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CHANGES IN GLYCEROLIPIDS AND THEIR FATTY ACID COMPOSITION DURING MATURATION OF TOBACCO SEEDS

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco seeds; development; triacylglycerols; polar lipids; fatty acid composition.

Abstract—Triacylglycerol, which was one of the minor lipid components in immature seeds of tobacco, accumulated dramatically between 7 and 27 days after flowering and, in mature seeds at 37 days, the fatty acid methyl esters of the triacylglycerols comprised 96.3% of those of the total lipids. Diacylglycerols and sterol ester also increased significantly during seed development. Phosphatidylcholine and phosphatidylethanolamine, which were major components in immature seeds, decreased constantly with increasing maturation as well as the quantities of phosphatidylinositol and phosphatidylglycerol. Monogalactosyldiacylglycerols, digalactosyldiacylglycerols and sulfoquinovosyldiacylglycerols also decreased and disappeared in mature seeds. In the triacylglycerols the percentages of palmitate, stearate and linolenate fell with increasing seed age, while that of linoleate increased up to 75.3% in mature seeds. A similar trend was observed in the fatty acid composition in the diacylglycerols and sterol ester. Generally, in the phospholipids the proportions of linoleate and linolenate decreased with concomitant increases of stearate and oleate.

INTRODUCTION

Seed lipids of most plants are preponderantly rich in triacylglycerols (TG) with small quantities of other glycerolipids such as phospholipids and glycolipids [1]. On the other hand, the fatty acids of TG or total lipids vary considerably among plant species. Linoleate is predominant in corn [2, 3], soybean [2] and safflower [4, 5], while oleate is in rape [6, 7], *Crambe* [6] and peanut [8]. Rape [6, 9] and *Crambe* [6, 10] are also rich in erucate, and flax [4] is in linolenate. Changes in glycerolipids and fatty acids in developing seeds have been extensively studied for these oil plants [3–7, 10], and the biosynthetic pathways of fatty acids and TG are being elucidated [5, 11–14]. Tobacco seed lipids are extremely rich in linoleate and also used as an edible oil in European countries [15, 16]. This paper deals with changes in individual glycerolipids and their fatty acid composition during maturation of tobacco seeds.

RESULTS AND DISCUSSION

Changes in dry wt and total lipid content of tobacco seeds during maturation are shown in Fig. 1. The dry wt per seed increased rapidly up to 27 days after flowering (DAF), followed by little change thereafter until maturity (37 DAF). The total lipid content on a dry wt basis of seeds began to increase dramatically at 7 DAF after a short lag, reaching a maximum at 27 DAF. A slight decrease was observed between 27 and 37 DAF.

Changes in individual glycerolipids and some lipid classes as their constituent fatty acid methyl esters (FAMES) are shown in Table 1. The major lipids at the early stage of seed development (5–7 DAF) were

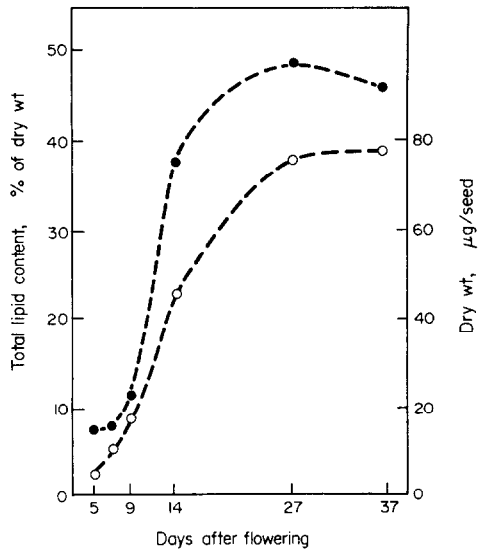

Fig. 1. Changes in dry wt and total lipid content of tobacco seeds during maturation. ○ = Dry wt; ● = total lipid content.

Table 1. Changes in individual glycerolipids and some lipid classes of tobacco seeds during maturation

Lipid	Fatty acid methyl esters (mg/g dry seeds) Days after flowering					
	5	7	9	14	27	37
TG	2.46	1.57	26.00	200	356	339
DG	2.15	1.70	1.82	5.49	9.78	5.51
MGDG	1.80	1.14	0.30	0.13	—	—
DGDG	2.04	1.21	0.88	0.31	—	—
SQDG	0.43	0.32	0.34	0.29	—	—
PG	2.53	1.62	1.31	0.97	0.57	0.41
PC	15.10	9.53	7.57	2.93	1.73	1.89
PE	7.69	5.28	3.57	1.75	0.57	0.54
PI	2.27	1.66	2.41	3.02	0.97	0.84
SE	1.12	1.39	1.40	3.16	3.33	2.92
FFA	2.31	1.44	0.83	1.38	2.18	0.85
Total	39.9	26.9	46.4	219	375	352

Table 2. Changes in the fatty acid composition of individual glycerolipids and some lipid classes of tobacco seeds during maturation

Lipid	Days after flowering	Fatty acid composition (% total)				
		16:0	18:0	18:1	18:2	18:3
Total lipids	5	23.3	1.6	4.3	52.6	18.2
	7	23.2	1.7	5.7	49.1	20.3
	9	16.0	2.2	9.2	60.3	12.3
	14	12.6	2.6	11.8	70.5	2.5
	27	9.7	2.9	12.4	73.6	1.4
	37	9.6	2.8	12.1	74.1	1.4
TG	5	34.8	9.3	10.2	35.1	10.6
	7	48.6	6.4	4.6	30.3	10.1
	9	20.3	2.9	10.7	62.9	3.2
	14	11.1	3.6	12.3	71.9	1.1
	27	9.3	2.8	12.0	74.8	1.1
	37	9.2	2.6	11.8	75.3	1.1
DG	5	51.6	12.5	14.0	17.4	4.5
	14	15.3	5.9	16.2	60.8	1.8
	37	15.5	5.7	16.6	61.7	0.5
MGDG	5	9.8	2.2	4.0	14.2	69.8
	14	37.5	12.5	10.9	14.9	24.2
DGDG	5	19.3	3.1	2.9	19.9	54.8
	14	27.7	11.6	8.7	15.5	36.5
SQDG	5	39.1	7.5	10.3	17.5	25.6
	14	50.5	12.9	9.8	18.1	8.7
PG	5	40.2	3.5	5.8	37.8	12.7
	37	51.8	13.9	15.8	16.1	2.4
PC	5	23.1	1.9	1.5	59.9	13.6
	37	18.4	5.8	18.0	56.8	1.0
PE	5	23.2	1.6	2.6	63.5	9.1
	37	32.4	7.8	13.9	45.0	0.9
PI	5	21.4	2.3	4.9	59.0	12.4
	37	38.1	10.1	12.6	37.1	2.1
SE	5	50.7	17.9	19.6	9.6	2.2
	37	16.8	6.1	10.0	65.3	1.8
FFA	5	45.2	14.4	14.6	19.1	6.7
	37	38.3	14.0	14.4	32.2	1.1

phosphatidylcholine (PC) and phosphatidylethanolamine (PE). These phospholipids as well as phosphatidylglycerol (PG) decreased constantly until 37 DAF. Phosphatidylinositol (PI) and free fatty acid (FFA) fluctuated significantly during seed development but remained in small quantities at 37 DAF. Considerable amounts were observed at the early stage in glycolipids such as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG). These glycolipids also decreased during development and disappeared almost completely in mature seeds. TG was one of the minor lipids in very immature seeds and the proportion of TG–FAMES was only 6.2% of total lipid-FAMES at 5 DAF. A dramatic accumulation of TG was observed between 7 and 27 DAF. This corresponds with the change in total lipid content. TG was by far the largest component by 14 DAF and, at maturity, TG-FAMES comprised 33.9% of seed dry wt and 96.3% of the total lipid-FAMES. Diacylglycerol (DG) and sterol ester (SE) also increased significantly between 9 and 27 DAF, though the quantities were small compared with TG.

The rapid accumulation of TG during seed development is generally observed in many oil plants, i.e. flax [4], safflower [4, 5], *Crambe* [6, 10], rape [6] and corn [3]. Also, the relatively large amount of phospholipids and glycolipids in very immature seeds, and the decreasing trend of these lipids during maturation have been reported for these plants [3, 4, 6]. These phenomena were ascertained in detail for tobacco seeds in the present experiments.

Changes in the fatty acid composition of lipids are shown in Table 2. There were large changes in the fatty acid composition of TG between 5 and 14 DAF. The percentages of palmitate, stearate and linolenate fell and that of linoleate increased. After 14 days the composition remained nearly constant and in mature seeds at 37 DAF the proportion of linoleate was 75.3% of the total fatty acids of TG. The fatty acid compositions of DG and SE showed similar patterns of change during seed development as seen in that of TG. In immature seeds at 5 DAF, all phospholipids had higher percentages of linoleate than those in TG. The percentages of linoleate of PG, PE and PI dropped in mature seeds with concomitant rises in those of palmitate, stearate and oleate. The fatty acid composition of PC change somewhat differently, where the proportions of palmitate, linoleate and linolenate decreased with concomitant increases of those of stearate and oleate as seeds matured. In MGDG, DGDG and SQDG, the general trend observed was that the percentages of palmitate and stearate increased until 14 DAF with a concomitant decrease in linolenate.

Since TG was by far the main component by 14 DAF, the fatty acid composition of total lipids was almost the same as that of TG thereafter. At the early stage of seed development, however, the distribution pattern of total lipid fatty acids differed considerably from that of TG fatty acids, and must be the reflection of fatty acid distributions of all lipid classes. The pattern of changes in the fatty acid composition of TG or total lipids during seed development for tobacco was found to be quite similar to that for safflower, where a rapid accumulation of linoleate also occurred [4, 5]. Thus, seed oil of tobacco resembles in its fatty acid composition that of safflower, which is one of the most important oil plants. On the other hand, distribution patterns of fatty acids in phospholipids and glycolipids during seed development differs considerably between tobacco (Table 2) and safflower [4, 5]. These lipids are minor components in mature seeds, therefore, may contribute little to the distribution pattern of fatty acids in total lipids.

Using developing cotyledons of linseed, soya bean and safflower, Slack *et al.* [14] suggested that oleate, initially esterified to PC, was first desaturated, and then polyunsaturated fatty acids transferred to TG via DG. The aim of the present work has been to reveal changes in glycerolipids and their fatty acid composition in developing seeds of tobacco, because no information is available on this subject.

EXPERIMENTAL

Materials. Tobacco plants (*Nicotiana tabacum* L. cv. Bright Yellow) were grown in a greenhouse at 28°. The developing seeds were obtained at desired stages of development and freeze-dried.

Lipid extraction. Seed samples (100 mg dry wt) were homogenized ×3 with $CHCl_3$–MeOH (1:1), and the combined homogenates filtered. The filtrate was washed with H_2O, the $CHCl_3$ layer evaporated and dried as described previously [17]. The total lipids obtained were weighed and dissolved in a small vol. of $CHCl_3$–MeOH (9:1).

Lipid separation. The total lipid soln was applied to Si gel TLC. Neutral lipids were separated by developing first with C_6H_6–Et_2O (49:1) and then by re-developing with C_6H_6–Et_2O–HOAc (50:50:1) [18]. Polar lipids were separated by developing with $CHCl_3$–MeOH–H_2O (65:25:4) in the first direction and with $CHCl_3$–MeOH–*iso*-propylamine–NH_4OH (13:7:0.1:1) in the second direction [17, 19]. Lipid spots were located under UV after spraying with Rhodamine 6G soln. Each lipid was identified by co-chromatography with known standards and by spraying with specific sprays for the various lipid classes [17, 18].

Lipid analysis. Individual lipids separated were quantitatively estimated as FAMES [17, 19] prepared by methanolysis with 5% H_2SO_4 in MeOH, and analysed by FID–GC using glass columns packed with 5% DEGS on Gaschrom Q. The column temp. was maintained at 200° with a He flow rate of 50 ml/min.

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