FORMATION OF FREE FATTY ACIDS IN SEED AND DEVELOPING EMBRYOS OF BRASSICA NAPUS

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ABSTRACT

Free fatty acid (FFA) content of seed from field-grown B. napus varied widely among several cultivars examined. The levels of FFA were markedly lower in developing zygotic and microspore-derived embryos than in mature seed, and cultivar differences seen in field-grown seed were not observed in developing embryos. Exogenously supplied ^{14}C-oleate labelled triacylglycerol (TAG) is readily degraded releasing FFA by crude cell-free homogenates of developing microspore embryos.

INTRODUCTION

Spring canola grown in Ontario, Canada frequently produces seed oil with levels of FFA higher than are acceptable to the oil refining industry (DeClercq and Daun, 1992; May et al. 1994). High levels of FFA in canola oil accelerate oil rancidity, reduce shelf-life of the oil, and alter its chemical properties. Furthermore, levels of FFA above 1% result in extra production costs for removing the FFA from the oil. We have initiated studies in an attempt to identify the as yet poorly understood biochemical mechanism(s) involved in the accumulation of FFA in canola seed.

EXPERIMENTAL

Microspore-derived and zygotic embryos of several cultivars of B. napus were obtained and total lipids extracted, as previously described (Pomeroy et al. 1991). FFA content of lipid extracts was determined by thin layer chromatography (TLC) of lipid extracts followed by methylation of the FFA band and GC analysis (Pomeroy et al. 1991). The capacity of microspore embryos to degrade complex lipids, thereby releasing FFA, was examined by incubating crude embryo homogenates or sub-cellular fractions

with oleate-labelled triacylglycerol (TAG). Radioactivity was quantified by liquid scintillation counting.

RESULTS AND DISCUSSION

The level of FFA in Ontario-grown spring canola varies appreciably among cultivars, and is modified by environmental stresses and various agronomic practices (May et al. 1994). In this preliminary investigation into the biochemical mechanism of FFA accumulation in canola seed, we have compared levels of FFA in seed and in developing zygotic and microspore-derived embryos of several B. napus cultivars (Table 1). FFA in seed from the field-grown plants varied from a high of 2.57% of total lipid (TL) in cv Triumph to a low of 0.57% TL in cv Kristina. The levels of FFA in late stage microspore and zygotic embryos were generally similar for all cultivars examined. These levels were comparable to those observed in cultivars with low seed FFA, but appreciably lower than in the high seed FFA cultivars. Even in the 37 DPF zygotic embryos, at a stage just prior to the onset of dehydration, there was no indication of increased FFA accumulation in the high seed FFA cultivars. These observations suggest that accumulation of FFA in canola seed does not occur at least until the final maturation stage of seed development.

Table 1. FFA content of seed from field-grown B. napus cultivars and from developing embryos of plants grown in growth chambers from seed of the field-grown plants.

	FFA Content (% TL)			
		28 day Microspore	Zygotic Embryos	
Cultivar	Seed	Embryos	20DPF[1]	30DPF
Triumph	2.57	0.59	0.44	0.40
Celebra	2.25	0.41	0.43	0.50
Global	1.37	0.33	0.52	-[2]
Legend	1.35	0.58	0.65	-
Stallion	1.28	0.46	0.58	-
Cyclone	0.68	0.49	0.47	-
WW1432	0.58	0.49	0.66	0.51
Kristina	0.57	0.55	0.51	0.39

[1] DPF - days post flowering [2] not determined

Exogenously supplied ^{14}C-oleate labelled TAG is readily degraded by crude cell-free homogenates of developing microspore embryos of B. napus cv Topas. Over the course of 24 hr, up to 200 pmoles of radioactive TAG per mg protein (representing about 10% of the offered TAG) is degraded and recovered primarily as FFA and DAG. The greatest total and specific activities for radiolabelled TAG degradation were consistently associated with supernatant fractions in comparison to their corresponding particulate fractions (Table 2). Overall, the 100,000g supernatant showed the greatest total and specific activities for TAG degradation, and all fractions gave FFA and DAG (up to 4% each in the 100,000g pellet and supernatant) as the main degradation products.

Table 2. [^{14}C] Triacylglycerol degradation by differential centrifugation-derived sub-cellular fractions of 28 day microspore-derived embryos of B. napus cv. Topas.[1]

	Crude Homogenate	500g Pellet	500g Super.	10,000g Pellet	10,000g Super.	100,000g Pellet	100,000g Super.
			Total Activity (nmoles TAG Degraded)[2]				
	9.6	0.6	12.2	0.1	8.5	0.2	23.1
			Specific Activity (pmoles/mg protein)[2]				
	61.4	29.0	151.9	15.8	189.1	26.0	754.2
Lipid Product			Distribution of Radioactivity (%)				
TAG	90.0	97.1	94.6	94.1	96.1	89.9	89.9
FFA	1.1	0.6	1.4	2.0	1.1	3.9	3.9
DAG	1.6	1.1	2.7	2.0	1.1	4.3	4.5
SE	0.9	0.9	0.9	1.5	1.2	1.3	0.9
Origin	0.4	0.3	0.4	0.4	0.5	0.6	0.8

[1] Assays were run for 3h in 0.5 mL of reaction mixture containing 50mM Bis Tris Propane (pH 7.5), 5mM DTT, 0.25% (w/v) gum arabic, approximately 107,000 dpm of 1,2,3[1-^{14}C-oleate] trioleoylglycerol, and 100μl of homogenates or sub-cellular fractions.

[2] Total and specific activities are based on the yield from 5g FW of embryos.

The amount of lipid degradation observed here appears to be sufficient to contribute to the problem of FFA accumulation in canola. Further work is necessary to determine the nature of the enzymes involved in this lipid degradation and to elucidate the role of such lipid degradation in the physiology of developing embryos.

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