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UNSATURATED FATTY ACIDS IN MATURING SEEDS OF SUNFLOWER AND RAPE: REGULATION BY TEMPERATURE AND LIGHT INTENSITY

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Key Word Index—*Brassica napus*; *Helianthus annuus*; Cruciferae; Compositae; rape; sunflower; seeds; lipid composition; temperature; light intensity.

Abstract—In rape seed, low temperatures and low light intensities increase the level of linoleic acid at the expense of oleic acid biosynthesis without change of the total lipid content. In sunflower seed, however, low temperatures and low light intensities decrease lipid accumulation, with little change in the fatty acid composition. The results are discussed in relation to the regulation of unsaturated fatty acid levels.

INTRODUCTION

The effect of temperature on the synthesis of unsaturated fatty acids in plant tissues, has been discussed for years. Recently [1–8], several studies have underlined again the importance of this subject. It is generally admitted that low temperature favours an accumulation of polyunsaturated fatty acids in plants [1, 6, 9]. At the present time, two hypotheses are under consideration to explain this general observation. (1) As suggested some years ago [9], the oxygen concentration in the medium is the only regulatory factor which acts directly on the 'desaturase' activity since oxygen is an obligatory cofactor of the desaturation system. (2) As proposed more recently for *Tetrahymena* [10–12], the fluidity of the membrane influences directly the activity of the 'desaturase' in the membrane to which this enzyme complex is bound. The last hypothesis advances a very interesting self-regulatory mechanism for the control of membrane fluidity.

Looking at several plants, it appears that unsaturated fatty acid biosynthesis may respond to temperature variation in several ways. While oleic acid biosynthesis is generally favoured by high temperatures [6, 13], more diverse responses are found for polyunsaturated fatty acids. In the roots of plants exposed to frost hardening (i.e. alfalfa) [14, 15] or to cold treatment (i.e. maize) [16], a stimulation of linoleic acid synthesis has often been observed, and in fungi [17], a stimulation by low temperatures of oleic and linoleic acid biosynthesis has been reported. On the other hand, the 'oleyl CoA-desaturase' activity of ageing potato tuber slices measured *in vivo* and *in vitro* has an optimal temperature of 30° [18]. The biosynthesis of linolenic acid is sometimes decreased by low temperatures [16]. Thus no synthesis is observed at temperatures lower than 10° in maize leaves and a five-fold stimulation of this activity takes place between 12° and 22°. The linolenic acid synthesis of *Pharbitis nil* L. cotyledons is also more rapidly induced in plants grown at 27° than in those grown at 17° (N. Salvia and A. Trémolières, unpublished work). On the other hand, various materials and especially blue-green algae are known to have their linolenic acid synthesis induced by a decrease of the temperature (i.e. 34–24° for *Anabaena* var.) [2] and 50–18° for *Cyanidium caldarium* [1]. In Sycamore cell cultures, Gawer *et al.* [7] observed an increased synthesis of all fatty acids at low temperatures, whereas on the same material grown in limiting oxygen conditions Rebeillé *et al.* [8] observed few changes in fatty acid biosynthesis with change in temperature.

Detailed studies on the effect of temperature on the fatty acid composition of oil seeds were reported and reviewed by Appelqvist [19]. In general, the seed oils produced by plants grown in a warmer climate have a lower percentage of polyunsaturated fatty acid than those grown in a colder environment [20] but an exception to this generalization has been reported [21].

These few examples show that (1) the three main unsaturated fatty acids of plants (oleate, linoleate, linolenate) are not under the same control by temperature, and (2) that different plants may respond in opposite ways to the same external variation of culture conditions. It seems clear that unsaturated fatty acid synthesis in plants is not controlled by a simple physical factor such as oxygen concentration or membrane fluidity, but more likely by complex genetic capacities varying from one plant (or tissue) to another. Very little is known of the influence of light intensity on the unsaturated fatty acid biosynthesis [13, 22].

In this paper, we describe two very different responses to temperature and light intensity. Thus in sunflower seed, temperature variations have a pronounced effect on fatty acid biosynthesis during the maturation of the seeds. Cold temperature enhances enormously the desaturation of oleate into linoleate. This effect, well known from field studies on sunflower crops of different geographic origin [23, 24] has now been reproduced in the greenhouse with fully controlled conditions. With the same plant a marked influence of light intensity on unsaturated fatty

acid biosynthesis has been observed. In the two zero-erucic acid varieties of rape seed (a Canadian spring variety and a French winter variety) different temperatures and light intensities do not markedly influence fatty acid synthesis. The Canadian spring variety however, forms twice as much linoleic acid than the French winter variety.

RESULTS

Sunflower seeds

Figures 1 and 2 show the effect of temperature on fatty acid and triglyceride synthesis during the last 4 weeks of maturation. The amounts of linoleic acid decrease from 125 mg/g fr. wt at 12° to about 75 mg at 27° and conversely the amount of oleic acid increases from 30 mg/g fr. wt to 115 mg. All these variations take place in the triglycerides which represent about 98% of the lipids in the mature seeds. As seen in Fig. 2, when the temperature increases there is a decrease in the relative content of linoleic acid-containing triacylglycerol and an increase in oleic acid-containing triacylglycerol. Figure 3 shows that light intensity also has an effect on fatty acid biosynthesis. This effect is significant only when the light intensity on the capitule is reduced by 90% or more (leaves always received full light intensity in these experiments). Under this very low light intensity, linoleic acid biosynthesis is enhanced in the seeds at the expense of oleic acid.

It is important to note that in all the conditions tested the total amount of fatty acid synthesized is about the same. So the results reported here may hide a true regulatory effect and may not simply represent independent variations of the synthesis of each fatty acid.

'High-linoleic' and 'jet-neuf' varieties of rape seed

As seen in Fig. 4. increase in temperature enhances markedly the amount of fatty acids synthesized by both varieties (this effect was not observed in sunflower seeds). However, temperature variations during maturation do not alter the composition of the oil as in the case with sunflower; only a slight increase in oleic acid is observed with increasing temperatures. In the Canadian spring variety ('high-linoleic'), oleic and linoleic acids increase together, but linolenic acid decreases slightly from 12 to 27°. In the French winter variety ('jet-neuf'), only oleic acid increases markedly with temperature. Figure 5 shows that even in rape the amount of polar lipids remains very low in mature seeds and as previously reported [4] most of the linolenic acid is found in the triacylglycerols. Fig. 6 shows that the effect of light intensity is qualitatively almost negligible. However, a 40% decrease of the total lipid content is observed when the light intensity is reduced.

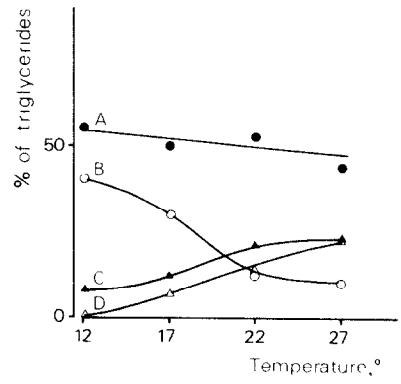

Fig. 2. Effect of temperature on the content of various triacylglycerols of sunflower seeds formed at different temperatures after flowering. Triacylglycerol composition (per mol): (A) one 18:1, two 18:2; (B) three 18:2; (C) two 18:1, one 18:2; (D) three 18:1.

DISCUSSION

In maturing seeds of sunflower, temperature and light intensity influence markedly polyunsaturated fatty acid synthesis and consequently the seeds accumulate a more fluid oil at low temperature. As total fatty acid synthesis remains fairly constant under all the experimental conditions, we attribute these results to a regulation of the

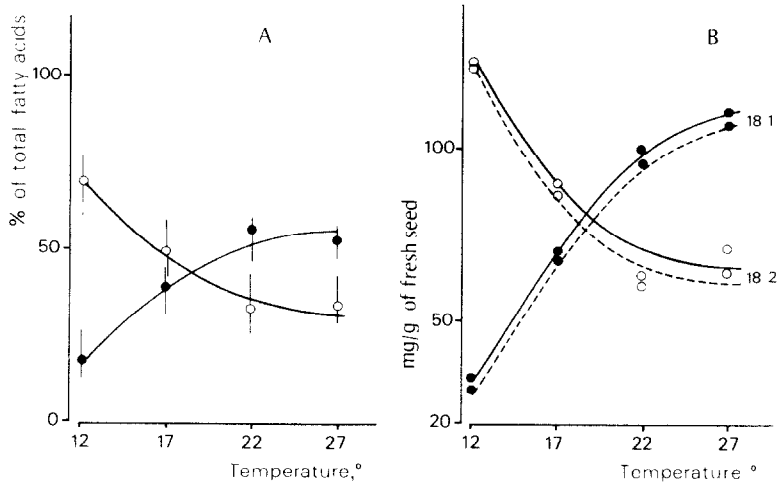

Fig. 1. Effect of temperature during seed formation on total fatty acid composition in sunflower seeds. (A) Percentages of total fatty acids in triacylglycerols: ●—●, oleic acid; ○ - -○, linoleic acid. (B) Absolute amounts of oleic and linoleic acids: —, in total lipids; - - -, in triacylglycerol fraction.

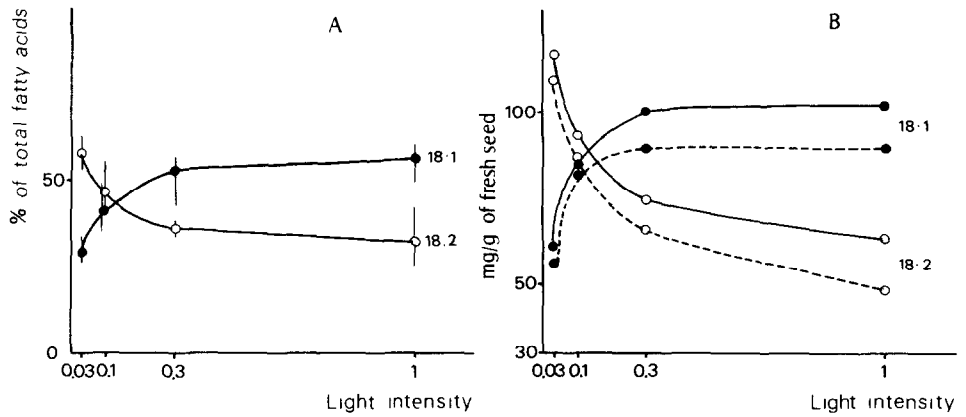

Fig. 3. Effect of light intensity on the fatty acid content of sunflower seeds. Light intensities applied to the capitules are indicated in values relative to full intensity (see Methods). (A) Percentages of total fatty acid in the seeds. (B) Amounts of oleic and linoleic acids per g of fresh seed. (Key to symbols, Fig. 1.)

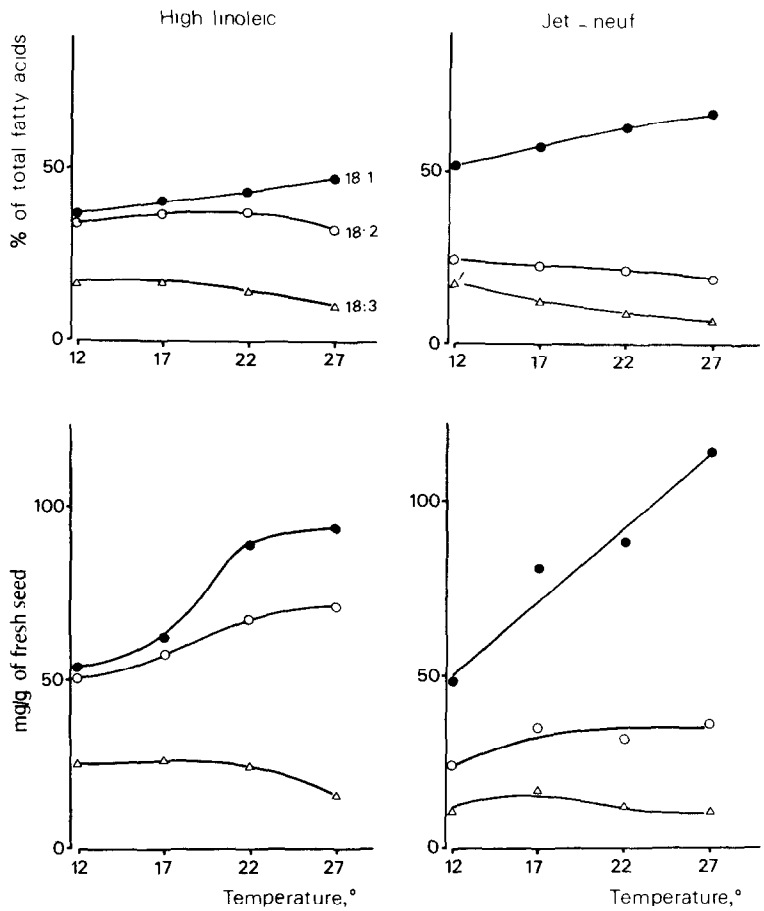

Fig. 4. Effect of temperature on the fatty acid content of rape seeds from two varieties (high linoleic and jet-neuf), when the development of the seeds occurred at four different temperatures. ●—●, Oleic acid; ○—○, linoleic acid; △—△, linolenic acid.

desaturases operating in the sequence: oleic → linoleic → linolenic acids. Clearly either the synthesis or the activity of the first enzyme (oleyl-CoA desaturase according to Kader *et al.* [18]) is enhanced by cold temperature or low light intensity on the seeds. In sunflower, the photosynthetic participation of the seeds is very low if not negligible for fatty acid biosynthesis, so that the oleic acid formed in this tissue is very probably synthesized by a different pathway from the well-documented plastidial pathway [25]. This synthesis seems to be independent of temperature and light intensity. As a conclusion, it appears that polyunsaturated fatty acid synthesis in sunflower is well regulated by external factors such as temperature and light intensity at

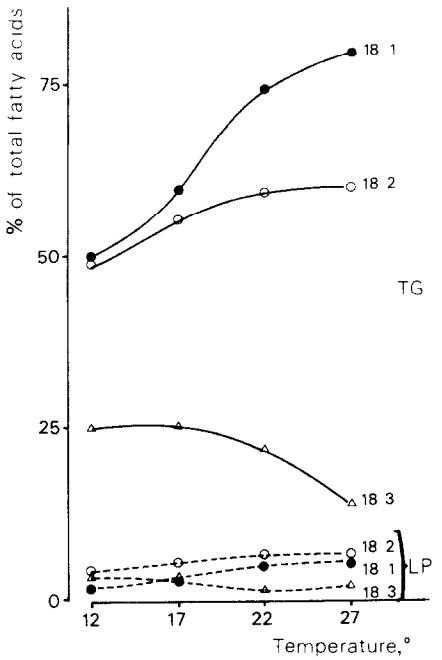

Fig. 5. Effect of temperature on fatty acid content of triacylglycerols (TG) and polar lipids (LP) of rape seeds (var. high linoleic) grown at four different temperatures. (Key to symbols, Fig. 4.)

the level of the desaturation of oleate to form linoleate. This regulation is sufficiently important to produce oils of radically different fatty acid compositions in relation to the climatic conditions of growth.

The situation is quite different in rape. First, the two varieties used are genetically very distinct and one of them (the Canadian spring 'high-linoleic' variety) produces much more linoleic acid than the other. Second, in these two varieties, temperature and light intensity have almost no influence on the formation of polyunsaturated fatty acids, but these factors influence markedly the total production of fatty acids and especially of oleic acid. This increase of oleic acid biosynthesis may be linked to the photosynthetic capacity of the seeds and siliqua. From the experiment (Fig. 6) with low light intensity on the siliqua but normal light intensity on the leaves, we can calculate that, at this stage of the growth, siliqua and seeds contribute about 40% in the energy needed for oil formation.

In conclusion, sunflower and rape display two very different responses to variations in temperature and light intensity: sunflower seeds are influenced by maturation conditions with respect to desaturase activity, rape seeds are not. So it seems difficult to explain the changes of desaturase activity only by a simple physical factor such as the increase of oxygen concentration at low temperature (for example, in sunflower a decrease in temperature can certainly increase the oxygen concentration in the cells but a reduction in light intensity probably cannot). Thus we suggest that in the two plants studied the regulation of polyunsaturated fatty acid biosynthesis does not result only from physical factors such as O_2 concentration or membrane fluidity but essentially from a genetically programmed activation of the enzyme or its biosynthesis. This programme may differ from one plant to another. Then, when the enzyme is present, physical factors may modify its activity and

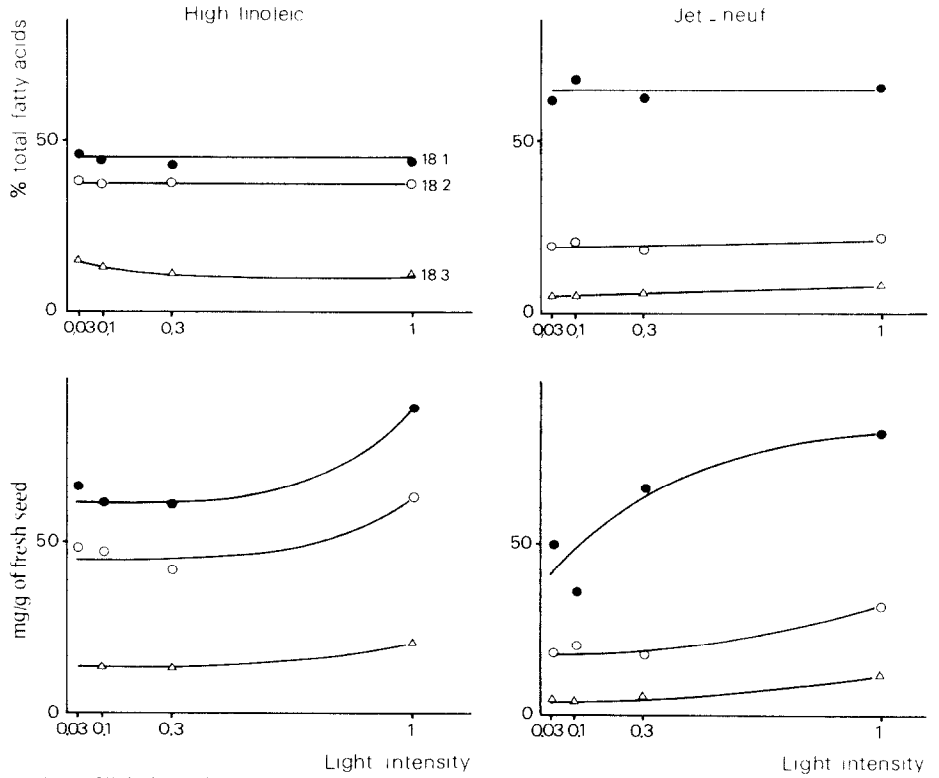

Fig. 6. Effect of light intensity on fatty acid composition of rape seeds. (Key to symbols, Fig. 4; light intensity units, see Experimental.)

consequently may interact with the genetically controlled programme.

The results presented here were obtained from *in vivo* experiments. *In vitro* experiments with fungi, however, have shown no correlation between desaturase activity and membrane fluidity in this organism [17]. Work on subcellular preparations of maturing seeds is now in progress in our laboratory, to check the hypothesis of a genetically controlled programme for the regulation of polyunsaturated fatty acid biosynthesis.

EXPERIMENTAL

Sunflower plants (*Helianthus annus* L. var. Clairsol) were supplied by Dr. Rolier (CETIOM, Paris), rape plants (*Brassica napus* L., var. 'jet-neuf' 0-erucic and 'high linoleic' 0-erucic) were supplied by M. Renard, Institut National Agronomique, Rennes. The plants were grown in the Phytotron of Gif-sur-Yvette.

Rape plants were grown at 22° under a 16-hr photoperiod until they had six or seven leaves. They were then transferred to a 5° cold chamber (with 9-hr photoperiod) for 7 weeks to induce vernalization (the Canadian spring variety 'high linoleic' was also treated in a cold chamber in order to standardize the climatic conditions applied to plants). The subsequent transfer back to 22° (16-hr photoperiod) led to complete flowering. Sunflower plants were grown at 22°, in 16-hr photoperiod until flowering without cold treatment.

Four weeks after completion of flowering, the plants were placed under various growth conditions: (1) 16-hr photoperiod with uniform full sun light intensity at 12°, 17°, 22° and 27° respectively; (2) 22° with a 16-hr photoperiod, the light intensity irradiating the capitule of sunflower, or the siliqua of rape plants, reduced by a filter applied around the fruits of the plants. The reduction of light intensity at the seed level was from 70 to 3%. The light intensity at the leaves was not reduced more than 10–20%. Over the 4 weeks of the experiments the mean full light intensity was about 104 W/m^2. Fatty acid analysis of seeds at the beginning of the experimental period showed that the accumulation of oil had just started. For each set of conditions, five plants of similar height and maturity were selected from the pool of maturing plants. At the end of the 4-week period, the seeds produced under each set of experimental conditions had reached the plateau for oil accumulation and had just started to dehydrate without changing their oil composition [24]. The batches of seeds obtained from each plant were separately extrd and analysed for lipid and fatty acid compositions. For sunflower as for rape, each plant yielded 20–30 g of seeds containing about 6 g of oil. In the figures, the mean of five values for the five plants analysed and the maximal variation recorded are plotted. In all cases a very good homogeneity of oil composition was observed for a given experimental condition.

Lipids were extrd according to [26]; fatty acid methyl esters were prepared according to [27] and analysed by GLC as previously described [28]. Triacylglycerols were sepd by TLC on Ag^{2+}–Si gel according to [29].

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