

## FATTY ACID SYNTHESIS IN MICROSPORE-DERIVED EMBRYOIDS OF RAPE-SEED (BRASSICA NAPUS)

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INTRODUCTION

Embryoids from microspores are well known to produce homozygous plants. In addition, this plant material offers a good chance for physiological studies, in particular on polyenoic fatty acid synthesis, since embryoids are available for observation and experimental manipulation even at very early stages of development. Therefore, we are interested in developing an in vitro-system to cultivate gametic embryoids similar to zygotic embryos in situ. For such purpose conditions are to be arranged to avoid precocious germination and to initiate the lipid storage process.

MATERIAL AND METHODS

Plants from the spring rape varieties "Duplo" (00-type) and "Janetzki's Sommerraps" with 40% erucic acid in their seed oil, were grown in the greenhouse. Plant cultivation and isolation of microspores was performed according the method of Mathias (1988). After two weeks embryoids were transferred from the induction medium, a modified Lichter medium, to either Lichter medium with 4%, 8% or 12% sorbitol, or Lichter medium with 0.1  $\mu\text{M}$ , 1.0  $\mu\text{M}$  or 10.0  $\mu\text{M}$  abscisic acid (ABA). Sorbitol and ABA treatment was assumed to prevent embryos from germination (Schopfer and Plachy 1984, Finkelstein and Crouch 1986). The development of the embryoids are classified morphologically throughout the cultivation. Fatty acid composition was determined by capillary gas chromatography. During the cultivation on ABA or sorbitol media embryos were shaken in light and at 18°C.

RESULTS

In the high concentrated sorbitol media embryoids show de-greening and a reduced growth. No precocious germination could be observed in any sample. From our experiments no effect of ABA on precocious germination or growth restriction can be reported. As demonstrated in Table 1, erucic acid (22:1) was produced on ABA and sorbitol medium in the embryoids of "Janetzki's Sommerraps" marking a storage process. The highest levels were reached on 4% sorbitol and 1.0  $\mu\text{M}$  ABA. The main fatty acid of "Duplo", oleic acid (18:1), does not show a similar reaction to osmoticum or hormone application, but it's amount reaches in vitro the same level as in the seed (65%), while 22:1 contents in "Janetzki's Sommerraps" embryoids are somewhat lower than in seed. No relation was found of linoleic acid (18:2) content to the treatment, but linolenic acid (18:3) increases when low amounts of 18:1 occur.

**Table 1. Fatty acid composition of gametic embryoids on sorbitol and ABA containing media**

Cultivar	Sorbitol %	Fatty acid %				ABA $\mu$ M	Fatty acid %			
		18:1	18:2	18:3	22:1		18:1	18:2	18:3	22:1
Duplo	0	65.4	15.6	6.6	-	0	64.4	15.7	7.1	-
	4	64.8	12.5	6.6	-	0.1	59.3	17.6	10.5	-
	8	57.5	14.8	11.5	-	1.0	56.8	19.3	12.4	-
	12	46.5	17.6	11.6	-	10.0	59.6	17.2	12.5	-
Janetzki	0	35.2	14.3	7.7	19.3	0	34.8	17.4	8.8	15.9
	4	31.4	17.5	12.4	28.6	0.1	27.1	13.6	10.5	28.7
	8	33.7	11.6	9.0	23.4	1.0	26.6	11.6	11.9	30.0
	12	36.7	16.5	9.2	8.8	10.0	36.0	14.5	7.2	19.0

DISCUSSION

Microspore derived embryoids of rapeseed are able to produce erucic acid, the marker of a storage process. Similar findings have already been reported by Taylor et al. (1990). During in situ embryogenesis, zygotic embryos accumulate storage products and at older stages of development they lose water. Precocious germination of zygotic embryos cultured in vitro can be inhibited by application of osmotica as observed by Finkelstein and Crouch (1986). ABA probably does not induce an osmotic effect, but inhibits water uptake by influence on the cell wall (Schopfer and Plachy 1985). Finkelstein and Somerville (1989) observed an increase of erucic acid in ABA- and sorbitol-treated zygotic embryos in vitro and they supposed a direct effect on gene expression. The present paper shows a comparable increase of erucic acid also in gametic embryoids. Similarly, Wilen et al. (1990) had observed a positive effect on the expression of storage protein genes after ABA and sorbitol treatment. Obviously water stress, mediated by ABA or sorbitol and simulating the natural process of seed ripening, may enhance the storage process.

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