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EFFECTS OF LIGHT AND TEMPERATURE ON FATTY ACID DESATURATION DURING THE MATURATION OF RAPESEED

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Abstract—In a winter variety of rapeseed, low temperatures enhance fatty acid desaturation as evidenced by ^{14}C-acetate incorporation into fatty acids or ^{14}C-oleate desaturation *in vivo*; similarly, low temperatures favour polyunsaturated fatty acid accumulation during the maturation of the seeds. Oleate desaturation was slightly higher under 16 hr daylight exposure than under 9 hr treatment.

INTRODUCTION

The effects of growth conditions (temperature and light) on plant fatty acid desaturases are not yet understood. In 1969, Harris and James [1] suggested that these enzymes were mainly controlled by oxygen concentration, but Cherif and Kader [2] established that potato oleoyl-CoA desaturase reaches its highest activity between 25 and 30° *in vitro*. Under these particular experimental conditions, the incubation medium was in equilibrium with air (about 250 nmol O_2/ml) and for higher oxygen concentrations the desaturation decreased. These results and others [3–5] have shown that plant desaturases are not controlled only by oxygen concentration but some genetic regulation of the desaturase content must exist in plants.

In the present work, we have essentially analysed the influence of temperature and daylength variations, during maturation, on the accumulation of linoleic and linolenic acids in rapeseed. This study was conducted on a French winter variety of rapeseed: *Brassica napus* L. var. Primor (O erucic) while up to now the consequences of cultural changes on oil composition have only been shown in spring varieties of rapeseed [3].

RESULTS

We have tried to analyse separately temperature long-term and short-term effects on fatty acid biosynthesis. To study the immediate influence of temperature on fatty acid desaturation, we have followed the incorporation of ^{14}C-acetate into the fatty acids of different portions from the same initial batch of seeds; the different portions were submitted to different temperatures during acetate incorporation. Temperature long-term effects were studied through the analyses of fatty acid compositions and biosynthetic activities of rapeseeds grown under different temperatures, during the whole maturation period of the seeds.

Microdroplets of a Na-^{14}C-acetate solution (see Experimental) were injected into the siliqua of plants grown at 22° for either 4 or 6 weeks after flowering. The seeds were then exposed for 20 hr to different temperatures (4, 22 or 33°) and the radioactive pattern of their fatty acids determined at the end of this period. From the results (Table 1), it can be observed that the lowest temperature (4°) promoted a larger incorporation into linoleic acid. The total acetate incorporation was not quantitatively reduced at low temperature (data not shown).

Table 1. Influence of temperature on the 1-^{14}C-acetate incorporation into the fatty acids of rapeseed

	% Total fatty acid radioactivity					
	4 weeks after flowering			6 weeks after flowering		
Incorporation temperature	4°	22°	33°	4°	22°	33°
$C_{16\,0}$	23.7	38.6	36.5	20.4	27	26
$C_{16:1}$	2.8	4	4.4	tr	tr	tr
$C_{18.0}$	5	14.4	11.9	5.3	12.4	15.5
$C_{18\,1}$	15.1	8.4	8.6	15.7	3	7.8
$C_{18\,2}$	46.6	21	28	52.4	46.5	39
$C_{18.3}$	6.8	13.6	10.7	6.3	11	11.7

Analyses were carried out 20 hr after the injection of the 1-^{14}C-acetate; incubations were performed at different temps. Before the experiment all plants were in the 22°, 16 hr daylength conditions (see text).

At the end of the flowering period, rape plants were exposed to different conditions of temperature and daylength: (a) some plants were grown under a 16 hr photoperiod at one of the following temperatures: 12, 17, 22, 27°; (b) other plants were grown under a 9 hr photoperiod either at 17 or 22°. In each set of these 5 growing conditions two samples of seeds were analysed during their maturation, either four weeks or eight weeks after the end of the flowering period. The results are presented in Tables 2 and 3.

At 12 and 17°, the rate of seed maturation was greatly reduced (siliqua and seeds remain rich in chlorophyll). Their content in polyunsaturated fatty acids was still very high after 8 weeks of maturation: 29% linoleic and 13% linolenic acid. (A period of 8 weeks after

Table 2. Influence of temperature on the fatty acid composition of rapeseeds, four weeks after flowering

	% Total fatty acid weight					
	Daylength 16 hr				Daylength 9 hr	
Maturation temperature	12°	17°	22°	27°	17°	22°
$C_{16:0}$	12.1	13.2	7.2	7.3	13.5	8
$C_{16:1}$	1.7	0.7	1.0	0.9	1.5	0.9
$C_{18:0}$	4.8	3	3	2.2	5	1.9
$C_{18:1}$	30	25.2	56	58	23.2	53.2
$C_{18:2}$	34.5	29.5	22.7	23.5	38	24
$C_{18:3}$	16.7	13	10.2	7.3	18.2	12

Average data for two separate samples collected as explained in Experimental.

complete flowering corresponds to the end of maturation time for a 22° culture temperature). For temperatures higher than 17°, we observed a very important increase of the oleic acid acid content (60% after 8 weeks). Table 4 shows that oleic acid increased not only in relative percentage but also in absolute amount with higher maturation temperatures.

The long-term effect of temperature on oleate-desaturase was then studied. NH_4-^{14}C-oleate was injected *in vivo*, at 22°, into different samples of siliqua taken from plants grown for 4 weeks after flowering at four temperatures. 24 hr later, the seed fatty acids from the different samples were analysed by radio-gas-chromatography (Table 5): it was observed that exposing plants to low temperatures (12–17°) during the maturation of the seeds induced higher oleate and linoleate desaturation in the siliqua. This increase of polyunsaturated fatty acid production at low temperature was observed for the two photoperiods used (9 and 16 hr).

The collected data (see Tables 1, 2, 3 and 5) showed that variation of daylength have little effect on fatty acid composition of the seeds. We noticed that under 16 hr daylength, at any temperature, oleate desaturation slightly increased its value as compared with a 9 hr daylength (see Table 5).

Table 3. Influence of temperature on the fatty acid composition of rapeseeds, eight weeks after flowering

	% Total fatty acid weight					
	Daylength 16 hr				Daylength 9 hr	
Maturation temperature	12°	17°	22°	27°	17°	22°
$C_{16:0}$	8.2	9	9.5	7	7.6	8.5
$C_{16:1}$	0.6	0.6	0.7	0.9	0.3	0.6
$C_{18:0}$	1.6	2	2.5	2	2.1	0.6
$C_{18:1}$	47.5	48.2	57.2	59	50	54
$C_{18:2}$	28.5	26.5	19.5	21.5	26	24.7
$C_{18:3}$	13.5	13.7	10	9	13.7	11.2

Average data for two separate samples collected as explained in methods.

Table 4. Fatty acid composition of seeds grown at different temperature during all the maturation

	Fatty acid composition (mg/g fr. wt)	
	17°	22°
$C_{16:0}$	8.2	22.8
$C_{16:1}$	0.3	1.2
$C_{18:0}$	1.0	2.9
$C_{18:1}$	43	151.7*
$C_{18:2}$	21.1	76.1
$C_{18:3}$	12.2	34.4

* After eight weeks of maturation at 22°, 90% of oleic acid is included in triglycerides.

DISCUSSION

The analyses reported here show that the change in temperature conditions during the period of seed maturation largely modifies fatty acid desaturation inside the seeds. Both short and long term effects were noted. There was an immediate effect of the temperature on seeds in the same physiological state, inducing a greater production of polyunsaturated fatty acids at low temperatures. This effect is consistent with the observations of Harris and James [1]. A difference was noticed between these *in vivo* experiments on rapeseeds and the *in vitro* assays of oleoyl-CoA desaturase of potato microsomes [2], the maximal activity of which was found for high temperatures (35°). A long-term effect was noted, which consists in an induction by low temperatures of some physiological state of the tissues, characterized by a higher desaturation activity. The immediate and the long-term effects add up to result in a higher percentage of polyunsaturated fatty acids in seeds at low growth temperature. At high temperature the oleic acid biosynthesis pathway is stimulated and as this acid is relatively less desaturated, it follows an enrichment of the rapeseed triglycerides in oleic acid.

We conclude that the fatty acid composition of rapeseed, winter var. Primor (O erucic), is controlled by the maturation temperature, both as a consequence of the modifications of oxygen concentration and/or temperature-dependent activities of enzymes. Furthermore, a genetic control of the fatty acid desaturase

Table 5. 1-*^{14}C-Oleate desaturation activities at 22° by seeds from plants grown at different temperatures during four weeks after flowering (24 hr incubation time)

	% Total fatty acid radioactivity					
	Daylength 16 hr				Daylength 9 hr	
Maturation temperature	12°	17°	22°	27°	17	22°
$C_{18:1}$	25.2	58.5	86.3	84	79	93.3
$C_{18:2}$	53.2	35.2	13.7	16	15	6.7
$C_{18:3}$	21.5	6.2	tr	—	5.5	—

content within rapeseed cells seems very likely (long-term effect).

The large variations of fatty acid composition of rapeseed induced by different artificial maturation temperatures are unfortunately of little practical or agricultural interest, because for usual maturation temperatures (above 17°) rape oil presents a fairly constant fatty acid composition.

EXPERIMENTAL

Rape plants (*Brassica napus* L., variety Primor, O erucic) were supplied by M. Renard of the 'Institut National Agronomique', Rennes, France. These plants were grown in the 'Phytotron' of Gif-sur-Yvette, France. They were first grown at 22° under a 16 hr photoperiod until they had 6 to 7 leaves, they were then transferred to 5°, in cold chambers (with a 12 hr photoperiod), for 7 weeks, in order to induce their vernalization. A subsequent transfer back to 22° (16 hr photoperiod) led to the complete flowering. Then plants were taken for experiments and placed under various growth conditions during their maturation period (from 4 to 8 weeks) as previously described. In each set of culture conditions two samples (about 3 g of fr. seeds from 3 different plants for each sample) were collected and analysed separately.

Incorporation studies. Na-(1^{14}C)-acetate (CEA, Saclay, 45 mCi/mM) was used in aq. solns (100 μCi/ml). NH_4-(1^{14}C)-oleate was prepared by heating 1^{14}C-oleic acid (CEA, Saclay, 57 mCi/mM) with 0.2 ml of NH_4OH (4.4% in H_2O). The radioactive precursor was subsequently dissolved in H_2O (100 mCi/ml). Microdroplets (10 μl) were injected into siliqua at the surface of the seeds. Lipids were extracted by the method of ref. [6]. Fatty acid methyl esters were prepared according to ref. [7] and analysed by radio-gas-liquid chromatography as previously described [8].

REFERENCES

1. Harris, P. and James, A. T. (1969) *Biochem. J.* **112**, 325.
2. Chérif, A. and Kader, J. C. (1976) *Potato Res.* **19**, 21.
3. Appelqvist, L. A. (1975) in *Recent Advances in the Chemistry and Biochemistry of plant lipids* p. 247. Academic Press, London.
4. Grenier, G., Mazliak, P., Trémolières, A. and Willemot, C. (1973) *Physiol. Veg.* **11**, 253.
5. Chérif, A. (1975) Thèse d'Etat, Université Pierre et Marie Curie.
6. Bligh, E. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
7. Metcalfe, L. D., Schmit, A. and Pelka, J. R. (1966) *Anal. Chem.* **38**, 514.
8. Trémolières, A. and Mazliak, P. (1974) *Plant Sci. Letters* **2**, 193.