FATTY ACID AND TRIACYLGLYCEROL BIOSYNTHESIS IN MICROSPORE EMBRYOS OF *BRASSICA NAPUS*

M.K. Pomeroy (1), S.A. Sparace (2), W.A. Keller (3)

(1) Plant Research Centre, Agriculture Canada, Ottawa, CANADA K1A 0C6
(2) Plant Science Dept., Macdonald College, Ste. Anne de Bellevue, P.Q. CANADA H9X 1C0
(3) Plant Biotechnology Inst., NRC Canada, Saskatoon, SK CANADA S7N 0W9

INTRODUCTION

Oilseed *Brassica* species are major sources of edible oils in many parts of the world, and they are also viewed as an important potential source of industrial oils in the production of such products as high temperature lubricants, drying oils, nylons and plasticizers. However, oils with very specific fatty acid compositions are required for many of these purposes. Traditional plant breeding has brought about major changes in the fatty acid profiles of rapeseed *Brassicas* (Downey et al. 1985; Rakow 1973; Stefansson 1983), but it would be of great benefit to the oilseed industry if technologies were developed to facilitate rapid modification of fatty acid compositions. Recent advances in microspore culture technology (Chuong and Beversdorf 1985; Keller et al. 1987) suggested that microspore-derived embryos of *Brassicas* provide a unique system to investigate and eventually to manipulate oil biosynthesis. The microspore embryo system has recently been utilized to examine the synthesis and metabolism of erucic acid moieties in *B. napus* cv Reston (Taylor et al. 1990). This paper describes certain aspects of lipid biosynthesis in microspore embryos, and compares the fatty acid compositions of developing zygotic and microspore embryos of *B. napus*.

MATERIALS AND METHODS

Seeds of *B. napus* L cvs Topas and Reston were sown in soil and seedlings grown for 6 weeks at 20°C day/15°C night followed by 10°C day/5°C night for 4 to 6 weeks (Huang et al. 1990). Microspores were isolated from flower buds 2-4 mm in length and embryos cultured as described by Huang et al. (1990). Zygotic embryos at various stages of development were dissected from maturing ovules using a microscope. Lipids were extracted either by the hexane/isopropanol method of Hara and Radin (1978) or the one-step extraction methylation procedure of Browse et al. (1986). Lipid classes were separated by thin layer chromatography of total lipid extracts using Kieselgel HPTLC plates. Fatty acid methyl esters were obtained by methylation of lipid extracts in acidic methanol at 90°C for 1 hour, and analyzed on a Varian 3400 GC equipped with a 30 m DB-Wax megabore column with a column temperature of 200°C and a carrier gas (He) flow rate of 12 ml/min.

RESULTS AND DISCUSSION

Microspores of *B. napus* cvs Reston and Topas initiated cell divisions within 3 to 4 days when placed in culture at 32.5°C, and over a period of 3 weeks, all stages of embryo development that occur during normal zygotic embryo development were observed in microspore embryos cultured in light and dark (Fig. 1). However, microspore embryos were generally larger than zygotic embryos at comparable stages of development. This observation was confirmed when FW (fresh weight) per embryo was compared for microspore and zygotic embryos (Table 1). Total fatty acids per embryo and per mg FW increased during embryo growth and development,

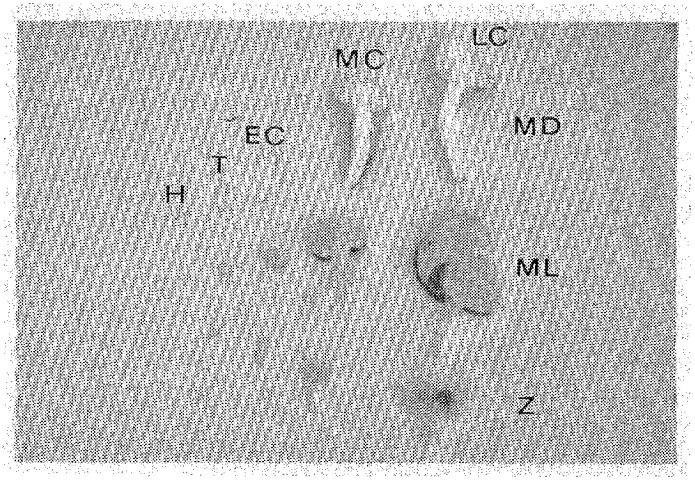

Fig. 1. Microspore embryos cultured in dark (MD) and light (ML) and zygotic (Z) embryos of B. napus cv Topas at various stages of development. H, heart; T, torpedo; EC early cytoledon; MC, mid cotyledon; LC, late cotyledon.

Table 1. Total fatty acids (FA) in zygotic and microspore embryos of B. napus cv Reston at various stages of development[1].

Developmental Stage	DPA[2] or Days in culture	FW per embryo (mg)	FA per embryo (µg)	FA per mg FW (µg)
Heart				
Zygotic	9	0.004	0.24	5.9
Microspore	8	0.012	1.13	5.5
Torpedo				
Zygotic	12	0.02	1.6	7.6
Microspore	10	0.05	3.0	6.0
Early Cytoledon				
Zygotic	16	0.06	6.1	7.9
Microspore	12	0.21	17.0	8.4
Late Cotyledon				
Zygotic	32	5.5	1490	28.5
Microspore	35	25.0	2590	13.5
Seed	-	5.0	1500	31.5

(1) Fatty acids were determined by the method of Browse et al. (1986)
(2) Days post anthesis

and the pattern of change was generally similar for microspore and zygotic embryos. The maximum level of fatty acids attained in late stage cotyledonary zygotic embryos was similar to that observed in seeds, whereas the level in microspore embryos at the same developmental stage

was significantly lower. This difference can be attributed, in part, to the higher moisture content of microspore embryos.

Triacylglycerols (TAGs) accumulated rapidly during development of microspore embryos (data not shown). They comprised approximately 15% of total lipids in freshly isolated microspores, and the level increased only slightly during the first week of culture. However, TAGs increased rapidly the next two weeks and by day 21, they represented about 80% of total lipid. The level of TAGs increased gradually during the final two weeks of culture, eventually comprising more than 90% of total lipids in late cotyledonary stage embryos.

The fatty acid compositions of total lipids from developing microspore and zygotic embryos of Reston are presented in Table 2. In freshly isolated microspores, the predominant fatty acid is 18:3, comprising nearly 50% of total fatty acids. The proportion of 18:3 and 16:0 declined consistently while 18:1 increased in both microspore and zygotic embryos during development. In the early stages of development, 22:1 was not

Table 2. Fatty acid composition of total lipids from zygotic and microspore embryos of B. napus cv Reston at different stages of development[1]

Developmental Stage	Fatty acid composition (wt %)						
	16:0	18:0	18:1	18:2	18:3	20:1	22:1
Microspores	15.3	3.9	12.5	15.8	47.6	0.9	0
Heart							
Zygotic	16.7	9.7	18.3	31.3	18.0	0	0
Microspore	7.8	7.3	33.4	31.6	14.4	0	0
Early Cotyledon							
Zygotic	9.7	4.8	25.3	34.2	17.3	1.8	1.2
Microspore	7.3	4.8	31.9	31.9	15.3	2.6	1.8
Late Cotyledon							
Zygotic	5.6	1.2	27.0	17.8	9.1	12.2	26.4
Microspore	4.8	1.3	33.3	15.6	10.4	11.8	21.8
Seed	3.3	1.0	23.7	17.2	8.5	11.2	32.7

(1) Fatty acids were determined by the method of Browse et al. (1986)

detected, but with the appearance of cotyledonary structures it started to accumulate and by late cotyledonary stage, represented more than 20% of total fatty acids. However, the proportion of 22:1 never attained levels in either zygotic or microspore embryos that were observed in seeds, and the level in microspore embryos was significantly less than observed in zygotic embryos. In contrast with the results obtained with Reston, the fatty acid compositions of late cotyledonary zygotic and microspore embryos and of seed of Topas (a zero 22:1 cultivar) were nearly identical (data not shown).

The proportion of 22:1 in cotyledonary stage microspore embryos of Reston is frequently quite variable and appears to be related to embryo development. Generally, 22:1 increases rapidly during development of cotyledonary-like structures in these embryos, but in some experiments, unusually low levels of 22:1 were observed. This phenomenon has been investigated further by re-plating embryos in fresh media at various stages of development to reduce embryo density and thereby enhancing

development. The results shown in Table 3 indicate that embryo development is severely inhibited at high plating densities and that 22:1 does not accumulate significantly in these cultures, even after 28 days. However, when embryos were re-plated to markedly reduce embryo density, the average FW of embryos and the level of 22:1 increased dramatically. When embryos were re-plated in fresh media without reducing culture density, embryo development and 22:1 content were not significantly different than observed in controls (Table 3). These observations demonstrate that the accumulation of 22:1 in microspore embryos of Reston is extremely sensitive to culture density, and that care must be exercised in selecting culture conditions to obtain microspore embryos with fatty acid profiles similar to those of zygotic embryos.

Table 3. Effect of culture density on embryo development and accumulation of erucic acid (22:1) in microspore embryos of B. napus cv Reston

Treatment	Embryo Density[1]	Days in Culture	FW (mg/embryo)	22:1 (%)
Standard plating density	3000	14	0.3	1.9
Standard plating density	3000	21	0.6	1.2
Standard plating density	3000	28	1.2	2.4
Re-plated day 14	360	21	5.9	11.9
Re-plated day 14, 21	15	28	54.0	14.5
Re-plated day 14, 21, 28	5	35	135.5	32.8
Fresh Media day 14	3000	28	1.4	2.1

(1) Number of cotyledonary stage embryos in 35ml of culture medium per 125x25mm Petri dish.

Microspore embryos of B. napus are currently being utilized in our laboratories to investigate lipid biosynthesis in this oilseed crop. Topas embryos at various stages of development, incubated with Na[14C] acetate for 3 hours at 24°C, readily incorporated label into the various classes of complex lipids (Data not shown). In 7 day embryos TAGs comprised approximately 30% of the total lipid mass and contained 30% of the radioactivity, while in 14 day embryos TAGs had increased to 75% and contained 50% of the label. Most of the remaining label was found in other neutral lipids, with only small amounts detected in the various polar lipid classes. Fatty acid analysis of various lipid classes revealed that nearly all of the label in phospholipids of both 7 and 14 day embryos was recovered in 16:0, 18:0 and 18:1, even though substantial mass of 18:2 and 18:3 was detected. In contrast, large amounts of label was found in very long chain saturated fatty acids (C-20, 22 & 24) of the neutral lipids, although significant mass of these fatty acids was observed only for monoacylglycerol (MAG). The presence of large proportions of long chain fatty acids in MAG and substantial amounts of label in the long chain fatty acids of all neutral lipid classes was unexpected since Topas is a low erucic acid cultivar. These observations suggest that two relatively distinct pathways of lipid biosynthesis may be operational in Brassica microspore embryos. One pathway is involved primarily in the biosynthesis of TAG with selectivity for very long chain fatty acids. A second pathway may be involved in synthesis of phospholipids with selectivity for the more typical C-16 & 18 fatty acids. Since we observe only slight accumulation of very long chain fatty acids, it appears that the latter pathway predominates in Topas microspore embryos.

SUMMARY

The results obtained in this investigation have shown that the patterns of fatty acid biosynthesis and triacylglycerol accumulation are generally similar for microspore-derived and zygotic embryos of B. napus. However, the synthesis and accumulation of erucic acid in Reston microspore embryos is extremely sensitive to embryo density and maximum levels of 22:1 can be attained only by re-plating and drastically reducing the number of embryos per plate. Microspore embryos readily incorporate radioactive acetate into all the major classes of complex lipids. The results obtained in this aspect of our studies suggest that the Brassicas may have a relatively distinct pattern of lipid metabolism.

REFERENCES

BROWSE, J., McCOURT, P.J. and SOMMERVILLE, C.R. 1986. Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Anal. Biochem. 152: 141-145.

CHUONG, P.V. and BEVERSDORF, W.D. 1985. High frequency embryogenesis through isolated microspore culture in Brassica napus L. and B. carinata Braun. Plant Sci. 39: 219-226.

DOWNEY, P.K., KELLER, W.A. and BEVERSDORF, W.D. 1985. Genetic manipulation in oil crops. In: Proceedings of the World Conference on Emerging Technologies in the Fats and Oils Industry. A.R. Baldwin (ed.). American Oil Chemists' Society, Champaign, IL. pp. 331-336.

HARA, A. and RADIN, N.S. 1978. Lipid extraction of tissues with a low toxicity solvent. Anal. Biochem. 90: 420-426.

HUANG, B., BIRD, S., KEMBLE, R., SIMMONDS, D., KELLER, W. and MIKI, B. 1990. Effects of culture density, conditioned medium and feeder cultures on microspore embryogenesis in Brassica napus L. cv. Topas. Plant Cell Reports, 8: 594-597.

KELLER, W.A., ARNISON, P.G. and CARDY, B.J. 1987. Haploids from gametophytic cells - Recent developments and future prospects. In: Plant Tissue and Cell Culture. C.E. Green, D.A. Somers, W.P. Hackett and D.D. Biesboer (eds.). A.R. Liss, Inc., New York. pp. 223-241.

RAKOW, G. 1973. Selektion auf Linol - und Linolensäureghalt in Rapssamen nach mutagener Behandlung. Z. Pflanzenzücjtg. 69: 62-82.

STEFANSSON, B.R. 1983. The development of improved rapeseed cultivars. In: High and Low Erucic Acid Rapeseed Oil. Production, Utilization, Chemistry and Toxicological Evaluation. J.K.G. Kramer, F.D. Sauer and W.J. Pigden (eds.). Academic Press. New York. pp. 143-159.

TAYLOR, D.C., WEBER, N., UNDERHILL, E.W., POMEROY, M.K., KELLER, W.A., SCOWCROFT, W.R., WILEN, R.W., MOLONEY, M.M. and HOLBROOK, L.A. 1990. Storage protein regulation and lipid accumulation of microspore embryos of Brassica napus L. Planta, 181: 18-26.