FATTY ACIDS OF ROOTS OF SELECTED SPECIES[1]

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ABSTRACT

In order to interpret the structural and functional roles of the lipids in roots, the composition of the lipids of these organs must be determined. A survey was made of the fatty-acid composition of roots of selected crop species. Seeds were germinated under sterile conditions; the lipids were extracted from the roots, methyl ester were prepared of the fatty acids, and the fatty acids were analyzed by gas chromatography. Two groups of plants were found—one which contained larger percentages of linoleic acid in the roots and another which contained larger percentages of linolenic acid. In both groups the most abundant saturated fatty acid was palmitic. The fatty acids of tissue samples taken at different distances from the tip of the root were determined. It was found that the amount and relative percentages of the individual fatty acids varied developmentally. The fatty acids of major lipid classes of corn roots (*Zea mays* L.) were determined. Most of the fatty acids were esterified in the phospholipids. Apparently the glycolipids are only minor constituents of the lipids of roots. This is in contrast to the lipid composition of photosynthetic tissues in which the glycolipids are major constituents.

INTRODUCTION

A considerable amount of effort has been expended to identify the lipids and fatty-acid composition of commercially valuable seeds and of photosynthetic tissues, especially leaves and chloroplasts (Hitchcock and Nichols, 1971). The most abundant fatty acid of green leaves is α-linolenic acid, followed by palmitic acid, the most abundant saturated fatty acid. In photosynthetic tissues (leaves), the palmitic acid is associated with a variety of parental lipids, whereas the α-linolenic acid is most commonly esterified in monogalactosyl diglyceride (Nichols and James, 1968). The fatty acids of the roots of a few species have been identified (Kaimal and Lakshminarayana, 1970; Holman and Nichols, 1972) where the most

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abundant saturated fatty acid is palmitic and the most abundant unsaturated fatty acid is generally linoleic (C18:2–18 carbon atoms and 2 double bonds).

This paper reports the results of efforts to identify the fatty-acid composition of young roots of some crop species, as well as to identify how these fatty acids of roots vary developmentally. The fatty acid composition of major classes of lipids was also identified. Because of the high concentration of lipids in biological membranes and the possible role of the fatty acids in the function of these membranes, identification of the fatty acid composition of these lipids is important.

MATERIALS AND METHODS

Growth of Roots

Eight different cultivars were used in this study. These were: squash (*Cucurbita maxima* Duchesne, Burpee's Blue Hubbard), bush bean (*Phaseolus vulgaris* L., Burpee's Tenderpod), pumpkin (*Cucurbita pepo* L., Burpee's Small Sugar), watermelon (*Citrullus vulgaris* Schrad., Burpee variety unknown), sweet corn (*Zea mays* L., Burpee's Honeycross-Yellow Hybrid), cucumber (two forms, both *Cucumis sativus* L., Burpee's A and C and Burpee's London), and oats (*Avena sativa* L., variety unknown; obtained from a local farm store).

All operations until the time of harvest were performed in a sterile transfer room. The seeds were first surface sterilized for 45 sec in a 10% Chlorox solution and were then rinsed with sterile distilled water. The seeds germinated at approximately 25°C in the dark in sterile Petri dishes containing sterile distilled water for a period sufficient to obtain enough root material (1–2 gm). In all cases a specific length of root measured from the apex was used for fatty-acid analysis. Segments of corn roots cut at different distances from the apex were used in the studies of development. A known weight (*ca.* 1–2 g) of tissue was harvested in each case.

Lipid Extraction and Fatty-Acid Analysis

The harvested root tissue was boiled for 3 min in chloroform-methanol (2:1, v/v). The tissue was then homogenized in chloroform-methanol, filtered, and the filter washed clean (three washings) with the solvent. The extract was then washed through an acidified aqueous layer and dried with anhydrous sodium sulfate. The fatty acids were transesterified with methanolic-HCl (2.5% HCl, w/w) under reflux for 1.5 hr in an atmosphere of nitrogen (Kates, 1964). The methyl esters were purified by passage through silicic acid columns. The fatty acid methyl esters were resolved by gas chromatography on a 10-ft column of 10% DEGS on HMDS-treated acid-washed Chromosorb W. Separations were made isothermally at 185°C. The detector was calibrated with known weights of fatty-acid methyl ester standards obtained from Applied Science Company, State College, Pa.

Column Chromatography of Lipids

Extracts from corn roots were chromatographed into general classes of lipids and then the fatty-acid composition of each of these classes was determined. The aliquot used for lipid analysis was washed with water, dried with anhydrous sodium sulfate, and was then placed on a 10-g column of Unisil silicic acid (Clarkson Chemical Company, Williamsport, Pa.). Neutral lipids were eluted with 500 ml of chloroform, monogalactosyl diglyceride was eluted with 100 ml of chloroform-acetone (1:1, v/v), digalactosyl diglyceride was eluted with 100 ml of acetone, and phospholipids were eluted with 100 ml each of chloroform-methanol (9:1, v/v), chloroform-methanol (1:1, v/v), and methanol.

The data given in the tables are all averages of three determinations except those in table 1, where the data are averages of only two determinations.

RESULTS AND DISCUSSION

The fatty-acid compositions of roots of seven species or varieties are given in table 1. The saturated fatty acid found in highest percentage was palmitic (C16:0), and the unsaturated fatty acid found in highest percentage was linolenic (C18:3). These two fatty acids are also most abundant in photosynthetic tissues, although photosynthetic tissues generally contain a higher percentage of C18:3. The relative amounts of the different fatty acids found in the roots of five of these species or varieties resemble the situation found in etiolated barley leaves (Newman *et al.*, 1973).

TABLE 1

Fatty-acid composition of roots of various species

Species	Fatty-acid composition in percent					
	C16:0*	C16:1	C18:0	C18:1	C18:2	C18:3
Squash	39.0†	0.40	0.69	3.01	8.76	48.0
Bush bean	17.7	0.63	2.05	4.76	26.6	48.7
Pumpkin	36.0	0	0.69	1.89	10.5	51.0
Watermelon	41.5	0	2.32	3.14	11.54	41.4
Cucumber A and C	33.8	0	6.56	2.79	4.79	52.0
Cucumber London	36.4	0.36	4.07	6.92	13.8	38.4
Oats	43.4	0	0.69	5.25	30.0	20.7

*First number (16 here) refers to the number of carbon atoms and second number (0 here) refers to the number of double bonds.

†Maximum variation in the samples was about ±4%.

Corn roots, in contrast to the roots of other species assayed, contained a high percentage of linoleic acid (C18:2; tables 2 and 3). As with the other species, the most abundant saturated fatty acid was C16:0. The relative amount of C18:2 remained about the same in all developmental stages assayed. The relative amount of C16:0 decreased and the relative amount of oleic (C18:1) increased with increasing stage of development. Apparently, it does make some difference how the tissue to be assayed is selected. This could possibly be explained by an increasing vacuolation of the cell in which there is a decline in the amount of fatty acid material per unit wet weight of the tissue (table 3). Generally, most of the fatty acids exhibited this decline in amount with increasing distance from the tip of the root.

TABLE 2

Fatty-acid composition of different developmental stages of corn roots

Section (measured from tip in cm)	Fatty-acid composition in percent				
	C16:0	C18:0	C18:1	C18:2	C18:3
0.0–1.0	31.6±3.9*	1.9±1.0	4.9±2.5	58.3±2.4	3.3±0.4
1.0–2.0	29.5±2.5	3.1±1.9	7.0±4.9	57.0±7.4	3.5±0.2
2.0–3.0	25.0±4.4	3.2±0.8	9.3±3.0	57.4±3.5	5.0±1.1
3.0–4.0	22.2±2.3	3.9±0.4	12.1±2.4	56.8±1.9	5.0±0.9

*Standard deviation.

TABLE 3
Fatty-acid composition of different developmental stages of corn roots

Section (measured from tip in cm)	Fatty acid composition in μmoles/g wet wt				
	C16:0	C18:0	C18:1	C18:2	C18:3
0.0–1.0	2.06±1.2*	0.14±0.13	0.26±0.23	3.7 ±1.7	0.21±0.14
1.0–2.0	0.76±0.28	0.08±0.04	0.16±0.11	1.5 ±0.5	0.09±0.02
2.0–3.0	0.41±0.35	0.05±0.03	0.13±0.06	0.86±0.6	0.07±0.03
3.0–4.0	0.31±0.19	0.05±0.03	0.16±0.09	0.77±0.4	0.06±0.03

*Standard deviation.

The phospholipid fraction contained the greatest abundance of fatty acids, of which C18:2 was found in highest concentration (table 4). The fatty acids of the neutral lipids consisted of both C18:2 and C16:0. Only minor amounts of fatty acids were found in the glycolipid fractions. Table 5 gives the relative percentages of each fatty acid from each column fraction. Linoleic acid (C18:2) was the most abundant fatty acid of each fraction (*i.e.*, it showed the highest relative percentage), but it was relatively more abundant in the phospholipid fraction than in the other fractions. The neutral lipid fraction contained, in addition to C16:0 and C18:2, significant amounts of C18:1. The glycolipid fractions, which are probably not abundant in roots, contained larger percentages of C18:3.

It is apparent from both the data and the literature (Holman and Nichols, 1972) that the fatty-acid composition of root tissues is not as uniform as is that of green leaf tissues (Hitchcock and Nichols, 1971). Of all the species studied, those reported in the literature (Holman and Nichols, 1972; Kaimal and Lakshminarayana, 1970) and those used for this study, two groups emerge. Both

TABLE 4
Fatty-acid composition of four classes of lipids from corn roots

Lipid class	Fatty acid composition in μmoles/g wet wt				
	C16:0	C18:0	C18:1	C18:2	C18:3
Phospholipids	0.646±0.133*	0.0316±0.0001	0.1180±0.0060	1.5010±0.263	0.0488±0.0090
Neutral lipids	0.170±0.026	0.0346±0.0110	0.0711±0.0550	0.2150±0.026	0.0156±0.0008
Monogalactosyldiglyceride	0.042±0.007	0.0138±0.0035	0.0184±0.0012	0.0577±0.016	0.0425±0.0120
Digalactosyldiglyceride	0.029±0.004	0.0123±0.0006	0.0155±0.0013	0.0518±0.0013	0.0296±0.0004

*Standard deviation.

TABLE 5
Fatty-acid composition of four classes of lipids from corn roots

Lipid class	*Fatty-acid composition in percent*				
	C16:0	C18:0	C18:1	C18:2	C18:3
Phospholipids	27.5±0.8*	1.3±0.3	5.1±0.6	64.0±0.0	2.1±0.1
Neutral lipids	33.5±2.9	6.7±1.6	14.1±1.0	42.5±3.1	3.1±0.4
Monogalactosyldiglyceride	24.4±2.8	7.8±0.8	10.8±1.8	32.7±2.4	21.5±0.4
Digalactosyldiglyceride	21.0±3.0	8.9±0.5	11.2±1.1	37.6±1.2	21.5±0.4

*Standard deviation.

contain C16:0 as the saturated fatty acid found in highest relative percentage. Some, however, contain C18:2 and also some C18:3 as the unsaturated fatty acid found in highest percentage. The fatty-acid profile also changes with distance from the root top. It is interesting to note that the lipid composition of developing corn grains also changes developmentally (Weber, 1969, 1970). The greatest percentage of fatty-acid material is found in the phospholipid fraction.

If the lipids in different non-storage roots have similar functions, as they may have in photosynthetic tissue, why is there not as much consistency in the fatty-acid composition in roots of different species as there is in that of photosynthetic tissues? It appears that most of the fatty-acid materials in the roots assayed are found in the phospholipids. If the phospholipids are mostly membrane constituents, then it is not critical whether the phospholipids contain more C18:2 or more C18:3. This slight degree of difference in unsaturation does not seem to be important.

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