EFFECTS OF TEMPERATURE VARIATIONS ON FATTY ACID COMPOSITION IN OLEIC SUNFLOWER OIL (*Helianthus annuus* L.) HYBRIDS

LAGRAVERE Thierry[(1)], CHAMPOLIVIER Luc[(2)], LACOMBE Séverine[(3)], KLEIBER Didier[(1)], BERVILLE André[(3)], DAYDE Jean[(1)]

[(1)] ESA-Purpan Laboratoire d'Agro-Physiologie, 75 Voie du TOEC, 31076 Toulouse Cedex 03.

Tel 33 5 61 15 30 78 ; Fax 33 5 61 15 30 60 ; Email : dayde@esa-purpan.fr

[(2)] CETIOM, Centre de Biologie Appliquée, Centre de Grignon BP4, 78850 Thiverval-Grignon

Tel 33 1 30 79 95 64 ; Fax 33 1 30 79 95 90

[(3)] ENSAM-INRA, UR Génétique et Amélioration des Plantes, 2 Pl. Viala, 34060 Montpellier Cedex 1

Tel 33 4 99 61 22 33 ; Fax 33 4 99 61 54 15 ; Email : berville@ensam-inra.fr

ABSTRACT

The influence of temperature on fatty acid composition of oil were studied for the three following hybrids, *cv.* Santiago (non-oleic hybrid), *cv.* Olbaril and *cv.* Trisun 870 (high oleic hybrids). Three conditions of diurnal/nocturnal temperatures (30°C/26°C [HTC] ; 24°C/20°C [MTC] ; 18°C/14°C [LTC]) during the first days after the flowering (flowering to flowering plus 300°C.day) were analyzed. Both oleic hybrids exhibited different biochemical behaviours regarding the accumulation of oleic acid. A strong effect of temperature was confirmed for non-oleic hybrids with large levels of variation among the three conditions : a decrease of 15 points of oleic acid content between MTC (48,69%) and LTC (33,64%). In addition for HTC, results indicated a decrease of oleic acid content compared to MTC : - 6,74 points. For oleic sunflower hybrids the influence of those temperature conditions on oleic acid was non-significant (even if the way of variation observed was the same as for the non-oleic genotypes). The hybrids studied seemed to be insensitive to temperature conditions. This result was not in agreement with those previously reported (Champolivier & Merrien, 1996), but the hybrids studied displayed a higher oleic acid content genetic potential than those of this above-mentioned study. The evolution of oleic acid content during seed maturation showed that it depends on time and not on heat sum (day-degree) after flowering. This observation was consistent with the postulate of a lack of temperature influence for oleic hybrids. At the molecular level, mRNA accumulation of Δ9- and Δ12- desaturase genes also seemed only influenced by time and not by temperature after flowering. Accumulation for Δ12- desaturase mRNA was very low for both oleic hybrids, with no difference between them. Our results suggest that both oleic hybrids were insensitive to the temperature conditions applied. Moreover, for the non-oleic hybrid we noticed that there is probably an optimum of temperature for oleic acid accumulation in the seed.

Key words : oleic sunflower, hybrids, oleic acid, temperature

1 - INTRODUCTION

Modification in fatty acid composition and oil content in sunflower seeds (*Helianthus annuus* L.) have been studied intensively these last years. Sunflower oil exhibits large amounts of unsaturated fatty acid mainly composed by linoleic acid (C18:2 ; more than 60% in oil from the South of France) and oleic acid (C18:1 ; 30% of oil). Nevertheless, an oleic sunflower mutant that exhibited a high level of C18:1, up to more than 90% (Garcés *et al.*, 1989a) has been created by chemical mutagenesis (Soldatov, 1976). If the genotype of the plant is the most important factor that defines the fatty acid composition (Knowles, 1988), the environmental factors and specially temperature during the seed-filling period can widely affect the oil percentage and the unsaturated fatty acid composition of oil. Indeed the C18:1/C18:2 sunflower ratio increases for high temperatures during seed maturation but, on the contrary, decreases in lower temperature conditions (Trémolières *et al.*, 1982). The action of temperature on C18:1/C18:2 sunflower ratio affect both Δ12- desaturase enzyme activity and the level of accumulation for Δ12- desaturase transcript. Elevated temperatures weakly enhance the level of transcript accumulation (Kabbaj *et al.*, 1996), but strongly inhibit the enzyme activity when temperature reaches more than 20°C (Garcès & Mancha, 1991). In those conditions it appeared that the oleic acid increases for high temperature conditions during seed-filling period. Nevertheless temperature effects on oleic sunflower remain unclear. For Garcés & Mancha, (1989b) the C18:1/C18:2 ratio is weakly influenced by growth temperatures, but in a more recent study Champolivier & Merrien, (1996) demonstrated that the effect of temperature during seed maturation significantly influences the final fatty acid composition (+7 points of oleic acid at 27°C/22°C -day/night- compared to at 16°C/10°C).

In order to understand the effects of temperature on fatty acid composition in oleic sunflower mutant oil from new generation hybrids, we developed an approach based on growth chamber experiment. The role of temperature in regulating the C18:1/C18:2 ratio in storage lipids will now be discussed.

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 cultivated in a growth chamber at "Centre de Biologie Appliquée - CETIOM " in Saint-Pathus (France) in 12-litre pots filled with field soil (alluvium-clayey soil). Plants were grown in 16h daily-light period with a light intensity of 300 $\mu E.m^{-2}.s^{-1}$. At early stage (B_4) (Merrien, 1992) each pot was thinned to contain only 2 plants at a similar stage of development. Water supply and fertilisation were performed according to the usual method.

In order to avoid the effect of temperature on fatty acid composition we used 3 temperature conditions from F1 stage (flowering) to 300 C°.day[1]. : a high temperature condition (HTC) 30°C/26°C (day/night : 22 C°.day), a middle temperature condition (MTC) 24°C/20°C (day/night : 16 C°.day) and a low temperature condition (LTC) 18°C/14°C (day/night : 10 C°.day). For both the vegetative growth and the post flowering stage (after 300 C°.day) we used identical temperature conditions : 26°C/18°C (day/night : 16 C°.day).

All inflorescences were bagged prior to anthesis, to prevent cross pollination between oleic and non-oleic sunflowers. In addition, only the three outer rows of each inflorescence were

[1] Daily sum of temperature was calculated as [(T°Max + T°Min) / 2]-6°C.

collected and pooled to conserve plant material of identical development stage. This was repeated for each pot at 200 C°.day, 300 C°.day, 500 C°.day and at maturity. Oil samples were obtained by hexane extraction. Fatty acids were analyzed by gas chromatography (GC) (Fisons Instruments GC 8000 Series - USA) according to the conventional method. For northern blot analyzes mRNA extraction and quantification were performed on immature embryos (200 and 300°C.day) according to the conventional method. A standard analyzis 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

The fatty acid composition of non-oleic sunflower oil is strongly dependent on temperature conditions during seed-filling period (Harris *et al.*, 1978). Nevertheless the influence of such a factor was still to be evaluated for new oleic sunflower hybrids.

Results are presented in ***Table 1***. The influence of temperature on saturated fatty acids appeared to elevate the level of C16:0 in high and low temperature conditions (4.07 % in HTC ; 3.48 % in LTC *vs.* Only 3.04 % in MTC).

Non-oleic hybrids showed a strong influence of temperature for oleic acid content according to the previous observation (Champolivier & Merrien, 1996). Indeed for the hybrid we observed an influence of temperature on oleic acid content during the first part of the filling period (until 300°C.day). The higher oleic acid content (48.69 %) was obtained for the MTC (*vs.* 41,95 % in HTC and 33,64 % in LTC). This observation showed that an optimum of temperature could exist for non-oleic hybrids. Nevertheless, this optimum cannot be related to the higher temperature condition as previously reported by Canvin (1965). This optimum of temperature probably acts on Δ9- and Δ12- desaturase gene expression or activity.

For oleic hybrids, on the contrary, we cannot observe a significant influence of temperature on oleic acid level (even if the level reached for both hybrids in the three conditions of temperature had the same behaviour than Santiago). Moreover both oleic sunflower hybrids exhibited the same oleic acid level (average for the three conditions of temperature 92.42 % *vs.* 92.44 % for Olbaril and Trisun 870).

Expressed in ($g.1000seeds^{-1}$) the quantity of oleic acid was not dependent on the sum of temperature received during the first days after the flowering (Data not shown). As for the oleic acid percentage the maximum was reached for the MTC for all the hybrids. On the contrary we observed a correlation between sum of temperature and linoleic acid content. This could permit in the future to determine the level of oleic acid in a field before harvest for a selective valorization, according to the quality of the oil (oleic acid content).

We followed the evolution of fatty acid during the seed-filling period according to the sum of temperature. Concerning the evolution of oleic acid we were surprised because at 200°C.day the highest level of oleic acid was reached for the lower temperature condition for all hybrids. This observation was in opposition with the results previously reported by many authors as Canvin (1965) and Trémolières *et al.*, 1982. (88.37 %, 73.22 % and 48.91 % for Olbaril in LTC, MTC and HTC ; 87.64 %, 82.38 % and 65.18 % for Trisun 870 in LTC, MTC and HTC *vs.* 47.11 %, 54.07 % and 55.22 % for Santiago in LTC, MTC and HTC).

In order to explain such a result, we compared the evolution of fatty acids according to day after flowering rather than sum of temperature (***Figure 1***). In such a case we observe for both oleic hybrids a superposition of the curves (for each temperature condition). These results confirmed in fact the lack of influence of temperature during seed-filling period on oleic acid accumulation in the case of oleic hybrids that was previously observed at maturity stage. On

the contrary the non-oleic hybrid exhibited a strong influence of temperature on fatty acid accumulation confirmed at maturity stage.
The level of hybridization signal obtained for the northern blot using either Δ9- desaturase cDNA, Δ12- desaturase or 18S rRNA as a probe are reported in ***Table 2***. The accumulation of desaturase transcript was ended before 500°C.day which corresponds to previous studies (Kabbaj *et al.*, 1996). The maximum accumulation was at 10 DAF for Δ9- desaturase and 20 DAF for Δ12- desaturase. For Δ9- desaturase we cannot see any difference between oleic hybrids, and the signal seemed weakly enhanced in comparison with non-oleic hybrid results. The level was more important at 300°C.day than 200°C.day excepted for the LTC where no signal can be measured at 300°C.day. Those results were once more time in agreement to the fact that the fatty acid accumulation was independent of sum of temperature. For Δ12- desaturase in oleic hybrids we observed very weak signals which were not measurable. On the contrary we noticed a strong accumulation for Santiago.

4 - CONCLUSION

The influence of temperature conditions during the first part of the seed-filling period confirmed the results previously reported for non-oleic hybrids. The level of oleic acid increased with temperature, but we notice in our case that this influence is stronger for MTC (24°C/20°C) than for HTC (30°C/26°C) suggesting the existence of an optimum for temperature condition. The variation of oleic acid level can reach more than 15 points oleic acid between MTC (48.69 %) and LTC (33.64 %).
In addition we were unable to demonstrate an effect of temperature on oleic acid level for both oleic hybrids, and the level of variation was limited to 1.5 points maximum.
Those results were not in agreement with those previously reported by Champolivier & Merrien (1996) who suggested an effect of temperature on oleic acid in oleic sunflower hybrids. This difference could be related to the difference in the hybrids studied. Those authors used other hybrids characterized by a lower oleic acid potential (about 80% oleic acid only). Those hybrids with lower oleic acid potential could be more sensitive to environmental conditions such as temperature, which would explain the limited level reached by those hybrids.
This insensitivity of oleic hybrids was confirmed by kinetic and molecular studies which suggested that the accumulation of oleic acid in sunflower seeds could be regulated by the development stage (DAF) rather than by the sum of temperature. The action of temperature on both desaturase activities (especially for Δ9- desaturase) is still to be studied in order to complete the possible level of temperature action.

5 - BIBLIOGRAPHIE

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	C16	C16:1	C18	C18:1	C18:2	C18:3
Hybrides (H)						
Santiago	4.28^{a}	0.07^{b}	2.90^{a}	41.43^{b}	51.21^{a}	0.11
Olbaril	3.07^{b}	0.10^{b}	2.25^{c}	92.42^{a}	2.05^{b}	0.10
Trisun 870	3.23^{b}	0.13^{a}	2.55^{b}	92.44^{a}	1.53^{c}	0.11
Temperature (T)						
18°C/14°C (LTC)	3.48^{b}	0.08^{b}	2.78^{a}	72.18^{c}	21.34^{a}	0.14^{a}
24°C/20°C (MTC)	3.04^{c}	0.06^{c}	2.55^{b}	78.67^{a}	15.61^{c}	0.08^{c}
30°C/26°C (HTC)	4.07^{a}	0.16^{a}	2.37^{b}	75.44^{b}	17.85^{b}	0.11^{b}
H x T						
Santiago LTC	4.75^{a}	0.07	2.78^{b}	33.64^{d}	58.62^{a}	0.13
Santiago MTC	3.57^{c}	0.03	3.35^{a}	48.69^{b}	44.27^{c}	0.09
Santiago HTC	4.51^{a}	0.12	2.57^{bc}	41.95^{c}	50.75^{b}	0.11
Olbaril LTC	2.83^{d}	0.09	2.40^{bcd}	91.30^{a}	3.25^{d}	0.13
Olbaril MTC	2.79^{d}	0.06	2.09^{d}	93.43^{a}	1.56^{d}	0.07
Olbaril HTC	3.59^{c}	0.14	2.27^{cd}	92.55^{a}	1.33^{d}	0.11
Trisun 870 LTC	2.85^{d}	0.09	3.16^{a}	91.59^{a}	2.15^{d}	0.15
Trisun 870 MTC	2.76^{d}	0.08	2.21^{cd}	93.88^{a}	0.98^{d}	0.09
Trisun 870 HTC	4.10^{b}	0.21	2.28^{cd}	91.84^{a}	1.46^{d}	0.11

Number followed by the same superscripts within the same group do not differ significantly at the 0.05 probability level according to the LSD (Newman & Keuls test) comparison of means ; the absence of letter indicates non significant effects.

Table 1 : Fatty acid composition at maturity stage for three sunflower hybrids, 2 oleic hybrids - Olbaril and Trisun 870 - and 1 non-oleic hybrid - Santiago - in 3 temperature conditions (diurnal/nocturnal : 18°C/14°C ; 24°C/20°C ; 30°C/26°C).

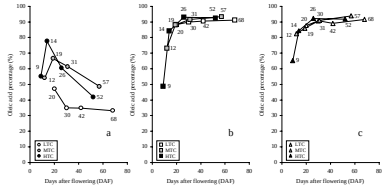

Figure 1 : Oleic acid evolution for three sunflower hybrids, 1 non-oleic hybrid – Santiago [**a**] - and 2 oleic hybrids - Olbaril [**b**] and Trisun 870 [**c**] - (diurnal/nocturnal : 18°C/14°C ; 24°C/20°C ; 30°C/26°C)

		Δ9- desaturase				Δ12- desaturase		
		Santiago	**Olbaril**	**Trisun 870**		**Santiago**	**Olbaril**	**Trisun 870**
LTC 14°C/18°C								
°C.day								
200	18S rRNA	46,33	62,37	91,18	18S rRNA	46,33		
	Δ9- desaturase	11,57	32,60	45,69	Δ12- desaturase	69,08		
	Δ9/18S	**0,25**	**0,52**	**0,50**	**Δ12/18S**	**1,49**	-	-
300	18S rRNA				18S rRNA			
	Δ9- desaturase				Δ12- desaturase			
	Δ9/18S	-	-	-	**Δ12/18S**	-	-	-
MTC 20°C/24°C								
°C.day								
200	18S rRNA	59,76	66,71	56,19	18S rRNA	59,76		
	Δ9- desaturase	24,00	18,50	16,98	Δ12- desaturase	76,71		
	Δ9/18S	**0,40**	**0,28**	**0,30**	**Δ12/18S**	**1,28**	-	-
300	18S rRNA	18,99	44,36	35,27	18S rRNA	18,99		
	Δ9- desaturase	6,95	27,38	19,17	Δ12- desaturase	16,33		
	Δ9/18S	**0,37**	**0,62**	**0,54**	**Δ12/18S**	**0,86**	-	-
HTC 26°C/30°C								
°C.day								
200	18S rRNA	46,61	49,63	53,57	18S rRNA	46,61		
	Δ9- desaturase	4,38	0,00	12,77	Δ12- desaturase	89,22		
	Δ9/18S	**0,09**	**0,00**	**0,24**	**Δ12/18S**	**1,91**	-	-
300	18S rRNA	48,58	21,98	36,33	18S rRNA	48,58		
	Δ9- desaturase	15,73	46,66	16,13	Δ12- desaturase	66,50		
	Δ9/18S	**0,32**	d.n.d.	**0,44**	**Δ12/18S**	**1,37**	-	-

d.n.d. : Data not determined

Table 2 : Hybridization signal (x 10000) for each specific cDNA Δ9- desaturase, Δ12- desaturase and 18S rRNA as a probe.