VARIATION OF FATTY ACID CONTENT IN SEEDS UNDER SCARCE WATER RESOURCES FOR OLEIC AND STANDARD SUNFLOWERS

J. Roche, A. Bouniols, and **T. Barranco,** INRA, Centre de Toulouse, UMR 1248, Chemin de Borde Rouge, BP 27, 31326 Castanet-Tolosan, France
E-mail: roche@toulouse.inra.fr

Z. Mouloungui, INRA, Centre de Toulouse, UMR 1010, Lipo-Oléo-Chimie, 118, route de Narbonne, 31077 Toulouse, Cedex 4, France

Abstract

Environmental factors, mainly temperature and water availability, have greatly influenced biochemical sunflower seed composition during reproductive development. To overcome scarce water resources, cultural practices such as delayed sowing and irrigation timing can be used. In this study the impact of water availability during the postflowering period on fatty acid content was investigated, in order to determine how to manage seed quality through cultural practices and genotype choice under water constraint. Two hybrids (oleic and standard) and one population were tested with conventional and late sowing dates associated with nonirrigated and irrigated treatments in field experiments at Toulouse during several years presenting contrasting climates. According to the intensity of water deficit during the F1-M0 period, the fatty acid pattern is modified under irrigation, particularly for standard hybrids which are more sensitive to water supply compared to oleic ones. Under irrigated treatments, major enhancement of oleic acid content in seeds is associated with a concomitant reduction of linoleic acid content and a decrease of saturated fatty acid content. The delay of sowing effect corresponds to an increase in oleic acid content in standard hybrid seeds irrespective of the climate sequences during reproductive phases, whereas in oleic hybrids the content remains relatively stable. Moreover, a high rate of oleic acid is reached under warmer mean temperatures during the F1-M0 period for standard and oleic genotypes. In conclusion, it appears there are several drought adaptation strategies for fatty acid accumulation according to the scarcity of water resources and to the genetic potential, in response to crop management.

Introduction

The sunflower crop has been expanding for 20 years (Pouzet and Delplancke, 2000) in the Mediterranean countries, where drought frequently occurs which is due to its ability to tolerate short periods of water deficit (Hattendorf et al., 1988). The main interest in sunflower is based on the diversity of fatty acid composition in seed oil. But the crop is in competition with other oil crops, such as rapeseed, which is less tolerant of drought. Therefore, the production quality presents a double interest in providing an added value to the yield for human nutrition as well as for industrial purposes, such as cosmetics and pharmaceuticals, and lubricants or paints. The fatty acid pattern of sunflower seeds depends on the genetic potential (Unger, 1980) and the environmental conditions.

The effect of high temperature during seed filling under day and night periods shows an increase of oleic acid content and a decrease of linoleic acid content (Izquierdo et al., 2002; Champolivier and Merrien, 1996; Piva et al., 2000). For standard hybrids, the reduction of oleic acid following a drop of mean temperature was recorded at different growth stages: at the beginning of flowering (Merrien et al., 1993), physiological maturity (Harris et al., 1978), and seed harvest (Nagao and Yamakasi, 1984). For oleic hybrids, the effect of temperature variation is not distinguishable (Lagravère et al., 2000). In the field, temperature impact could be evaluated using delayed sowing date by monitoring mean temperature and duration of reproductive phases. In a study over several years, Roche et al. (2004) demonstrated that delayed sowing of one month between mid-April and mid-May induces an increase of the oleic and linoleic acid ratio in standard genotypes (hybrids and populations) due to the longer duration of the F1-M0 period. In the case of early sowing (mid-April), Flagella et al. (2002) found the same assumptions in seeds of two oleic hybrids.

Data on the effect of water availability on seed oil composition are often inconsistent due to various experimental conditions (intensity and timing of water stress) that modify the crop response and foliate transpiration and development (Flénet et al., 1996). Baldini et al. (2000) showed that water stress induces an increase of oleic acid in standard hybrid (about 5%) and a decrease of its percentage in oleic hybrids (15%), with no variation in stearic and palmitic acids. In contrast, an increase of these saturated fatty acids may be associated with a water deficit during seed-filling (Piva et al., 2000).

Thus, cultural practices are a way to manage biochemical composition of seeds by taking into account soil and climate conditions during lipid accumulation. The goal of this present study is to analyze the influence of delayed sowing and water availability during the reproductive period on fatty acid pattern and to test it in years presenting contrasting climate sequences.

Materials and Methods

Experiments were carried out in Toulouse-Auzeville on Rotation-Quality tests at the "Institut National de la Recherche Agronomique" (INRA) in southwestern France (43.551°N latitude) during two years which presented contrasting climate sequences (year I (YI): 2002 and year II (YII): 2003) on a deep calcareous clay silt soil with high water-holding capacity (Cabelguenne et al., 1999). Three genotypes were investigated: i) one standard hybrid (Santiago II) provided by North Company Krups (now Syngenta), ii) an oleic hybrid (Proleic 204) from Rustica (now Euralis Soltis), and iii) a population (Ichraq) from INRA-Morocco.

Field Setup and Cultural Practices. The treatments and genotypes used are presented in Table 1. Sunflower was sown in mid-April (conventional sowing date, SD1) and one month later for the late sowing date (mid-May, SD2). Two contrasted water regimes were applied corresponding to: i) a nonirrigated treatment, and ii) an irrigated treatment. Irrigation with automatic sprinklers was applied after flowering during the seed ripening period in order to satisfy nearly 70 % of all sunflower water requirements. Irrigation amount was determined via a meteorological approach considering variations of cultural coefficients with sunflower phenological stages (Picq, 1989) and based on transpiration potential (TP), rainfall, mean temperature and total radiation collected from the meteorological station of INRA.

Experiments were conducted in a split-plot design with three replications, (elemental plot: 126 m sq. in YI and 180 m sq. in YII) were associated with water regimes. Seeds were dried in a ventilated vacuum oven for 48 h at 80C in order to obtain 0% moisture.

Table 1. Cultural practices and genotypes tested during the two experimental years.

Year	Treatments		Genotypes		
	Sowing dates	Water regimes	Populations	Standard hybrid	Oleic hybrid
I	Conventional	Non irrigated	Ichraq	Santiago II	Proléic 204
	Late	Irrigated		Santiago II	Proléic 204
II	Conventional	Non irrigated		Santiago II	Proléic 204
	Late	Irrigated	Ichraq	Santiago II	Proléic 204

Years I (YI) and II (YII) corresponding to 2002 and 2003, respectively.

Climate Sequence Characterization. With regard to the total rainfall amount, YII was drier than YI (200 mm more in YI) during all phenological phases, accentuated during the reproductive phases (F1 to M2) (Table 2). According to the mean temperature, YII was

Table 2. Rainfall, mean temperature, temperature sums and water deficits (WD) in the two experimental years at different physiological phases.

Years	Sowing dates	Parameters	Physiological phases				Total
Year I			S-F1	F1-M0	M0-M2	M2-Maturity	
	SD1	Rainfall (mm)	191.0	31.0	33.0	40.5	295.5
		Mean temperature (°C)	16.7	20.4	20.6	18.6	
		Temperature sum (°Cd)	834.7	220.1	406.9	332.3	1793.9
		WD Nirr (mm)	-41.5	**-40.7**	-59.9	-19.3	
		WD Irr (mm)	-41.5	**-0.7**	-59.9	-19.3	
	SD2	Rainfall (mm)	184.5	31.5	40.5	15.0	271.5
		Mean temperature (°C)	19.1	20.1	18.6	18.3	
		Temperature sum (°Cd)	801.2	308.1	311.9	136.9	1558.0
		WD Nirr (mm)	-73.6	**-54.6**	-22.4	-8.8	
		WD Irr (mm)	-73.6	**-24.6**	-22.4	-8.8	
Year II							
	SD1	Rainfall (mm)	71.0	2.5	0.0	21.0	94.5
		Mean temperature (°C)	18.8	22.8	25.3	25.9	
		Temperature sum (°Cd)	872.3	258.0	292.9	654.8	2077.9
		WD Nirr (mm)	-92.1	**-74.9**	-80.9	-105.5	
		WD Irr (mm)	-92.1	**-19.5**	-92.0	-90.9	
	SD2	Rainfall (mm)	21.5	0.0	9.5	41.0	72.0
		Mean temperature (°C)	23.3	24.2	27.3	21.6	
		Temperature sum (°Cd)	895.1	278.7	324.9	469.1	1967.8
		WD Nirr (mm)	-149.9	**-87.5**	-66.1	-41.9	
		WD Irr (mm)	-149.9	**-20.8**	-65.0	-33.3	

SD1: conventional sowing date, SD2: late sowing date, Nirr: non irrigated treatment, Irr: irrigated treatment, WD: water deficit, S: sowing date, F1: flowering onset, M0: seed ripening onset, M2: end of seed ripening. Water deficit is expressed for irrigated treatment by deviation in mm from the water regime corresponding to 70% of all requirements of sunflower growth cycle.

warmer whatever the sowing date, and was characterized by a constant increase of 2C from flowering stage (F1) to maturity. In term of temperature sums, only the M2-maturity period was significantly higher in YII compared to YI. The greatest difference of temperature sums between the two sowing dates were recorded in YI during the beginning of seed filling until M2 (F1-M0: 88 and 21C d; and M0-M2: 95 and 32C d in YI and YII respectively). Consequently, water deficits were stronger in YII compared to the YI. The nonirrigated treatment presented stronger water deficits in YII compared to YI. Besides, water constraints appeared more severe in sowing date SD2 compared to SD1.

Analysis of Fatty Acids. Analyses were carried out on three samples/plot/genotype/treatment (12 replications). Fatty acid contents were analyzed with preferred methods of the "Oléo-Lipo-Chimie" Laboratory of "INRA-Transformation des Végétaux." Total fatty acid content was analyzed by gas chromatography (Varian GC-3800) directly on dried and ground seed according to the ISO 5509: 1990 Norm, and by comparing the retention time of FAME Mix identified integration peaks of rapeseed oil (SUPELCO, USA).

Statistical Analysis. Collected data were analyzed by ANOVA performed on the SigmaStat statistical package (version 2.0, USA). Within a year, the different genotypes, sowing dates and/or water regime treatments and their interactions on major fatty acid contents were separately analyzed. Differences between mean values were obtained using Student-Newman-Keuls test.

Results

The analysis concerns mainly two saturated (palmitic and stearic) and two unsaturated (oleic and linoleic) fatty acids and other detected fatty acids (myristic, arachidic, linolenic, and behenic), which did not exceed 2 % of total fatty acids, showed modest variations and were not taken into account.

Impacts of sowing date, water regime and their interactions with genotype on fatty acid contents are shown in Table 3.

Table 3. Effects of genotype (G), sowing dates (SD), water regimes (WR) and interactions within years (I and II).

Variable	Year		Genotype	Sowing date	Water regime	G*SD	G*WR	SD*WR	G*WR*SD	Residual
C16 :0 (%)	I	MS	84.150	2.369	0.0187	2.857	0.00304	0.0247	0.00634	0.0218
		P	***	***	NS	***	NS	NS	NS	
C18 :0 (%)	I	MS	49.795	0.473	0.924	0.0216	0.224	0.0241	0.00120	0.0384
		P	***	***	***	NS	NS	NS	NS	
C18 :1 (%)	I	MS	65791.387	236.756	4.594	792.810	2.509	1.938	1.893	4.571
		P	***	***	NS	***	NS	NS	NS	
C18 :2 (%)	I	MS	58643.258	181.583	1.342	725.395	1.758	0.907	1.231	4.088
		P	***	***	NS	***	NS	NS	NS	
C16 :0 (%)	II	MS	9.461	0.632	2.608	0.00576	0.203	0.492	0.0684	0.00707
		P	***	***	***	NS	***	***	***	
C18 :0 (%)	II	MS	1.303	1.408	0.884	0.0701	0.0779	0.109	0.149	0.0190
		P	***	***	***	NS	NS	NS	***	
C18 :1 (%)	II	MS	7231.499	64.187	186.914	34.092	29.972	2.050	5.010	2.123
		P	***	***	***	***	***	NS	NS	
C18 :2 (%)	II	MS	6687.188	84.028	115.061	29.336	22.822	0.127	5.382	2.016
		P	***	***	***	***	***	***	NS	NS

* : significant for p<0.05, ** : significant for p<0.01, *** :significant for p<0.001, NS: nonsignificant, MS: Mean Square, p: probability value.

Sowing Date* x *Genotype Interaction. Very high significant effects of sowing date are detected in palmitic, oleic and linoleic acid contents that may be reversed according to the experimental year (Table 3). In YI, SD2 corresponds to a reduction of the palmitic acid content for the standard genotype (-10.8%) whereas the content in Proléic 204 seeds remains unaffected (Table 4). Concerning stearic acid content, the oleic genotype presents a decrease (-5.6%) in SD2 whereas the content in Santiago II seeds does not fluctuate. In YII, delayed sowing involves an increase in the two saturated fatty acids for all genotypes (from +3.8% for Santiago II to +7.2% for Proléic 204 and from +6.8% for Santiago II to +15.8% for Proléic 204 in palmitic and stearic acid content respectively). Generally, delay of sowing is associated with the accumulation of oleic acid content in seeds of the standard genotype Santiago II (+26.2% and +14.6% in YI and YII respectively), whereas the rate in oleic genotype seeds is unchanged in YII or slightly decreased in YI. The linoleic content presents the inverse variations because of the close correlation between the oleic and linoleic acids (Roche et al., 2004).

Table 4. Effects of sowing date, water regime treatments for each genotype on palmitic (C16:0), stearic (C18 :0), oleic (C18 :1), and linoleic (C18 :2) acid content in Y I a) and II b).

a)

Year I	Genotypes	Santiago II			Proléic 204			Ichraq	
Variables	Sowing dates	Nirr	Irr	Average	Nirr	Irr	Average	Nirr	Irr
C16:0 (%)	SD1	5.54 a	5.57 a	5.55 a	3.35 a	3.33 a	3.34 a	5.60 a	nd
	SD2	4.93 a	4.86 a	4.90 b	3.40 a	3.34 a	3.37 a	nd	5.67 a
Average		5.23 a	5.22 a		3.37 a	3.33 a			
C18:0 (%)	SD1	4.50 a	4.36 a	4.43 a	3.18 a	2.86 a	3.02 a	3.73 a	nd
	SD2	4.35 a	4.29 a	4.32 a	2.98 a	2.71 a	2.85 b	nd	3.28 a
Average		4.42 a	4.32 a		3.08 a	2.79 b			
C18:1 (%)	SD1	24.74 a	25.42 a	25.08 b	82.80 a	83.57 a	83.19 a	24.97 a	nd
	SD2	34.19 a	33.74 a	33.97 a	80.20 a	80.96 a	80.58 b	nd	22.05 a
Average		29.47 a	29.58 a		81.50 a	82.26 a			
C18:2 (%)	SD1	63.44 a	63.06 a	63.25 a	8.56 a	8.08 a	8.32 b	64.01 a	nd
	SD2	54.77 a	55.23 a	55.00 b	11.34 a	10.80 a	11.07 a	nd	67.26 a
AAverage		59.11 a	59.14 a		9.95 a	9.44 a			

b)

Year II	Genotypes	Santiago II			Proléic 204			Ichraq		
Variables	Sowing dates	Nirr	Irr	Average	Nirr	Irr	Average	Nirr	Irr	Average
C16:0 (%)	SD1	5.72 b	5.26 c	5.48 b	3.88 b	3.82 b	3.85 b	5.52 b	5.13 c	5.32 b
	SD2	6.03 a	5.38 c	5.70 a	4.38 a	3.91 b	4.15 a	6.20 a	5.01 c	5.61 a
Average		5.87 a	5.32 b		4.13 a	3.86 b		5.86 a	5.07 b	
C18:0 (%)	SD1	4.06 a	3.60 b	3.83 b	3.03 c	3.04 c	3.04 b	3.46 b	3.30 b	3.38 b
	SD2	4.21 a	4.02 a	4.11 a	3.76 a	3.45 b	3.61 a	4.10 a	3.33 b	3.71 a
Average		4.13 a	3.81 b		3.39 a	3.25 a		3.78 a	3.31 b	
C18:1 (%)	SD1	34.94 d	40.76 b	37.85 b	84.82 a	83.84 a	84.33 a	38.14 b	45.54 a	41.84 a
	SD2	41.39 c	47.24 a	44.32 a	82.91 a	85.80 a	84.35 a	40.18 b	46.54 a	43.36 a
Average		38.16 b	44.00 a		83.86 a	84.82 a		39.16 b	46.04 a	
C18:2 (%)	SD1	53.45 a	48.70 b	51.07 a	6.34 a	7.40 a	6.87 a	51.03 a	44.35 b	47.69 a
	SD2	46.90 c	41.97 d	44.43 b	6.93 a	5.05 a	5.99 a	48.17 a	43.91 b	46.04 a
Average		50.17 a	45.33 b		6.64 a	6.22 a		49.60 a	44.13 b	

SD1: conventional sowing date. SD2: late sowing date; Nirr: nonirrigated treatment; Irr: irrigated treatment. For each year, mean values with the same letter are not significantly different at the threshold of 5 %. Nd: not determinate.

Water Regime* x *Genotype Interaction. The effect of water regime x genotype interaction on fatty acid content is highly significant (Table 3); but within the genotype, the response to water management depends on the years. Globally, in seeds of the oleic genotype, no variation is observed under different water regime treatments, whatever the experimental year; fatty acid content is quite stable except for stearic acid content in YI (-9.4%) and palmitic acid content in YII (-6.5%) for which water supply induces a decrease of the rate (Table 4). Concerning standard genotypes, in YI no modification in fatty acid content is noticed whereas in YII, the water supply is associated with a reduction of the saturated fatty acid (C16:0 and C18:0) content (from -8.6 to 13% average for Santiago II and Ichraq respectively). Oleic acid percentage is significantly enhanced under the irrigated treatment (from +13.3% to +14.9% for Santiago II and Ichraq respectively) while the linoleic acid rate is depressed.

Sowing Date* x *Water Regime* x *Genotype Interaction. The major effect appears only on saturated fatty acid content and in YII. Globally, significant higher rates of palmitic acid are obtained in SD2 under irrigated treatment irrespective of the genotype (from 4.38 to 6.20% for Proléic 204 and Ichraq respectively). In Proléic 204 seeds, stearic acid content is higher under SD2 associated with irrigation. Concerning the unsaturated fatty acids and standard hybrids grown in YII, SD2 and water supplies combine to increase oleic acid content from 10 to 15% compared to SD1 in the nonirrigated treatment. The higher rate of linoleic acid (63%) is available in SD1 and nonirrigated regime. The maximum percentage in oleic genotype seeds is recorded also under SD2 and the irrigated treatment.

Discussion

Based on temperature variation and water deficit occurring during the reproductive stages, the experimental conditions in this study generated contrasting climate sequences within the years and the planting dates. Therefore, data are discussed according to the differences in climate sequences in the experimental years and their impact according to critical stages of the crop.

Changes in Major Fatty Acid Contents Affected by Planting Date. The delayed sowing corresponded to an increase of the C18:1/C18:2 ratio in mature seeds of standard hybrids. These results were confirmed by Roche et al. (2004), who reported on the main modifications obtained under several others climate sequences. Changes in the level of oleic acid in seeds were modulated by mean temperature during F1-M0 and M0-M2 in YII (enhancement of 2C). According to Garcès and Mancha (1991) an increase of temperature leads to the reversible inhibition of the enzyme Δ-12 desaturase which is involved in the transformation from oleic to linoleic acid (Garcès et al., 1989). The maximum activity was observed during the F1-M0 period (Lagravère et al., 2000). Rondanini et al. (2003) pointed out that a brief period of a temperature increase higher than 35C during the same phase induces an irreversible increase of oleic acid content. So, the increase of oleic acid percentage observed over two years in standard hybrid mature seeds resulted on one hand, in the increase of mean temperature during F1-M0, and on the other hand, in the lengthening of the phase, because delayed sowing also induced an enhancement of the oleic acid content (+9 points) in YI. Regarding the oleic genotype, in YI and YII few variations were recorded as observed by Lagravère et al. (2000). The content of saturated fatty acids varied according to the year. In YI, delayed sowing led to a reduction of saturated fatty acid content (reduction of palmitic acid content in Santiago II seeds and of the stearic acid content in Proléic 204 seeds), whereas in YII, late planting date involved an increase in their percentage irrespective of the genotype. With early sowing, Flagella et al. (2002) also found an increase in the palmitic acid percentage.

Changes in Major Fatty Acid Content Affected by Water Supply. In unsaturated fatty acid content, water supply generated a significant increase in oleic acid content and a concomitant decrease in linoleic acid content only in YII which presented the most contrast in water deficit between irrigated and nonirrigated treatments. Our experiment pointed out the great stability of whole fatty acid composition in the oleic genotype while irrigated management largely modulated the composition in standard genotypes (Ichraq and Santiago II). However, Flagella et al. (2002) observed some fluctuations of C18:1 and of C18:2 in oleic hybrids sown earlier in the growth season. The different level of water deficit in the experiments could explain the divergences. Besides, in our experimental conditions, a strong water deficit during seed filling superior to -70 mm (in YII) is associated with a loss between 10 and 16% of oleic acid. A concomitant increase of 9 to 11% of linoleic acid in standard hybrid seeds was observed and a water depletion of mean intensity (around -50 mm) was conducted to the same but nonsignificant weaker variations (< -3% for oleic acid and < to -1% for linoleic acid contents). These data could be the consequence of different genotype adaptation strategies in regard to producing quality seeds under drought conditions.

Conclusions

The study shows the ability to modify fatty acid composition in sunflower seeds using cultural practices such as delay of sowing and irrigated conditions. This may involve temperature and water stress effects on physiological fatty acid accumulation during the reproductive periods of the sunflower crop (F1-M0 and M0-M2). Regarding the ratio of unsaturated fatty acids (oleic/linoleic acids), the effects of the factors depend on the typology of genotype (standard and oleic) (Figure 1). The combination of a temperature increase higher than 20C during the grain filling period and a moderate water deficit improves the C18:1/C18:2 ratio on standard hybrids and does not affect this ratio in oleic hybrids. In oleic hybrids, only temperature may influence the balance. Our results may support the hypothesis that genotypes develop different adaptation methods for fatty acid elaboration to alleviate scarce water resources and that crop management could be used to modulate fatty acid composition of sunflower seeds. Further works are planned to characterize the differential level of gene expression in lipid metabolism and in drought tolerance of these genotypes.

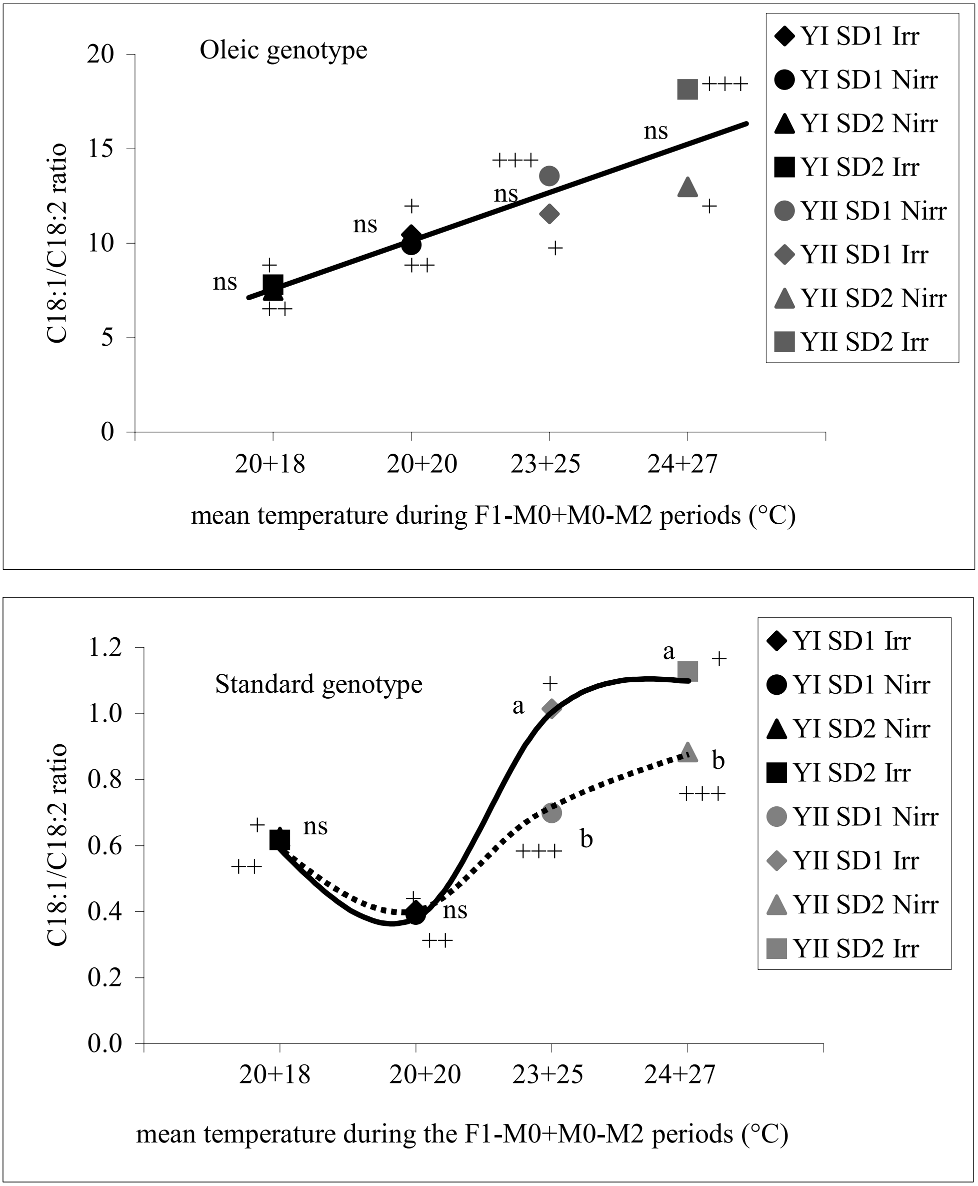

Figure 1. **Top**: An oleic hybrid (Proléic 204) according to the mean temperature during F1-M0 physiological period for the first quantify and M0-M2 physiological period for the second quantify in the two experimental years. **Bottom:** Ratio of unsaturated fatty acids (oleic [C18: 1]/linoleic [C18: 2] acids) for a) a standard hybrid (Santiago II). The symbol + corresponds to a water deficit < -40 mm, ++ corresponds to a water deficit comprising between -40 and -70 mm and +++ corresponds to a water deficit >-70 mm. The different letters correspond to a significant difference between points into the same range of temperature. Ns: nonsignificant. YI: year I, YII: year II, SD1: conventional sowing date, SD2: late sowing date, Nirr: nonirrigated treatment, Irr: irrigated treatment.

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References

Baldini, M., Giovanardi, R., and Vannozzi, G.P. 2000. Effects of different availability on fatty acid composition of the oil in standard and high oleic sunflower hybrids. Proc. 15th International Sunflower Conference. Toulouse (France). Tome I, A. p. 79-84.

Cabelguenne, M., Debaeke, P., and Bouniols, A. 1999. EPICphase, a version of the EPIC model simulating the effects of water and nitrogen stress on biomass and yield, taking account of developmental stages: validation on maize, sunflower, sorghum, soybean and winter wheat. Agricultural Systems. 60:175-196.

Champolivier, L., and Merrien, A. 1996. Evolution de la teneur en huile et de sa composition en acides gras chez deux variétés de tournesol (oléique ou non) sous l'effet de températures différentes pendant la maturation des graines. OCL, 3. p. 140-144.

Flagella, Z., Rotunno, T., Tarantino, E., Di Caterina, R., and De Caro, A. 2002. Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to sowing date and the water regime. European Journal of Agronomy. 17:221-230.

Flénet, F., Bouniols, A., and Saraiva, C. 1996. Sunflower response to a range of soil water contents. European Journal of Agronomy. 5(11):1198-1210.

Garcès, R., Garcià, J.M., and Mancha, M. 1989. Lipid characterization in seeds of a high oleic acid sunflower mutant. Phytochemistry. 28 (10):2597-2600.

Garcès, R., and Mancha, M. 1991. In vitro oleate desaturase in developing in sunflower seeds. Phytochemistry. 30(7):2127-2130.

Harris, H.C, William, J.R., and Mason, W.K. 1978. Influence of temperature on oil content and composition of sunflower seed. Aus. J. Agric. Res. 29:1203-1212.

Hattendorf, M.J., Redelfs, M.S., Amos, B., Stone, L.R., and Gwin, R.E. Jr. 1988. Comparative water use characteristics of six rows crops. Agron. J. 80:80-85.

Izquierdo, N., Aguirrezábal, L., Andrade, F., and Pereyra, V. 2002. Night temperature affects fatty acid composition in sunflower oil dependeing on the hybrid and the phenological stage. Field Crops Research. 77:115-126.

Lagravère, T., Champolivier, L., Lacombe, S., Kleiber, D., Bervillé, A., and Daydé, J. 2000. Effects of temperature variations on fatty acid composition in oleic sunflower oil (*Helianthus annuus* L.) hybrids. Proc. 15th International Sunflower Conference. Toulouse (France). Tome I, A. p. 73-78.

Merrien, A., Champolivier, L., Raimbault, J., and Evrard, J. 1993. Tournesol oléique: premiers facteurs de variation de la composition. Oléoscope . 15:17-9.

Nagao, A, and Yamazaki, M. 1984. Effect of temperature during maturation on fatty acid composition of sunflower seed. Agric. Biology Chemistry. 48(2):53 555.

Picq, G. 1989. Résultats de l'observatoire du tournesol. Ed. CETIOM. p. 105-124.

Piva, G., Bouniols, A., and Mondiès, M. 2000. Effect of cultural conditions on yield, oil content and fatty acid composition of sunflower kernel. Proc. 15th International Sunflower Conference. Toulouse (France). Tome I, A. p. 61-66.

Pouzet, A., and Delpancke, D. 2000. Evolution comparée de la production et de la compétitivité du tournesol dans différentes aires de production. Proc. 15th International Sunflower Conference. Toulouse (France). Tome I, A. p. 1-9.

Roche, J., Essahat, A., Bouniols, A., El Asri, M., Mouloungui, Z., Mondiès, M., and Alghoum, M. 2004. Diversified composition of sunflower (*Helianthus annuus* L.) seeds within cultural practices and genotypes (hybrids and population). Hélia. (in press).

Rondanini, D., Savin, R. and Hall, A. J. 2003. Dynamics of fruit growth and oil quality of sunflower (*Helianthus annuus* L.) exposed to brief intervals of high temperature during grain filling. Field Crops Research. 83:79-90.

Unger, P.W. 1980. Planting date effects on growth, yield, and oil of irrigated sunflower. Agron. J. 72:914-916.