FATTY ACID COMPOSITION OF THE TRIACYLGLYCEROL FRACTION IN DEVELOPING BRASSICA RAPA SEEDS.

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ABSTRACT

The fatty acid composition of triacylglycerols of developing seeds was measured in the turnip rape (Brassica rapa) low erucic acid (LEAR) cultivar 'HORIZON' and high erucic acid (HEAR) cultivar 'R500'. Seed tissue was sampled every 3 days starting the fifthteenth day after pollination (DAP) until seed maturity. The seed development of both lines can be divided into different stages based on fatty acid composition of the triacylglycerol fraction. Other researchers have shown that little fatty acid accumulation occurs during the first 15 DAP. A drastic change in fatty acid composition occurs during the second stage, which lasted from 15 to 36 DAP. The percentage of oleic acid in the LEAR line increased rapidly during this period. In the HEAR line, the proportion of oleic acid changed at the same rate as in the LEAR line, until eicosenoic and erucic acid appeared at 24 DAP; subsequently the proportions of both fatty acids increased rapidly. During the last stage which lasted from 36 DAP to seed maturity very small changes occurred in the percentages of the major fatty acids in both lines. At maturity, the predominant fatty acids were erucic acid (52.0%) for the HEAR line and oleic acid (60.3%) for the LEAR line.

INTRODUCTION

Rapeseed (Brassica napus, B. rapa) is grown worldwide as a source of vegetable oil (Anonymous, 1989). Rapeseed can be grown either as an edible or an industrial oil crop, depending on the fatty acid composition of the mature seed (Appelqvist, 1972). The majority of the oil produced contains low levels of erucic acid (22:1) and is used for cooking oil, margarine or salad oil (Vles, 1974). Unlike some other major oilseed crops, rapeseed contains limited amounts of saturated fatty acids, a relatively high level of monounsaturated fatty acids, and an intermediate level of polyunsaturated fatty acids, giving the consumer of this oil a nutritional advantage (McDonald, B.E., et al, 1974).

In order to clarify the timing of storage oil synthesis, several studies of fatty acid changes in rapeseed during seed development have been made (Appelqvist, 1975; Fowler and Rakow, 1970; Rakow and McGregor, 1975). One study has followed changes in triacylglyceride composition during rapeseed development (Norton and Harris, 1983). Such studies have used the amphidiploid species B. napus; to date no experiments have been made using the diploid species B. rapa. This study presents information on the triacylglycerol fatty acid

composition of developing seeds from turnip rape (B. rapa) grown under controlled environmental conditions.

MATERIALS AND METHODS

In order to assure the fatty acid composition of the plant material twenty mature seeds of the B. rapa cultivars 'Horizon' and 'R500' had a portion of their cotyledons removed. The oil from the excised portion was extracted and transmethylated as previously described (Christie, 1982), prior to analysis for fatty acid composition. Methyl esters were separated on a 15m X 0.250 mm ID capillary column (50% cyanopropylphenyl) maintained isothermally at 190° C.

The remainder of the seed was planted in soil mix in 2 inch pots. At flowering, plants were transferred to growth chambers prior to pollination with the sister plants. Growth chamber temperature was 20° C for the 14 h light photoperiod and 15° C for the 10 h dark period. Approximately 50 developing seeds from each cultivar were used for the fatty acid composition analyses at each sample date. Samples were taken at 3 day intervals beginning at 15 DAP and stored at -80° C until ready for fatty acid analysis. Total lipids were extracted by homogenizing in 2.0 ml diethyl ether; individual lipid classes were separated by TLC on silica gel H, using hexane: diethylether: acetic acid, 70:30:1 (v/v/v). The neutral fraction (triacylglycerides) was methylated and the fatty acid composition determined as described above.

RESULTS & DISCUSSION

The objective of this study was to investigate storage lipid fatty acid composition in developing seed embryos of HEAR and LEAR turnip rape (B. rapa). Developing seeds were first visible within a week after pollination. Because of the small size of the embryo in these seeds, no samples were collected until 15 DAP, the first time point at which fatty acid composition could be measured. Changes in fatty acid composition occurred in similar stages as reported previously (Murphy and Cummings, 1989)(Tables 1 and 2).

On the 15th DAP, the LEAR cultivar 'Horizon' showed an oleic acid (18:1) proportion of 34.6%, which was almost equal to that of linoleic acid (18:2) (31.5%)(Table 1). Linolenic acid (18:3) and palmitic acid (16:0) were 15.5 and 12.0%, respectively, with only minor amounts of stearic acid (18:0). The fatty acid composition changed rapidly in the subsequent 14 days (Table 1). By 36 DAP, oleic acid reached proportions approaching those observed in the mature seed. Palmitic and stearic acid proportions declined after 15 DAP. These results are consistent with previous work in B. napus (Murphy and Cummings, 1989). The low ratio of linolenic acid observed in the early stage of development are lower than those reported in similar studies, where analyses were not confined to the neutral lipid fraction. Therefore, the reported results may include the contribution of other seed lipids to the linolenic

acid total (Appelqvist, 1975; Norton and Harris, 1975). Linoleic acid was constant from 21 DAP to maturity and linolenic acid content was unchanged from 18 DAP to maturity. The fatty acid composition of the mature seed is shown on Table 1.

Table 1. The triacylglycerol fatty acid composition of developing seed of B. rapa cultivar 'Horizon'.

	Fatty Acid Composition						
DAP[1]	16:0	18:0	18:1	18:2	18:3	20:1	22:1
	- - - - - - - - % weight - - - - - - - - -						
15	12.0	6.1	34.6	31.5	15.5	0.2	0.0
18	9.2	5.5	45.1	28.5	11.3	0.1	0.0
21	8.5	4.8	52.8	22.6	10.8	0.5	0.0
24	7.9	4.9	52.6	23.4	10.5	0.7	0.0
27	7.5	4.7	53.3	23.5	10.5	0.5	0.0
30	7.3	4.5	54.6	22.3	10.7	0.6	0.0
33	6.2	3.9	55.5	22.5	11.5	0.4	0.0
36	6.1	3.1	58.1	20.8	11.3	0.6	0.0
39	5.6	3.0	58.7	20.5	11.3	0.9	0.0
42	5.1	2.5	59.7	21.1	10.8	0.8	0.0
45	4.9	2.1	59.4	21.0	11.5	1.0	0.0
48	3.6	1.5	62.9	20.2	10.9	0.8	0.0
51	3.5	1.5	62.5	19.6	12.2	0.7	0.0
54	3.6	1.1	62.0	20.6	12.1	0.6	0.0
57[2]	3.8	0.9	60.3	22.0	12.0	1.0	0.0

[1] DAP = Days After Pollination.
[2] Mature seed.

In the HEAR line 'R500', the percentages of oleic acid during the period 15 to 27 DAP were very similar to those of the LEAR line (Table 2). Trace amounts of eicosenoic (20:1) and erucic (22:1) acids were first observed at the end of the 21st DAP. During the following three weeks, the percentage eicosenoic and erucic acid increased rapidly. At 42 DAP, the proportion of erucic acid was comparable to that found in the later stages of seed development. Palmitic acid proportions were high initially and decreased during seed development. Stearic acid percentages were low and decreased slightly during seed development. The proportion of linoleic acid decreased from 30.6% at 15 DAP until 42 DAP, when the ratio was equal to that found in mature rapeseed (Table 2). The percentage of oleic acid declined from 24 DAP to 42 DAP, while those of eicosenoic and erucic acid increased. The pattern observed is consistent with the stepwise elongation of newly synthesized oleic acid to eicosenoic and then erucic acid, as was demonstrated by in vitro fatty acid biosynthesis studies with labelled ^{14}C acetate, and radioactive labeling of immature pods (Stumpf, 1980).

Although the experiment reported here did not monitor the

accumulation of fatty acids during the initial 15 DAP, the similarities of the data between this study and the findings in B. napus by other researchers suggest that fatty acid biosynthesis in B. rapa transpires in several stages. A stage takes place between 15 and 36 DAP when rapid changes in fatty acid composition occurred in the LEAR line. In the HEAR line, eicosenoic and erucic acid first appeared at 24 DAP, and increased in proportion until 42 DAP. The last stage of maturation, as defined by no further changes in fatty acid composition, occurs after 36 DAP for the LEAR, and after 42 DAP for the HEAR line.

Table 2. The triacylglycerol fatty acid composition of developing seed of B. rapa cultivar 'R500'.

	Fatty Acid Composition						
DAP[1]	16:0	18:0	18:1	18:2	18:3	20:1	22:1
	% weight						
15	20.1	3.5	32.4	30.6	12.8	0.4	0.2
18	18.9	3.6	35.4	29.9	11.5	0.3	0.3
21	18.2	3.5	40.1	25.2	11.9	0.5	0.5
24	14.9	2.9	43.2	21.3	11.2	3.5	3.0
27	10.0	2.8	45.2	19.8	11.0	4.9	6.3
30	7.1	2.5	39.8	18.1	9.7	6.3	16.5
33	5.5	2.0	30.0	17.1	9.8	8.5	27.0
36	4.8	2.1	27.2	16.8	11.1	8.7	29.1
39	4.1	1.5	20.2	14.8	10.2	9.0	40.1
42	4.0	1.5	14.9	11.7	10.3	12.1	45.5
45	2.8	1.1	14.0	13.3	10.4	9.3	49.1
48	1.6	0.9	13.5	14.5	9.8	9.3	50.4
51	1.8	1.0	13.2	14.6	9.0	9.9	50.5
54	1.6	0.9	12.5	14.5	8.5	9.5	52.5
57[2]	1.4	0.9	12.6	14.3	9.1	9.7	52.0

[1] DAP = Days After Pollination.
[2] Mature seed.

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