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COMPARISON OF FATTY ACID COMPOSITION IN TISSUES OF LOW LINOLENATE MUTANTS OF SOYBEAN

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Abstract—Phosphatidylcholine and monogalactosyldiacylglycerol are the proposed substrates for fatty acid desaturation to form linolenic acid (18:3) in plant tissues. A comparative analysis of the compositions of PC and MGDG molecular species, together with determination of total fatty acids, was conducted in the soybean cv. Century and in C1640, a low 18:3 mutant, to evaluate the expression of this mutation in the two classes of lipids among various tissues. Seeds of C1640 and A5, another low 18:3 mutant, had decreased 18:3 levels in cotyledons and axes but not in seed coats. Vegetative tissues of C1640 and A5 had lower 18:3 levels in roots but not in leaves and stems. The amount (mol%) of 16:0/18:3 and 18:3/18:3 PC species in cotyledons and 18:3-containing PC species in axes, decreased in C1640 compared to Century. The reduction of 16:0/18:3 and 18:3/18:3 species in both PC and MGDG also occurred in roots but little alteration existed in leaves and stems of C1640. The present results suggest the existence of a possible common mechanism for the formation of 18:3 in roots and seeds which is different from leaves and stems.

INTRODUCTION

The existence of at least two sites has been proposed for the desaturation of linoleic acid (18:2) to linolenic acid (18:3) in higher plants [1]. One site, termed 'prokaryotic', is in the chloroplast and the other termed 'eukaryotic', is extra-chloroplastic, presumably in the endoplasmic reticulum (ER) [2]. In developing oilseeds, the synthesis of 18:3 has been considered to occur mainly in the ER and phosphatidylcholine (PC) has been shown to be a precursor for the desaturation of 18:2 to 18:3 [3]. In leaves, monogalactosyldiacylglycerol (MGDG) in chloroplasts is a proposed site of 18:3 formation [3]. However, it is not yet known how the different sites contribute to 18:3 formation and how they are regulated in various tissues.

A number of genetic mutants with low 18:3 content have been isolated from several plant species [4–8]. One of the uses of these mutants is to elucidate the regulation of 18:3 formation. In soybean, previous work has shown changes in PC molecular species in cv. Century compared to C1640, a mutant with low 18:3 level in seeds and derived from cv. Century [9]. In order to further investigate the mechanism of control of 18:3 levels in soybean, a detailed comparative analysis of the molecular species of PC and MGDG was conducted in various tissues of Century and C1640. The major objective of the present study was to evaluate the expression of the low 18:3 mutation in the two major classes of lipids, PC and MGDG, among various tissues.

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RESULTS

PC molecular species in the various tissues of Century and C1640

The composition of PC molecular species from cotyledons and axes of mature seeds of Century and C1640 were compared (Table 1). Decreases in 16:0/18:2, 16:0/18:3 and 18:3/18:3 species and increases in 18:2/18:2 and 18:2/18:3 occurred in the cotyledons of C1640. In axes, C1640 exhibited reduced levels of the 18:3-containing species, 16:0/18:3 and 18:2/18:3, with corresponding increases in 16:0/18:2 and 18:2/18:2.

In the vegetative tissues of 21-day-old seedlings (Table 2), there were few differences in PC compositions in the leaves and stems of Century compared to C1640. However, in roots of C1640 there were substantial decreases in 16:0/18:3 and 18:3/18:3 and increases in 16:0/18:2, 18:2/18:2, and 18:2/18:3. The PC molecular species of stems, leaves, and roots from mid-maturation plants were also examined and the same trends seen in the younger plants (Table 2) were observed (data not shown). The combined data showed that the low 18:3 phenotype is clearly expressed in PC of cotyledons, axes and roots in C1640 but not in PC of leaves and stems.

The composition of PC molecular species in the cotyledons differed considerably from that of axes in mature soybean seeds (Table 1). Fewer PC species were detected in axes than in cotyledons. 18:2/18:2 was the most abundant PC species in axes; whereas 16:0/18:3 was the major one in cotyledons. The vegetative tissues were found to have PC compositions more similar to those of cotyledons than axes. Interestingly, 18:3/18:3 was not

Table 1. Molecular species of PC in cotyledons and axes of Century and C1640

Molecular species	Cotyledons		Axes	
	Century	C1640	Century	C1640
16:0/14:1*	3.48†	3.17	—‡	—
16:0/14:2	4.55	6.31	—	—
16:0/18:2	19.24	9.34	23.9	27.4
16:0/18:3	32.46	26.78	15.8	13.1
18:1/18:1	9:38	10.99	5.4	5.7
18:1/18:2	7.85	6.87	tr§	tr
18:1/18:3	3.43	3.99	tr	tr
18:2/18:2	4.70	17.86	37.5	40.7
18:2/18:3	4.45	7.21	17.4	13.1
18:3/18:3	10.46	7.48	tr	tr

*Pairs of numbers representing fatty acids and separated by a slash refer to the *sn*−1 and *sn*−2 positions, respectively of the molecular species.

†Values reported are a percentage by weight of the total PC fraction.

‡Not detected.

§Trace, < 1–2% of total.

detected in axes of either Century or C1640 in spite of the high abundance of this molecular species in cotyledons, stems, leaves, and roots. The decline of the 18:3 content in cotyledons and roots of C1640 occurred at the *sn*-1 position of C18/C18 species and at the *sn*-2 position of C16/C18 species; whereas corresponding changes in axes occurred at the *sn*-2 position of both C18/C18 and C16/C18 and no 18:3 was detected at the *sn*-1 position in axes (Tables 1 and 2).

MGDG molecular species in vegetative tissue of Century and C1640

MGDG is a major class of lipids in vegetative tissues and is a proposed substrate for 18:2 desaturation [3]. In leaves and stems there was little difference between Century and C1640 in the composition of MGDG molecular species (Table 3). However, in the roots of C1640 there were substantial decreases in 16:0/18:3 and 18:3/18:3 MGDG with corresponding increases in 16:0/18:2 and 18:2/18:2 molecular species (Table 3), indicating that the low 18:3 phenotype is also expressed in root MGDG.

The predominant MGDG molecular species in leaves and stems was 18:3/18:3; whereas 18:2/18:3 was the major species in roots. Low levels of the characteristic prokaryotic lipid components 18:3/16:3 and 18:3/16:2 MGDG were found in leaves and stems, confirming that soybean is predominantly eukaryotic in terms of MGDG synthesis. C1640 was not different in the prokaryotic MGDG molecular species, and these lipids were not detectable in roots.

A substituted pyridazinone herbicide, San 9785, was previously reported to have a differential effect on the 18:3 content of developing cotyledons of Century and C1640 [9]. This compound has been shown to reduce the levels of 18:3 in plant tissues [10]. Treatment with this compound was progressively less effective in reducing 18:3 of cotyledons during seed development in C1640 than in Century [9]. However, the analysis of leaf MGDG species from plants grown in the presence of 50 μM of San 9785 showed similar increases of 18:2/18:3 and 18:2/18:2 and reductions in 18:3/18:3 in both genotypes (data not shown). This further supports that different pathways are operative in regulating 18:3 levels in leaves and roots of Century and C1640.

Fatty acid composition in various tissues of Century, C1640 and A5

A second low 18:3 mutant of soybean, A5 [6], was included in these studies. The low 18:3 phenotype of C1640 and A5 were both expressed in cotyledons and axes but not in seed coats (Table 4), indicating tight developmental specific regulation of 18:3 formation. C1640 displayed a decline in the 18:3 level with a corresponding increase in 18:2 in both cotyledons and axes. However, compared to Century, A5 had an elevated level of 18:1 and a decreased level of 18:2 and 18:3

Table 2. Molecular species of PC in roots, stems, and leaves* of Century and C1640†

Molecular species	Roots		Stems		Leaves	
	Century	C1640	Century	C1640	Century	C1640
16:0/14:1	2.36‡	2.77	0.95	1.34	1.19	1.25
16:0/14:2	3.69	2.30	1.87	1.51	2.46	2.70
16:0/18:2	22.97	29.87	11.46	10.71	8.75	9.18
16:0/18:3	38.62	20.12	43.26	42.56	39.27	38.45
18:1/18:1	6.62	6.0	3.21	2.40	0.91	0.58
18:1/18:2	6.70	6.48	2.87	3.13	2.23	2.71
18:1/18:3	4.40	2.96	2.98	2.76	1.96	1.21
18:2/18:2	2.20	12.32	14.23	15.68	17.21	16.76
18:2/18:3	3.97	12.41	6.62	7.99	10.58	12.85
18:3/18:3	8.52	3.77	12.55	11.92	15.44	14.31

*First trifoliate.

†21-day old seedlings.

‡Value reported on a percentage by weight of total PC molecular species.

Table 3. Molecular species of MGDG in roots, stems, and leaves of Century and C1640*

	Roots		Stems		Leaves	
Molecular species	Century	C1640	Century	C1640	Century	C1640
16:0/14:2	3.16	3.27	—	—	—	—
16:0/18:2	8.46	12.32	1.96	2.06	5.20	5.0
16:0/18:3	10.15	5.20	2.45	2.47	2.36	3.07
18:1/18:1	9.88	10.44	1.72	2.00	1.45	2.0
18:2/18:2	18.41	23.21	1.98	1.85	1.06	1.15
18:2/18:3	45.43	43.61	7.21	10.45	5.49	11.65
18:3/16:2	—†	—	1.42	1.59	2.01	1.98
18:3/16:3	—	—	0.97	1.12	3.27	3.01
18:3/18:3	4.41	1.95	82.29	78.46	79.16	72.14

* 21-day-old seedlings.
† Not detectable.

Table 4. Fatty acid composition in cotyledons, axes, and seed coats of mature seeds and in roots, stems, and leaves of seedlings (21-day-old) of Century, C1640, and A5

Tissues	Genotypes	Fatty acid (%)				
		16:0	18:0	18:1	18:2	18:3
Cotyledons	Century	13.55	3.53	15.46	58.61	8.85
	C1640	13.88	3.05	14.54	64.58*	3.95*
	A5	14.93	2.91	30.54*	47.95*	3.67*
Axes	Century	16.82	2.82	1.93	53.75	24.69
	C1640	15.51	3.09	1.95	67.04*	12.41*
	A5	17.64	2.36	8.10*	64.8*	7.16*
Seed coats	Century	32.33	11.72	10.06	34.75	11.14
	C1640	31.78	10.93	7.97	38.38	10.94
	A5	33.01	13.66	12.54	30.26*	10.52
Roots	Century	36.05	5.88	2.54	25.67	29.87
	C1640	34.87	5.82	2.71	31.50*	25.12*
	A5	34.83	4.61	1.87	35.36*	23.32*
Stems	Century	28.41	6.69	2.60	26.68	35.62
	C1640	27.34	6.77	3.52	26.29	36.09
	A5	26.56	7.02	3.48	29.65	33.04
Leaves	Century	20.83	4.82	3.11	17.57	53.67
	C1640	21.56	4.15	3.03	18.46	52.81
	A5	19.98	3.01	2.17	16.89	58.95

*Numbers significantly different from Century at the 5% level based on the Protected Least Significant Difference Test [9].

in cotyledons; whereas in axes both 18:1 and 18:2 increased.

There was a decrease in the total 18:3 level and an increase in the 18:2 level in the roots of C1640 and A5 but not in the leaves or stems. Pods (30 days after flowering) also did not show differences in fatty acid composition (data not shown). Roots of both low 18:3 genotypes had higher 18:2 than Century and there was no difference in 18:1 level among Century, C1640 and A5 roots (Table 4).

DISCUSSION

The present study indicates the existence of an associated decline of 18:3 levels in both seeds and roots of two low 18:3 mutants of soybean, C1640 and A5. The decrease in C1640 was seen in terms of both total fatty acid composition and in levels of individual PC molecular species. The decrease of 18:3 in seeds and roots of A5 is consistent with that reported previously for A5 and two other low 18:3 mutants of soybean [11]. In mutants isolated from other plant species, a similar expression of 18:3 levels has also been observed. For instance, the seed 18:3 in a low 18:3 mutant of linseed was almost eliminated but the 18:3 level in leaves was not altered [12]. In contrast, the 18:3 levels in roots and seeds were not changed in a mutant of *Arabidopsis* [4] with low leaf 18:3 and 16:3. Together with the observations reported here, it seems likely that seeds may share common mechanisms for 18:3 formation with roots, which differ from leaves and stems. Different desaturation systems (or their control) may predominate in photosynthetic versus non-photosynthetic plant tissues. However, whether photo-

synthetic activity of a tissue plays a role in the differential regulation among the various tissues still remains to be elucidated.

The results of the different fatty acid compositions among Century, C1640, and A5 also indicate that the low levels of 18:3 can result from the decreased desaturation at different steps of the sequence 18:1→18:2→18:3. A5 was reported to have reduced 18:1 desaturase activity [13]. Based on the analysis of total fatty acid composition, and that of PC and MGDG molecular species, the low 18:3 content in C1640 appears to be the result of decreased 18:2 desaturation. Studies in which ^{14}C-labelled 18:2 was fed to developing cotyledons (30 days after flowering) revealed that the conversion of 18:2 to 18:3 in C1640 was 20% lower than in Century [14], again indicating decreased 18:2 desaturation activity in C1640.

EXPERIMENTAL

The soybean genotypes Century, C1640, and A5 were grown in a greenhouse in the spring and summer of 1987 in full sunlight with *ca* 27° and 21° day/night temperatures. Plants were harvested after growth for 21 days and lyophilized. Total lipids from *ca* 15 mg tissue, were extracted with 2 ml of $CHCl_3$–MeOH (2:1). Subsequent preparation of fatty acid methyl esters, analysis of fatty acid composition, and statistical analysis of data were as reported previously [9].

In analyses of PC and MGDG molecular species, total lipids were extracted from lyophilized tissues and separated into neutral lipid, galactolipid, and phospholipid classes using silica Sep Pak cartridges (Waters Assoc.) as described in ref. [15]. TLC procedures were utilized for recovery of MGDG and PC [15]. Samples of these lipids (0.2–0.4 μmol) were resolved into constituent molecular species by HPLC utilizing an ACS Model 351 solvent delivery system (Applied Chromatography Systems Ltd., Luton, England), a Rheodyne Model 7125 syringe-loading sample injector and a 25 cm × 4.6 mm i.d. Beckman Ultrasphere (5 μm) reversed-phase column. The molecular species were directly quantified using a Tracor 945 Flame Ionization LC Detector (Tracor Instruments, Austin, TX) operated as previously defined [15]. In analysis of MGDG, the HPLC solvent was MeOH–H_2O (24:1 delivered at a flow rate of 1 ml/min. PC molecular species were resolved using MeCN–MeOH–HOAc–H_2O-1-ethylpropylamine (898:68:15:10:9) at 1.1 ml/min [9, 15].

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