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OLEATE DESATURATION IN SEEDS OF TWO GENOTYPES OF SUNFLOWER

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Abstract—*In vivo* oleate incorporation and desaturation in developing seeds of normal and the high oleic acid mutant of sunflower have been studied. In seeds less than 15 days after flowering (DAF) of both genotypes, incorporation and desaturation was similar and took place mainly in polar lipids. Seeds 15–35 DAF incorporated fatty acids preferently into triacylglycerols. During this period mutant seeds lacked oleate desaturation capacity but it was recovered after the cotyledon started a special process of differentiation.

INTRODUCTION

A high oleic acid (18:1) sunflower mutant, with high amounts of 18:1 and very low content of linoleic acid (18:2) in the seed lipids has been characterized [1]. In this mutant the expression of the 18:1 character takes place exclusively in the developing seeds, during the synthesis and accumulation of reserve lipids. The fatty acid composition of seed lipids from both the normal genotype and the high oleic mutant depends on growth temperature although the normal genotype is more strongly affected than the mutant. High 18:1 seeds obtained at low temperature (21/14°) contained 3–9% of 18:2 and those obtained at high temperature (34/22°) had less than 0.5% [1, 2]. Exogenous [1-^{14}C] 18:1 is taken up by developing sunflower seeds and incorporated into acylthioesters and lipids and desaturated to 18:2 [3]. Studies on 18:1 desaturation in high 18:1 mutants from soybean [4] and safflower [5] showed that 18:1 is readily incorporated into lipids but 18:1 desaturase activity was reduced as compared with normal seeds.

In the present paper, developing sunflower seeds of both normal and mutant genotypes, obtained at low temperature in order to have the maximum 18:1 desaturase activity, have been incubated *in vivo* with [1-^{14}C] 18:1 and the incorporation into lipids and the desaturation of 18:1 to 18:2 studied. The reversion of the high 18:1 character in special seeds in which the developing cotyledon grows out after breaking the hull while the synthesis of 18:2 is switched on has also been studied.

The high 18:1 mutant of sunflower should be a valuable material for biochemical and enzyme regulation studies on fatty acid desaturation and tissue differentiation as, apparently, there is a reversible lack of 18:1 desaturase activity in a very specific tissue.

RESULTS AND DISCUSSION

The fatty acid composition of total lipids from seeds of both normal and high 18:1 genotypes used in this work are shown in Table 1. In agreement with previous results [1] very young seeds had similar fatty acid compositions in both genotypes while in the more developed and mature seeds the composition was very different. The growth temperature (21/14°) was selected in order to increase the percentage of 18:2 and consequently of 18:1 desaturase activity in the normal seeds and, if possible, to have some activity in the mutant.

During seed development [1-^{14}C] oleate was incorporated into polar lipids, diacylglycerols and triacylglycerols at different rates. No differences were observed in the incorporation into lipids of both genotypes. The percentages of incorporation into polar lipids and triacylglycer-

Table 1 Fatty acid composition of total lipids from normal and high 18:1 sunflower seeds obtained from plants cultivated at 21/14°

	G8 mutant High oleic acid mol %					RHA-274 Normal genotype mol %				
Age (DAF)	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
8	19.7	5.8	41.9	28.8	3.7	17.0	3.9	31.5	40.2	7.3
25	4.1	7.0	81.2	7.1	0.5	6.5	8.0	44.9	39.8	0.8
40	3.3	8.2	84.2	3.5	0.8	5.7	6.5	36.1	50.9	0.6

Mean of data from two plants

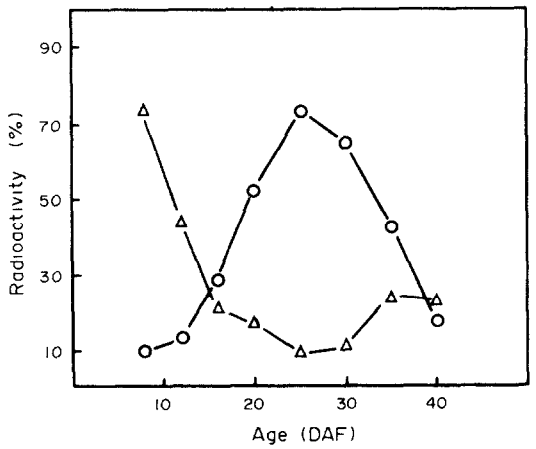

Fig 1 Incorporation of [1-^{14}C] 18 1 into polar lipids (△) and triacylglycerols (○) during development of normal sunflower seeds Results are expressed as % of radioactivity

ols of normal developing seeds are shown in Fig. 1. In young seeds the incorporation took place preferently into polar lipids. The active synthesis of triacylglycerols started at *ca* 12 DAF and reached a maximum at 25 DAF

The *in vivo* desaturation of 18:1 associated with structural and reserve lipids of developing seeds is shown in

Table 2 Fatty acid composition of total lipids from cotyledon (inner and emerging tissue) of high 18 1 sunflower special seeds obtained at 21/14° (for details see Experimental)

Age (DAF)	Tissue	Fatty acid (mol %) 16 0	18 0	18 1	18 2	18·3
25	Inner	4 2	8 6	82 2	4 4	0.6
	Emerging	7 9	6 3	65 3	19 3	1 2
40	Inner	4 4	1 5	32 1	11 4	0 5
	Emerging	5 4	3 3	77 5	13 0	0 7

Mean of data from two plants

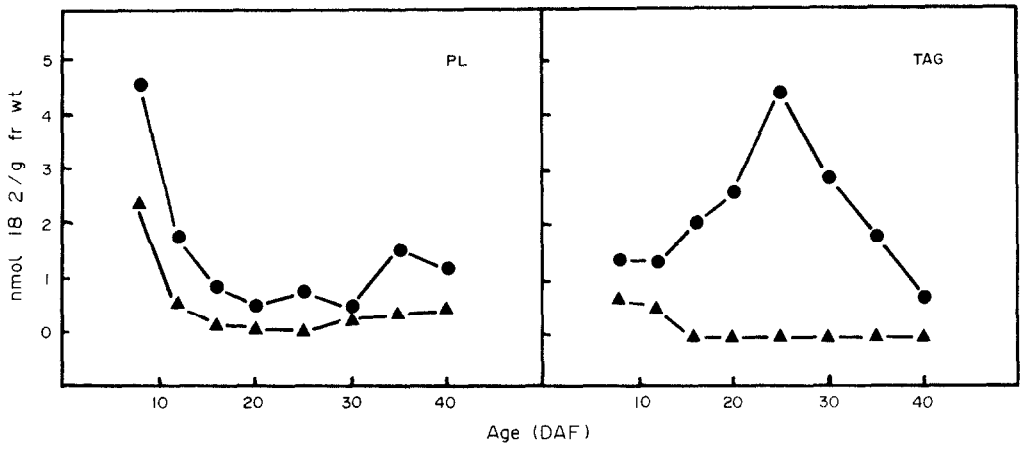

Fig 2 18 1 desaturation by normal (●) and high 18.1 (▲) developing sunflower seeds. Seeds were incubated with [1-^{14}C] 18 1 and the incorporation of newly synthesized 18 2 into polar lipids (PL) and triacylglycerols (TAG) determined Results are the mean of four experiments

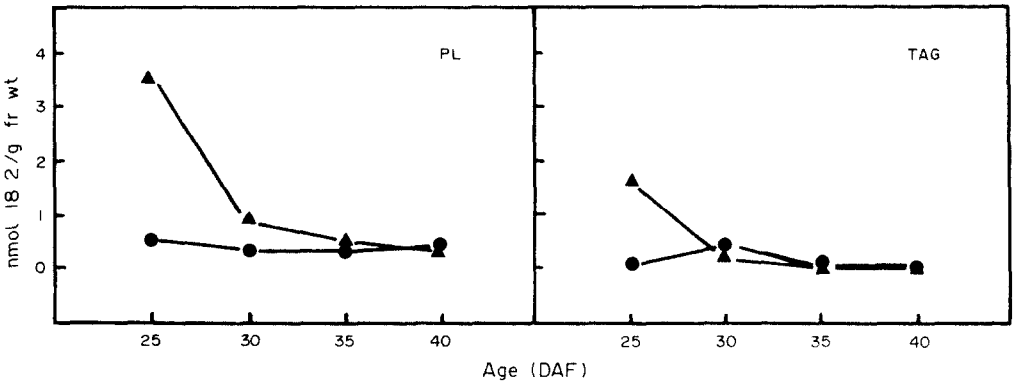

Fig 3 18 1 desaturation by inner (●) and emerging (▲) tissue of high 18 1 special seeds Tissues were incubated with [1-^{14}C] 18 1 and the incorporation of the newly synthesized 18 2 into polar lipids (PL) and triacylglycerols (TAG) determined. Results are the mean of two experiments

Fig. 2. The amount of newly synthesized 18·2 in polar lipids was very similar in both genotypes during the developing period Young seeds showed a higher 18·1 desaturation capacity and also preferential incorporation into polar lipids (Fig. 1), the minimum level of desaturation corresponded to seeds of 15–30 DAF being 0.5–0 8 nmol of linoleate/g fr. wt in the normal genotype and less than 0 3 nmol of linoleate/g in the high 18:1 mutant. On the contrary, the amount of 18:2 incorporated into triacylglycerols was very different. In normal seeds the maximum of desaturation took place at 25 DAF (Fig. 2) corresponding to the maximum of incorporation into triacylglycerols (Fig. 1). In the mutant, no desaturation was observed in triacylglycerols after 15 DAF. All these results are in agreement with previous results [1] which showed that the expression of the high 18:1 character is restricted to the synthesis of reserve lipids in the developing seeds.

In the self-pollination programme, designed to provide a permanent supply of both normal RHA-274 and high 18:1 G8 seeds, we came across and maintained lines which produced a number of seeds in which the cotyledon emerged out of the hull This tissue grows actively from 20 to 30 DAF and then gets dry. When this occurred in the high 18·1 mutant the synthesis of 18:2 was increased A similar effect has been reported [6] in immature safflower cotyledons in which 18:2 synthesis was induced by light. The fatty acid composition of the lipids from the inner part of the seed and the emerging tissue of sunflowers is shown in Table 2 The composition of the inner tissue in seeds 25 DAF corresponded to that of the high 18.1 seeds (Table 1). However, the emerging tissue contained a higher amount of 18:2(19 3%). In adult seeds (40 DAF), even the inner part contained more 18:2 than expected for high 18 1 seeds (Tables 1 and 2). The emerging tissue was produced by a special differentiation resembling a etiolated germinating seed. Interestingly enough, this reversion of the high 18 1 character took place in the same tissue where it was expressed. In the initial step of development of emerging tissue a high 18·1desaturase activity was observed, 18:2 being incorporated into both polar lipids and triacylglycerols (Fig 3). Probably the synthesis of 18·2 was due to polar lipid requirements for the new tissue, the incorporation of 18:2 into triacylglycerols being a secondary effect On the contrary, the inner part showed little desaturase activity in agreement with the results obtained for the mutant seeds between 25 and 40 DAF (Fig. 2) At present it is not known if the increase of 18:2 in the inner part of mature seeds (Table 2) is due to the diffusion of 18 2 from the emerging tissue or the induction of 18:1 desaturase activity.

EXPERIMENTAL

Plant material Sunflower plants were cultivar RHA-274 and mutant G8 (high 18 1 acid content in seed oil) [1] Plants were cultivated in a growth chamber with 14 hr photoperiod at 21/14° and light intensity of 300 $\mu E/m^2$ sec

Incubations Peeled seeds (~50 mg) at the appropriate DAF were incubated in an uncapped test tube with 16 KBq [1-^{14}C] NH_4- 18·1, 1 9 GBq/mmol (New England Nuclear) in 200 μl of H_2O for 15 hr in a shaking bath at 20°. Reactions were stopped by submerging the test tube in H_2O at 90° for 5 min, the sample was then rinsed with H_2O and the residual isotope removed For the special seeds inner and emerging parts were sectioned with a razor blade before incubations

Lipid extraction and analysis Methods for total lipid extraction, isolation of lipid classes and determination of fatty acid composition were as described in ref [1] Total radioactivity incorporated into sample lipids was measured using a calibrated scintillation counter To determine the desaturation of [1-^{14}C] 18 1 into [1-^{14}C] 18 2, polar lipids and triacylglycerols were scraped off from prep TLC and the corresponding fatty acids converted into Me esters [3] and then sepd into monoenes and dienes on $AgNO_3$–silica gel plates developed with C_6H_6. Radioactivity in the different lipid classes and in fatty acid Me esters was measured using a TLC-linear analyzer

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