0	-	64.1
Label in 1-C position	L		-	-	-	-	7.9	7.4

*12:0, 18:0 and 18:1 were incubated for 20 hrs.,16:3 and 18:2 for 3 hrs. in light (20 Klux) and air. L = Leaves, S = Seeds
+Determined by Schmidt decarboxylation.

TABLE 2

EFFECT OF EXOGENOUS HEXADECATRIENOATE AND OXYGEN SUPPLY ON INCORPORATION OF [1-14C] ACETATE INTO FATTY ACIDS OF SEEDS AND LEAVES OF RAPESEED. INCUBATIONS WERE FOR 3 HRS. IN 20 KLUX LIGHT AT 20°C

Treatement	Organ	% substrate unincorporated	% radioactivity in 16:3	18:0	18:1	18:2	18:3
Air	Leaves	26.8	12.8	4.5	27.0	36.4	19.3
	Seeds	27.6	4.4	3.5	72.3	18.0	1.8
Air and 16:3	L	33.2	10.1	4.7	24.7	41.1	19.3
	S	25.3	5.1	2.5	67.1	20.1	5.2
N_2 and 16:3	L	39.0	17.8	11.2	30.7	19.2	21.1
	S	37.8	9.1	4.5	67.5	12.6	6.3
Label in 1-C position	L		8.6	-	-	12.6	12.4

To determine the presence or absence of an alternate pathway involving desaturation of short-chain fatty acids and their subsequent chain

elongation, [1-14C] 12:0 and [U-14C] 16:3 were fed to leaves and were readily incorporated into linolenic acid without any appreciable breakdown and re-synthesis (Table 1). In leaves incubated with [1-14C] acetate, exogenous 16:3 and nitrogen, the synthesis of linolate, but not of linolenate, was greatly inhibited (Table 2). Chemical α-oxidation of the isolated linolenate and 16:3 (Table 3) showed that the distribution of label in the carbon chain was uniform in both of these acids and only 1.7% of the label was present at the 1-C position of linolenate (Table 1). The results established that [U-14C] 16:3 was directly elongated to linolenate.

In almost all studies on biosynthesis of linolenic acid in plants thus far, only leaves or their chloroplast preparations have been used (Harris and James, 1965; Kannangara et al., 1973). The seed embryos of many oilseed crops, however, contain linolenate as well as chloroplasts during their development (Thies, 1971). Rapidly developing rapeseed embryos (20-24 days old) fed [1-14C] acetate, [U-14C] 16:3 or [U-14C] 18:2 incorporated large quantities of label into linolenate (Table 1 and 2). The pattern of incorporation of substrate label into linolenate was similar to that found in the leaves (Table 1). Exogenous 16:3 enhanced incorporation of [1-14C] acetate into linolenate of embryos, but not in leaves which already contain 10-12% of 16:3 (Table 2).

Table 3

RADIOACTIVITY IN DEGRADED FRAGMENTS OF FATTY ACIDS ISOLATED FROM LEAVES FED (i) [U-14C] 18:2 AND (ii) [U-14C] 16:3 IN LIGHT AND AIR FOR 3 HRS. CHEMICAL α-OXIDATION AFTER HARRIS ET.AL., (1965)

Fragments obtained from		C_{18}	C_{17}	C_{16}	C_{15}	C_{14}	C_{13}	C_{12}	C_{11}
(i) Linolenic acid	% R*	42	13	11	18	8	3	-	-
	% M	36	11	22	14	12	4	-	-
Linoleic acid	% R	81	12	3	-	1	-	2	1
	% M	77	5	10	-	4	1	2	-
(ii) Linolenic acid	% R	36	12	13	6	11	8	7	5
	% M	40	8	15	7	10	8	7	3
16:3	% R			39	18	21	12	8	3
	% M			32	8	23	14	16	6

*R = radioactivity M = mass

Remarkably, the biosynthesis of linoleate from [1-14C] -acetate + 16:3 was inhibited in the absence of oxygen, but the biosynthesis of linolenate was not (Table 2). These data suggest that in rapeseed embryos linolenate is synthesized by direct desaturation of 18:2, as well as by chain elongation of 16:3.

CONCLUSIONS

Alpha-linolenate, in young leaves as well as in 20-24 days old embryos of rapeseed, was synthesized both by direct desaturation of linoleate and by chain elongation of hexadecatrienoate. In rapeseed leaves, [U-14C] 16:3 was a more effective substrate for linolenate than [U-14C] 18:2, while for seeds the reverse seemed to be true (Table 1).

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