Unsaturated Fatty Acid Synthesis in Sunflower (Helianthus annuus L.) Seeds in Response to Night Temperature

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ABSTRACT

The incorporation of 14C02 into unsaturated fatty acids during seed development was measured in sunflowers grown in controlled environments with day temperatures of 28°C and night temperatures of 15°C or 22°C. While the average temperatures to which the plants were exposed did not differ greatly, the ratio of linoleic acid to oleic acid synthesized was much greater at a night temperature of 15°C than at 22°C. These results support the proposal (Harris et al. 1978) that the mean minimum temperature experienced during seed development is the major environmental factor influencing the unsaturated fatty acid composition of sunflower seed oil.

Abbreviations: DAG, diacylglycerol; DAP, days after pollination; TAG, triacylglycerol; TLC, thin-layer chromatography

INTRODUCTION

The influence of temperature during seed development on the unsaturated fatty acid composition of sunflower oil is well documented. The proportion of linoleic acid in the oil can vary from over 70% of the total fatty acids to less than 40%, depending on the growth temperature (Hilditch and Williams 1964). In controlled temperature experiments, Canvin (1965) found that increasing temperature during seed development led to a decrease in the linoleic acid content of oil. Field studies in Australia have confirmed this effect by comparing the oil composition of a single variety grown in different regions (Bridge et al. 1951) and in serially grown crops exposed to changing climatic conditions (Keefer et al. 1976).

Further studies on sunflowers grown under controlled environment and field conditions have indicated that the mean minimum temperature experienced by the plant in the period between mid-flowering and harvest maturity may be the critical factor affecting the linoleic acid content of the oil (Harris et al. 1978). It was projected that a mean minimum temperature less than 16°C would be required to obtain a linoleic acid content of at least 64% in sunflower oil. This paper reports a study of the effect of night temperature on the incorporation of 14CO2 into the unsaturated fatty acids of developing seeds of sunflowers grown under controlled environmental conditions.

MATERIALS AND METHODS

Sunflower plants (Helianthus annuus, cultivar Sunfola 68-3, derived from Peredovik) were grown in pots under field conditions, and hand pollination was performed when the first 4-5 rows of florets in each head were fully developed. All plants were pollinated within 48 hours of each other, and were subsequently transferred to growth cabinets 4-6 days after pollination (DAP).

The plants were divided into two groups, which were subjected to night temperatures of either 22°C or 19°C in humidified cabinets providing equal day temperatures of 28°C, with a 12 hour photoperiod and day/night temperature regime, and light intensities of approximately 500 mol m−2 s−1. At various stages during development, plants were removed from the cabinet and the highest available leaf was completely enclosed in a transparent chamber and sealed around the petiole. The leaf chamber was artificially illuminated at a light intensity of 400-500 mol m−2 s−1, while air was circulated at 6 l min−1. Attached to the chamber, which formed part of a closed system, were silica gel drying columns and an acid bath containing 0.5 M HCl. After 10 minutes, two injections of 0.5 MBq Na214C3 (740 MBq/m mol) into the acid bath were each followed by a period of 30 minutes to allow for 14CO2 uptake by the leaf. The circulating air was then diverted to pass through a CO2 trap solution of 0.5N NaOH for 30 min. The temperature inside the leaf chamber did not exceed 32°C during the procedure. The plants were then returned to the controlled temperature cabinets for 48 hours, after which time seeds of the appropriate age were collected from the head and stored at 4°C after steam killing.

Embryos were removed from samples of 10-15 seeds from each plant and homogenized in a Ten Broek homogenizer with 10 ml CHCl3-CH3OH (2:1). Lipids were partitioned into the CHCl3 phase by addition of 0.9% NaCl (1.8 ml) and 0.5 mg of the antioxidant butylated hydroxytoluene was added. Both the aqueous methanol and chloroform extracts were stored at -20°C. The distribution of radioactivity between lipid and non-lipid products was determined by scintillation counting.

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Lipid classes were then analyzed for radioactivity after separation into TAG, DAG, and polar lipids by TLC on Kieselgel G (Merck) plates using as solvent, hexane-diethyl ether-acetic acid (70:30:1, v/v/v). Lipid bands, detected by staining with dichlorofluorescein and identified by co-chromatography with standards, were scraped from the plates and eluted with CHCl₃-CH₃OH (2:1, v/v). The distribution of radioactivity between the lipids was determined by scintillation counting. Portions of the eluents containing TAG samples were evaporated under N₂ and fatty acid methyl esters were prepared using 14% BF₃/CH₃OH reagent (4 ml) at 75 °C for 60 min (Morrison and Smith 1964), in the presence of 1.0 ml benzene. Methyl esters were extracted into petroleum spirit (b.p. 40°-60°C) and separated into saturated, mono-unsaturated and di-unsaturated classes by TLC on Kieselgel G containing 10% (w/w) AgNO₃, using hexane-diethyl ether (92:8, v/v) as solvent. The distribution of radioactivity between the methyl ester classes was determined by scraping gel bands stained with dichlorofluorescein into vials and counting directly in 10 ml of scintillant. Gas-liquid chromatography analysis of methyl esters of total lipid was performed at 175°C on a 1.8 m glass column packed with 10% SP-222-PS (Supelco).

RESULTS AND DISCUSSION

When sunflowers were exposed to a night temperature of 22°C approximately 63% of the radioactivity incorporated into the embryos from ¹⁴CO₂ at 12 DAP was found in the lipid fraction. At 15, 18 and 21 DAP this amount increased to approximately 88% of the incorporated label. Similar results were obtained with plants grown at a night temperature of 15°C. Net incorporation of radioactivity into embryo lipid varied between seed samples from different plants receiving replicate treatment by up to a factor of 4, but the distribution of label was comparable in each case.

Under both night temperature conditions, TAG contained 92-95% of the label incorporated into embryo lipid. Only 2-5% was found in DAG or polar lipid and no free fatty acids were detected.

Despite these similarities in the incorporation of ¹⁴CO₂ into embryo lipids, the pattern of labelled fatty acids found in the TAG component differed markedly between the two treatments. A night temperature of 22°C led to the label being incorporated predominantly into oleic acid, which constituted 66% of labelled fatty acids at 12 DAP, increasing to approximately 80% at 15-21 DAP. During this period only 15% of the incorporated radioactivity was found in linoleic acid (Figure 1). At a night temperature of 15°C, oleic acid contained only 33-56% of the label in TAG during the 12-21 DAP period, while 44-29% appeared in linoleic acid. The relative amount of label incorporated into saturated fatty acids (palmitic and stearic acids) ranged from approximately 10% to 20% under both temperature regimes.

The different night temperature conditions under which the plants were kept therefore affected the distribution of label from ¹⁴CO₂ between oleic and linoleic acid during TAG synthesis in the developing sunflower embryos. This effect was also observed in the fatty acid composition of the accumulated embryo lipid. In plants held at a night temperature of 22°C, embryo lipid contained 66-82% oleic acid and 8-16% linoleic acid (Figure 2). With a night temperature of 15°C, oleic acid constituted 37-39%, and linoleic acid 40-49% of the embryo lipid fatty acid. The relative amount of saturated fatty acids present was little affected by the night temperature difference (data not shown).

Field studies on this cultivar (Harris et al. 1978) have shown that the most rapid accumulation of oil and linoleic acid occurs between 250 and 500 degree days after pollination. The sampling times between 12 and 21 DAP used in this study fall within this period for both groups of plants. With equal day temperatures and the respective night temperatures of 15 °C and 22°C, the average temperature in the two chambers differed by only 3.5°C. Any difference in rate of development between the two groups of plants was therefore minimal over the duration of the experiment.

Changes in the fatty acid composition of sunflower seed lipid such as those observed here, are also produced when plants are grown at constant temperatures between 10 °C and 27°C (Canvin 1965; Tremolieres et al. 1982). However in the range of average temperatures used in the present work, 21° to 25°C, constant temperature growth produced no significant difference in the synthesis of linoleic acid (Tremolieres et al. 1982).