OIL COMPOSITION AND ACCUMULATION OF FATTY ACIDS IN NEW OLEIC SUNFLOWER (*Helianthus annuus* L.) HYBRIDS

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ABSTRACT

Studies on oleic sunflower (*Helianthus annuus* L.) were carried out in order to determine which mechanism produces oleic and linoleic acids in seeds of "new generation" oleic hybrids. Fatty acid composition for different lipid fractions (structural and storage forms) was followed during the seed development of three hybrids including *cv*. Santiago (non-oleic hybrid), *cv*. Olbaril and *cv*. Trisun 870 (high oleic hybrids). In addition, a simple physiological model of fatty acid accumulation ($g.1000seeds^{-1}.day^{-1}$) was derived from computer-aided analysis. In order to complete this biochemical approach, we developed a molecular study of desaturase gene accumulation in developing seed of sunflower hybrids.
Results obtained on fatty acid composition of oil at maturity, indicated differences in the oleic acid content as regards the allo-pollen pollution.
Moreover, the evolution of oleic and linoleic acid contents in seed revealed the lack of Δ12-desaturase activity for oleic hybrids when compared to the non-oleic hybrids but indicated also differences between oleic genotypes related to linoleic acid content in the first days following pollination and until maturity. Molecular studies confirmed that the Δ12- desaturase transcript was not accumulated for oleic hybrids (using northern-blot analyses).
All these observations were confirmed by studies on the selective dispatching of oleic and linoleic acids in different lipid fractions (phosphatidylethanolamine and triglycerides) during the seed development. Trisun 870 showed a very low content of linoleic acid in triglycerides all along seed maturation whereas Olbaril presented an initial higher content followed by a rapid decrease. Finally, these results enabled us to propose theoretical mechanisms for fatty acid biosynthesis in these oleic sunflower hybrids.

Keys words : oleic sunflower, hybrids, oleic acid, linoleic acid, accumulation.

1 - INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the major oilseeds grown in temperate climates. Some years ago, sunflower hybrids producing oil with high oleic acid content were obtained by chemical mutagenesis on normal sunflowers (Soldatov, 1976). Oleic sunflower oil is characterised by a high level of oleic acid in the seeds (up to 90 % ; Lagravère *et al.*, 1998), but not in vegetative tissues. The lack of vegetative tissue modification regarding fatty acid composition can be related to a mutation concerning a seed specific gene of Δ12- desaturase which converts oleic acid into linoleic acid (Garcés *et al.*, 1989 ; Heppard *et al.*, 1996). Nevertheless, the presence of up to 10 % of linoleic acid in the oil seed leads us to wonder about the origin of this linoleic acid biosynthesis. The present work was undertaken to provide a better understanding of the mechanism by which oleic and linoleic acids are produced in oleic and non-oleic sunflower seeds of hybrid genotypes. We thus report results on fatty acid accumulation in oil and phospholipids and Δ9- and Δ12- transcript accumulation during seed maturation.

2 - MATERIALS AND METHODS

Three common cultivated sunflower (*Helianthus annuus* L.) hybrids, including Santiago (non-oleic cv. provided by Novartis Seeds - earliness ½ early / ½ late), Olbaril (high oleic cv. provided by Pioneer Hi Bred – earliness ½ late / late) and Trisun 870 (high oleic cv. provided by Mycogen - earliness ½ early / ½ late) were grown in experimental fields at the INRA Research Centre of Toulouse-Auzeville, France during 1996 and 1997. All inflorescences were bagged prior to anthesis, to prevent cross-pollination between oleic and non-oleic sunflowers. Seeds obtained under bag will be designated SPC (self-pollination conditions) and seeds obtained in normal pollination conditions OPC (open-pollination conditions). In addition, only the three outer rows of each inflorescence were collected and pooled to conserve plant material in the same development. This was repeated for each bloc. Oil sample was obtained by hexane extraction. Lipid classes were separated by Thin Layer Chromatography (TLC) according to Christie, (1982). Fatty acids were analysed by gas chromatography (GC) (Fisons Instruments GC 8000 Series - USA) according to the conventional method. Phospholipids were determined by microphosphorus determination on the lipid extract (Morrison, 1963). For Northern blot analyses RNA extraction and quantification were performed on immature embryos (8, 10, 16, 18 et 26 DAF) according to the conventional method. A standard analysis of variance was carried out by using Newman and Keuls method based on the least significant difference (LSD) at the 0.05 probability level.

3 - RESULTS AND DISCUSSION

High oleic “new generation” hybrids like Olbaril or Trisun 870 reached up to more than 90 % of oleic acid in SPC, with great stability through two-year trial studies. Nevertheless Trisun 870 oleic acid content was higher for both years than Olbaril indicating significant differences in genotype potential for oleic acid percentage between these two oleic hybrids (91.32 % *vs.* 89.83 % average of 1996/1997 for Olbaril *vs.* Trisun 870 respectively). In OPC, Olbaril reached only 84.91 % average of 1996/1997, 4 to 5 points under the level reached using SPC (89.83 %) according to the non-oleic pollen environment as described previously (Fernandez Martinez *et al.*, 1993). Nevertheless, Trisun 870 exhibited no significant difference between OPC and SPC for oleic acid percentage for both years (91.34 % *vs.* 91.32 % : average 1996/1997 for OPC *vs.* SPC, respectively). In contrast, Santiago exhibited an opposite behaviour in OPC ; the presence of oleic pollen increased the oleic acid level (39.23 % vs. 28.28 % : average 1996/1997 for OPC vs. SPC respectively). Saturated fatty acid, especially palmitic acid (C16:0) appeared also to be significantly greater for non oleic than oleic genotypes (Santiago : 5.16 % compared to Olbaril : 2.94 % and Trisun 870 : 2.88 %).

The fatty acid composition of lipids extracted was determined during the seed filling period in 1997 (***Figure 1***). Oleic acid was the most abundant fatty acid newly synthesised in the maturing seed between 10 and 20 DAF. This result is consistent with those concerning the expression of Δ9- desaturase which is mainly expressed at 12 DAF (Kabbaj *et al.*, 1996 ; Lacombe *et al.*, 2000). During this period the percentage of linoleic acid decreased (Data not show). This decrease can be explained by a dilution of linoleic acid already present by the newly synthesised fatty acids (mainly oleic acid). The difference observed at maturity stage for the oleic acid content already appeared at 12 DAF. For Santiago the percentage of oleic acid started to decrease after 20 DAF while the Δ12- desaturase was mainly expressed after 18 DAF (Kabbaj *et al.*, 1996), and begun to convert the oleic acid into linoleic acid. On the contrary, for both oleic hybrids we did not observe this conversion. Nevertheless a difference appeared between oleic hybrids (***Figure 1***). For Olbaril, the major increase of oleic acid occurred within 9 days (14.52 % at 10 DAF, 80.43 % at 19 DAF) whereas for Trisun 870 the same increase appeared within 3 days (19.83 % at 10 DAF, 79.34 % at 13 DAF). This result indicated a new significant difference of behaviour between the two high oleic hybrids. This difference could be related to Δ9– desaturase activity or the different remaining activities of Δ12- desaturase between both oleic genotypes.

Oleic acid accumulation expressed in $g.1000seeds^{-1}$ (***Figure 2***) showed strong differences between hybrids. For Santiago accumulation occurred only within the first 30 days after pollination (DAF), and stopped when Δ12- desaturase was fully active (after 20 DAF). On the contrary Olbaril and Trisun 870 accumulated oleic acid until physiological maturity (about 40 DAF) when all accumulations (*i.e.* oil) stopped in the seed and only desiccation occurred. Linoleic acid accumulation showed strong differences especially for oleic genotypes. All the hybrids exhibited a synthesis of linoleic acid at early stages (*i.e.* until 13 to 20 DAF), but we noted that a difference between the two oleic sunflower hybrids occurred. For Trisun 870, the quantity of linoleic acid synthesised, reached a maximum after 20 DAF, on the contrary Olbaril showed a continuous increase of linoleic acid accumulation like Santiago but with a much lower intensity and reached an always higher level than Trisun 870. This observation was confirmed by a simple physiological model of fatty acid accumulation ($g.1000seeds^{-1}$) derived from computer-aided analysis and used for calculated the daily accumulation rate ($g.1000seeds^{-1}.day^{-1}$) of linoleic acid (***Figure 3***). Those results confirm that daily accumulation rate of Trisun 870 was less important than Olbaril and shorter (only 20 days).

The selective dispatching of oleic and linoleic acids in the triglycerides (TG) and the phosphatidylethanolamine (PE) at three stages of the seed filling period is presented ***Table 1***. With all of the sunflower varieties we observed a continuous increase of the oleic acid percentage in the PE, whereas the percentage of C18:2 decreased especially for oleic hybrids. Nevertheless, the final percentage of oleic acid in the PE was higher in the mutants, and the percentage of linoleic acid was higher in the non-oleic hybrid. This result suggested that structural phospholipids form (PE) was also affected in mutant types. In sunflower mutants there was a specific dispatching of C18:2 in the structural lipids. This last result was not totally in agreement with those obtained by Garcés *et al.* (1989) who did not underline drastic differences in fatty acid composition between TG and polar lipids. This is understandable, since the total polar lipid fraction used by these authors includes the PC fraction which is well known to be involved in the synthesis of TG (Stymne & Stobart, 1987) and PC is more and more abundant in the polar fraction all along the maturation phenomenon. Regarding the composition of fatty acids in TG, we observed a strong difference between both mutants : Trisun 870 exhibited a lower percentage of C18:2 all along the seed maturation, whereas Olbaril had more than 20% of linoleic acid at 13 DAF. This result could explain the difference in final fatty acid composition for both mutant hybrids. This suggested also that both hybrids appeared to be different in genetic background for desaturases, for example the

percentage of C18:1 is much higher in Trisun 870 than in Olbaril at early stages of seed filling period.

At the molecular level results obtained for northern blot (***Figure 4***) were in agreement with results previously reported by Kabbaj *et al.*, 1996. Δ9- and Δ12- desaturase transcript accumulation occurred at least between 10 and 18 DAF for non-oleic hybrid and there was a lack of Δ12- desaturase transcript accumulation for both oleic hybrids. While southern studies have demonstrated that the Δ12- gene was present (Lacombe *et al.* 1998, Hongtrakul *et al.*, 1998) the lack of Δ12- transcript accumulation could be due to a non expression of this gene or a faster degradation of the mRNA. At this point we can not choose between both hypotheses.

4 - CONCLUSION

First we observe that between 35 DAF and the maturity the oleic acid percentage was quite stable and could permit an early prevision of final oleic acid concentration in oil. This prevision could be used to separate seeds with a high or very high percentage of oleic acid in the oil. Regarding oleic sunflower mutants, the accumulation of oleic acid is due to a deficient Δ12- desaturase (Garcés *et al.*, 1989). Nevertheless, some linoleic acid is still present in the seeds, which suggests an other phenomenon. In soybean seeds, it has been reported by Heppard *et al.*, (1996) that two desaturase systems are involved in the biosynthesis of unsaturated fatty acids. The first one is a constitutive system present in the whole plant, and the second one is specially devoted to storage metabolism in the seeds. Thus a mechanism comparable to soybean metabolism could be acceptable in our case. During the first part of the maturation, the constitutive desaturase could be responsible for a low synthesis of linoleic acid in all kinds of hybrids. Then the specific desaturase is involved in the accumulation of high quantities of linoleic acid in the standard hybrids. However, we can suppose that oleic sunflower mutants are only deficient in seed specific desaturase. Nevertheless, there was an accumulation of linoleic acid after 20 DAF in one of the sunflower mutant. This result is probably due to a residual activity of the seed specific desaturase system in the case of Olbaril hybrid. This was partially confirmed by investigations performed on lipid classes typical of storage (TG) or of structural metabolism (PE). Linoleic acid was more abundant in the PE fraction of Olbaril hybrid than in Trisun 870 and synthesised at an early stage in the TG of Olbaril. Furthermore, linoleic acid was systematically present in the PE fraction of sunflower mutants, whereas it was negligible in the TG fraction. These last results underlined two specific metabolisms for storage or structural metabolism for desaturation of fatty acids in sunflower.

5 - ACKNOWLEDGEMENTS

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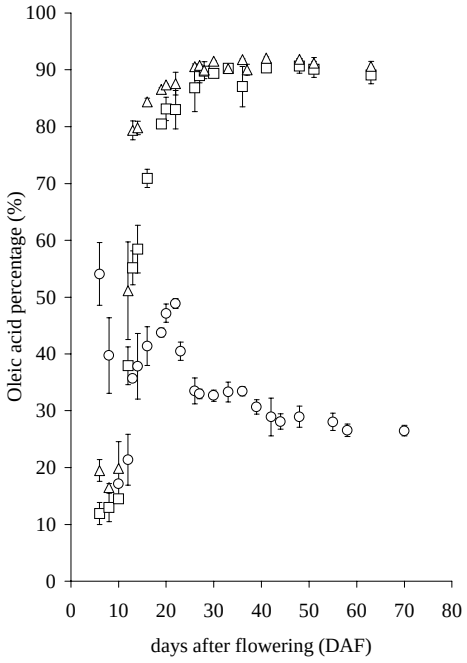

Figure 1 : Evolution of oleic acid percentage (%) in 1997 for three sunflower hybrids , 2 oleic (Olbaril [□] and Trisun 870 [Δ]) and 1 non oleic (Santiago [○])

Table 1 : Percentage of oleic acid (C18:1) and linoleic acid (C18:2) in triglycerides (TG) and phosphatidylethanolamine (PE) in seeds of 2 oleic sunflower hybrids (Olbaril and Trisun 870) and 1 non oleic hybrid (Santiago) at three stages of seed filling period.
Each value is the mean of three determinations. CV was less than 5%

		Santiago			Olbaril			Trisun 870		
DAF	**Lipid class**	**C18:1**	**C18:2**	**Others**	**C18:1**	**C18:2**	**Others**	**C18:1**	**C18:2**	**Others**
13	TG	2.08	42.26	55.66	32.87	**21.17**	45.96	15.48	**0.50**	84.02
	PE	15.50	20.42	64.08	11.27	34.20	54.53	13.11	37.23	49.66
20	TG	74.79	7.53	17.68	64.71	**3.11**	32.18	87.60	**1.07**	11.33
	PE	23.00	17.02	59.98	11.65	22.95	65.40	25.69	14.05	60.26
55	TG	32.90	51.30	15.80	90.58	**1.54**	7.88	90.28	**1.48**	8.24
	PE	39.69	21.36	38.95	53.24	15.83	30.63	52.54	13.26	34.2

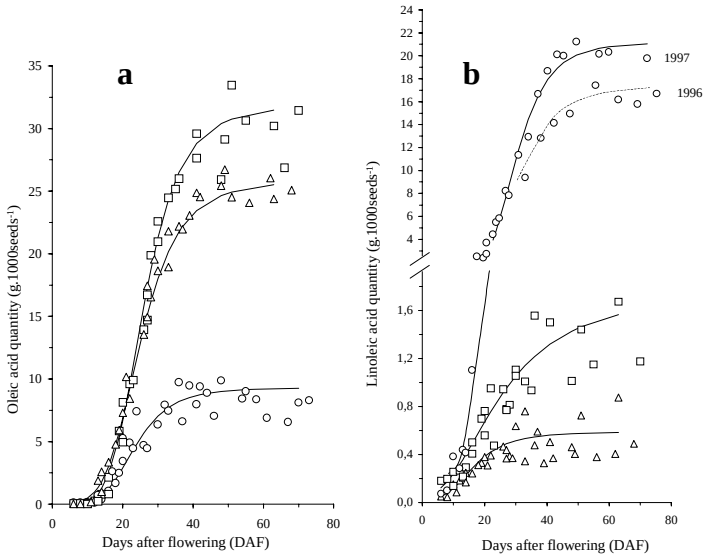

Figure 2 : Evolution of oleic acid (Fig. 3a) and linoleic acid (Fig. 3b) quantity (g.1000seeds^{-1}) during seed filling period for 2 years 1996 and 1997 (data mixed) and for three sunflower hybrids, 2 oleic hybrids : Olbaril (□), Trisun 870 (Δ) ; and 1 non oleic hybrid : Santiago (○).

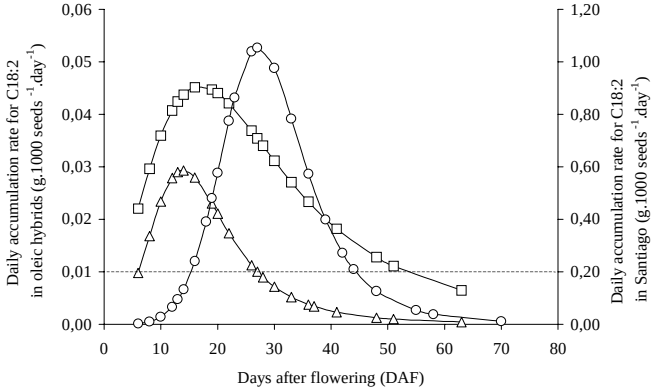

Figure 3 : Daily accumulation rate of oleic acid (g.1000seeds^{-1}.day^{-1}) during seed filling period in 1997, and for three sunflower hybrids, 2 oleic hybrids : Olbaril (□),Trisun 870 (Δ) ; and 1 non oleic hybrid : Santiago (○).

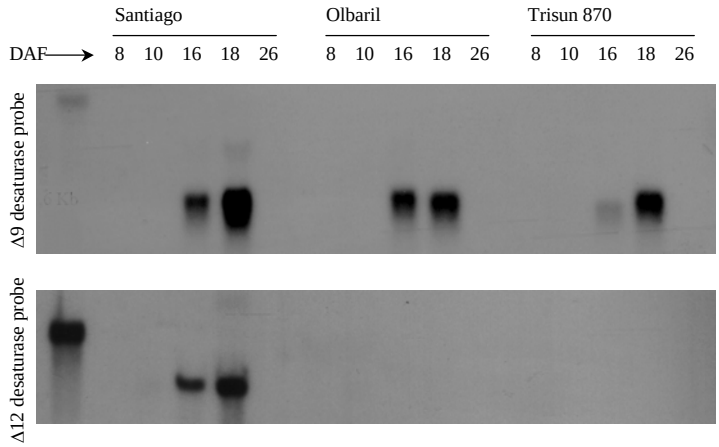

Figure 4 : Δ9- and Δ12-desaturase accumulation transcript during seed filling period in 1997 for three sunflower hybrids, 2 oleic hybrids : Olbaril and Trisun 870 and 1 non oleic hybrid : Santiago. 5 development stages : 8, 10, 16, 18 et 26 DAF