BIOCHEMICAL ASPECTS OF OIL DEPOSITION IN DEVELOPING RAPESEED

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INTRODUCTION

Several reports have appeared on the lipid and compositional changes occurring in developing rapeseed (summer and winter varieties) and related species (Fowler and Downey, 1970; Gurr, Blades and Appleby,1972; Norton and Harris, 1975). A feature common to all these reports is that oil deposition and dry matter accumulation (when measured) follows a sigmoid pattern. Further, the onset of storage reserve deposition appears to be controlled by the development stage of the embryo(Norton and Harris, in preparation). Shortly after anthesis (14 d) the embryo accounts for less than 2 % of the total seed volume whereas the latter has already attained 50 % of its final volume. At this time, the embryo (early torpedo stage) is in a rapid phase of cell division and over a period of less than 14 d the full cell complement is achieved. No cell enlargement occurs during this period and no storage reserves are deposited. After the completion of cell division, cell enlargement (hence embryo volume) commences. Cell enlargement proceeds with the accumulation of storage reserves in the form of oil and protein almost until maturity.

LIPIDS IN DEVELOPING RAPESEED

During the period of rapid cell division the lipid content of the seed was low but as cell enlargement proceeded, the total lipids increased proportionately. Very little of the lipid present in seeds prior to cell enlargement was triglyceride. Approximately 80 % of the total lipid was phospho- and glycolipid. This fact was reflected in the gross fatty acid composition of the lipid which was high in $C_{16:0}$, $C_{18:2}$ and $C_{18:3}$ (Norton and Harris, 1975). Fatty acids predominant in the storage triglycerides of the mature seed $C_{22:1}$, $C_{20:1}$ and $C_{18:1}$ were either completely absent or present in relatively small quantities. Detailed analyses of the individual phospho- and glycolipids will be presented elsewhere (Norton and Harris, in preparation). The onset of rapid lipid formation, largely triglyceride, coincided with the cell expansion phase. This phase of development was manifested by dramatic changes in the fatty acid composition of the lipids. ($C_{22:1}$ is the major fatty acid of the storage triglyceride in the mature seed of the rapeseed variety (Panter) employed in the investigations in this laboratory and this acid has been used as a marker for the onset and progress of oil deposition throughout development). With time, an increasing proportion of the rapeseed lipids was triglyceride and at maturity this accounted for over 90% of the total seed lipid whereas phospho- and glycolipid only accounted for 7%. The fatty acid composition of the lipid reflected this functional change in the seed over this period (Table 1).

TRIGLYCERIDES IN DEVELOPING RAPESEED

In the period from three weeks after anthesis to maturity the quantities of triglycerides in the seed increased almost 700 fold (1.5×10^{-3}-1.06 μmoles). Much of this increase occurred over a relatively short period of time (weeks 5 - 8 after anthesis). Seven molecular species of triglyceride were found to be present in the mature seed but the contribution of individual species to the neutral lipid fraction varied considerably with time (Table 2). The fatty acid composition of the individual

triglyceride species changed substantially throughout development. These changes occurred even in the three major triglycerides (species 4, 6 and 8 having 3, 4 and 5 double bonds respectively) but were more marked in the minor species (1, 3, 5 and 7 having 2, 2, 3 and 4 double bonds respectively) particularly in the early stages of oil deposition. Such compositional changes pose considerable problems in the interpretation of the analytical data. Indeed, it may be argued that individual triglycerides at weeks 3 or 10 cannot be equated on compositional grounds alone. For the purpose of this report, it will be assumed that the individual triglycerides from seeds of different stages of development having the same mobility on TLC plates, are the same molecular species. Detailed fatty acid compositional data of the individual triglycerides will be presented elsewhere (Norton and Harris, in preparation).

The seven triglyceride species persist in different amounts throughout development. Species 4, 6 and 8 which accounted for almost 80% of the total triglycerides in the mature seed predominate throughout development apart from the earliest stages. The other species (3, 5 and 7) which made up 20% of the total triglycerides in the mature seed were either entirely absent or present in trace amounts in seeds at week three. In the earliest sampling, species 1 accounted for 30% of the total triglyceride (relatively small amounts in absolute terms) and the remainder was made up of species 4, 6 and 8. With time, the contribution of species 1 to the seed triglycerides declined until at maturity it only accounted for 1% of the total. In terms of absolute amounts there were condisderable increases in species 3, 4, 5, 6, 7 and 8 during the rapid phase of oil deposition. These increases were accompanied by the fatty acid compositional changes mentioned above but even at the earliest sampling date the six major triglyceride species were easily identifiable. One of the most prominent fatty acid compositional changes occurring with time in the triglycerides was an increase in the proportion of $C_{22:1}$ and to a lesser extent $C_{20:1}$ at the expence of $C_{18:1}$. From week 6 onwards only minor compositional changes were observed.

Although stereospecific analyses of the triglycerides were not performed in this work, the compositional data for five of the triglycerides from mature rapeseed were very similar to that obtained by Gurr, Blades and Appleby (1972) for the triglycerides of Crambé abyssinica. These workers isolated five major molecular species of triglycerides possessing 2, 3, 3, 4 and 5 double bonds respectively from the mature seed of Crambé. Stereospecific analyses of the triglycerides revealed that position 2 was always occupied by C_{18} fatty acids with Δ 9 unsaturation ($C_{18:1}$, $C_{18:2}$ and $C_{18:3}$). No $C_{22:1}$ or $C_{20:1}$ was found in position 2. Position 1 was occupied entirely by saturated fatty acids or $C_{22:1}$ while position 3 was occupied by $C_{22:1}$ (or $C_{20:1}$). Based on this work the structures of the triglycerides in mature rapeseed have been tentatively proposed (Table 2).

The presence of a major triglyceride in rapeseed oil (species 8) with five double bonds possessing a high content of $C_{18:3}$ is important since this is the only triglyceride containing this technologically undesirable fatty acid. The high content of $C_{18:3}$ in some rapeseed oils cannot be ascribed to the presence of chloroplasts in the cells of the cotyledons since at maturity the functional lipids only contained a small proportion of the total linolenic acid in the seed. Further the pattern of accumulation of triglyceride containing $C_{18:3}$ is not consistent with the involvement of functional chloroplasts as has been suggested by Thies (1971). Although this particular triglyceride is present throughout development its synthesis is more rapid in the later stages of the oil deposition phase.

At this stage of development it is questionable whether chloroplasts in the cotyledonary cells are functional at all. The biosynthesis of this triglyceride and its constituent fatty acids is of considerable importance and interest since if this can be elucidated (mechanism and site) a more effective means of reducing the level of $C_{18:3}$ in rapeseed oil may be devised. Studies on the biosynthesis of individual fatty acids and triglycerides in rapeseed are in progress in this laboratory.

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TABLE 1

LIPIDS OF DEVELOPING RAPESEED

Week number‡‡	Total lipid per seed (mg) *	Percentage distribution of lipid classes+					
		NL	PC	PE	MGDG	DGDG	Acidic lipid
3	0.05	21.5	16.8	7.0	-	14.4	40.3
4	0.09	60.6	5.8	0.6	2.7	4.9	25.4
5	0.25	76.9	7.5	0.1	2.6	5.3	7.5
6	1.06	88.9	3.3	1.0	1.2	2.0	3.6
10	2.42	92.9	0.8	0.6	0.7	1.3	3.7

* Lipid material soluble in $CHCL_3/CH_3OH$ (2:1, v/v).

+ Lipids fractionated by chromatography on DEAE cellulose (Nichols and James, 1964). Individual lipids assayed by the hydroxamate procedure (Gurr, Blades and Appleby, 1972).

‡‡ Weeks after anthesis. Full details of compositional changes in developing rapeseed have been described previously (Norton and Harris, 1975).

TABLE 2

MOLECULAR SPECIES OF TRIGLYCERIDES IN DEVELOPING RAPESEED

Species*	Relative amount mol/100mol			Number of double bonds	Molar proportion;+ S,M,D and T/mol triglyceride	Possible triglyceride structure ‡		
	Weeks					Positions		
	3	5	10			1	2	3
1	30	4	1	2	S,M,M	16:0 18:0	18:1	18:1 20:1 22:1
3	Trace	12	5	2	S,M,M	16:0 18:0 20:0	18:1	22:1
4	22	22	22	3	M,M,M	22:1	18:1	22:1
5	0	9	10	3	M,M,D/S	16:0 /18:2	18:1	22:1
6	25	33	30	4	M,M,D	22:1	18:2	22:1 /20:1
7	0	8	7	4	M,M,D	22:1	18:2	22:1
8	13	12	27	5	M,M,T	22:1	18:3	22:1

* Separation of triglycerides on thin layer pates of 10% $AgNO_3$ - silica gel HF developed in 0.5% ethanol in $CHCl_3$. The numbering of these species is according to the mobility on the plate.

\+ Triglycerides found in the mature seed. Fatty acid composition of similar species from seeds of different developmental stages are different: S = saturated, M = monoene, D = diene, T = triene.

‡ Stereospecific analyses have not been performed. Structures based on the analyses of the molecular species of triglycerides in Crambé (Gurr, Blades and Appleby, 1972).