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Variability of Seed Fatty Acid Composition to Growing Degree-Days in High Oleic Acid Sunflower Genotypes

Abstract: High temperature enhances the oleic acid content in the oil of normal cultivars but conflicting results are reported on temperature effects on oleic acid content in HO cultivars: either no effect or an increase in oleic acid content with temperature. To investigate the effects of temperature on HO genotypes under natural field conditions, a three-year field trial was conducted using two sowing dates and three HO genotypes (two inbred lines and one hybrid). To compare our results with previous works, growing degree-days (GDD) were computed (base temperature = 6°C). GDD accumulated during the “flowering – 25 days after flowering” period influenced fatty acid composition of seed. Oleic and linoleic acid contents were affected by accumulated GDD in two HO genotypes (one inbred line and the hybrid). There was an increase of about 3% in oleic acid content as response to more high GDD accumulated. Their content was not modified by GDD in the other inbred line. There was a genotype × environment interaction that we suppose depending on modifier genes. These genetic factors affected oleic acid content. This indicated the importance of breeding targeted to select hybrids with a stable oleic acid content and higher than 90%. Saturated fatty acids (palmitic and stearic) were also influenced by temperature, and there was genetic variability among genotypes.

Keywords: *Helianthus annuus*, planting date, seed oil content, growing degree-days, fatty acids composition, high oleic hybrids

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in the world. Sunflower oil has a wide range of applications, both in the food industry (e.g. margarine production) and as raw material for non-food application (biofuels, oleochemicals). The quality of sunflower seed oil is determined by the content and ratio of fatty acids. It contains both saturated (palmitic and stearic acid) and unsaturated fatty acids (up to 90–95%), either monounsaturated (oleic acid) or polyunsaturated (mostly linoleic and traces of other minor fatty acids). Depending on unsaturated fatty acid composition, sunflower cultivars can be divided into traditional or normal or low oleic (LO $-<40\%$ oleic acid) types, with an oil composition characterized by a majority of linoleic acid, mid-oleic (MO – 65–75% oleic acid) and high oleic (HO $->80\%$ oleic acid).

Previous research has demonstrated that the fatty acid composition of sunflower oil depends on genotypes (LO, MO and HO) and environmental conditions during seed-filling phase (Harris *et al.*, 1978; Champolivier and Merrien, 1996; Roche *et al.*, 2006; Izquierdo and Aguirrezábal, 2008). HO sunflower was obtained by chemical mutagenesis with dimethyl sulfate (DMS) of LO sunflowers (Soldatov, 1976). HO genotypes cumulate the Pervenets mutation effect and other independent factors acting on oleic acid content such as modifier genes (Miller *et al.*, 1987; Fernandez *et al.*, 1999; Velasco *et al.*, 2000; Lacombe *et al.*, 2004). Modifier genes are minor genes having no known effect except to intensify or diminish the expression of a major gene (Briggs and Knowles, 1967). Some genetic variation for oleic acid content among HO genotypes depends on these independent genetic factors (genetic background of parental lines) from Pervenets mutation, and they fixed the genetic oleic acid content potential of HO genotypes. Phenotypic expression of some of these modifiers can be modified by temperature, and some of them have no phenotypic expression at higher temperatures (Velasco *et al.*, 2000).

It has long been known that temperature is the main environmental factor affecting the fatty acid composition in the oil of LO sunflower (Canvin, 1965), mainly regulating the ratio of oleic and linoleic acids. High temperature enhances the oleic acid content of normal cultivars but conflicting results are reported about temperature effects on oleic acid content of high oleic acid cultivars: either no effect (Lagravére *et al.*, 2000) or an increase in oleic acid content with temperature (Champolivier and Merrien, 1996; Triboï-Blondel *et al.*, 2000; Izquierdo and Aguirrezábal, 2008). These differences could be related to differences in the studied hybrids as well as their genetic backgrounds.

Lagravére *et al.* (2000) suggested that hybrids with low oleic acid potential could be more sensitive to environmental conditions such as temperature, while hybrids with a higher genetic oleic acid content potential were insensitive to temperature conditions. Under natural field conditions, the effect of temperature on HO genotypes through a delay in sowing has not been extensively studied. Connor and Sadras (1992) reported that sowing date influences the fatty acid composition by modifying the ontogenesis. The same variation is observed for oleic hybrids (Flagella *et al.*, 2002; Roche *et al.*, 2004, 2006) but the variation between dates of sowing was less (a variation in oleic acid content of about 2%).

Another topic of interest is quantifying the effects of temperature on oleic acid content at different stages in the seed-filling phase in sunflower and thus selecting the critical period. Critical periods are those when the sensitivity to an environmental variable is highest. Izquierdo *et al.* (2002, 2006) and Izquierdo and Aguirrezábal (2008) found increments in oleic percentage with higher night temperatures applied during early stages of grain development (100–300 degree days after flowering, base temperature 6°C), with standard genotypes showing the greatest change and high oleic hybrids the least. Roche *et al.* (2006) hypothesized that changes in the level of oleic acid in seeds are modulated by the mean temperature during the flowering period and also by the temperature sums of all phases. It is not known whether the period in which temperature has maximum effect on fatty acid composition differs among HO genotypes.

The first issue, therefore, in selecting HO hybrids with a very high content in oleic acid content (>94%) is to avoid the effects of modifier on oleic acid content, or in other word the interaction genotype by environment, to maximize its content. The second issue is concerning the ratio oleic–saturated fatty acid content. The saturated fatty acids, palmitic and stearic, are less influenced by environmental conditions than unsaturated fatty acids with small variations between years and locations (Lajara *et al.*, 1990; Izquierdo *et al.*, 2002, 2006; Vick *et al.*, 2004). However, to select HO hybrids with a high and stable oleic acid content, it is important to detect all factors that may reduce or increase its concentration in the seeds.

In this study, we compared two HO inbred lines, the first with a high genetic oleic acid content potential (R978) while the second had a low potential in oleic acid content (342mt) and their hybrid. This work aimed at assessing the grain fatty acid composition of three HO sunflower genotypes in response to growing degree-days (GDD) accumulation and to study variability in seed fatty acid composition among HO genotypes as affected by temperature.

Materials and methods

Plant material

The sunflower (*Helianthus annuus* L.) seeds used in this work were from the inbred lines 342mt and R978, both high oleic inbred lines, and from their hybrid 342 × R978. Line 342mt is a selection, made at the University of Udine, derived by Ha 342 USDA, and it is a male sterility maintainer with a single head. Line R978, selected by the University of Udine, is a fully branched type and is a male fertility restorer. The lines are maintained at the University of Udine, and they have been used in a high oleic breeding program for 20 years. The behaviors, concerning seed fatty acids composition, of these HO lines are well established in the environmental conditions of North-East Italy. R978 inbred line has a high genetic potential in oleic acid content while 342 has a low potential.

Field trials

Inbred lines and hybrid were grown in 2009–2011 on the experimental farm of the University of Udine, Azienda Agraria Universitaria "A. Servadei" (46°04′N, 13°22′E, 109 m a.s.l.), in North-East Italy. The experiment was designed as a completely randomized block scheme, with three replications, using two sowing dates. Plot size was 5 m × 2 m. The seeds were sown with a spacing of 0.75 m between rows. Plants were thinned after seedling emergence from 10 to 7.5 plants m^{-2}. Nitrogen was applied at 100 kg ha^{-1}. Weeds and diseases were controlled, and regular watering throughout the experiment ensured that plants were not subjected to water deficit during the entire growing period. At the R4 stage, all plants were covered with paper bags (Schneiter and Miller, 1981) to prevent cross-fertilization. Five plants were studied per plot.

Meteorological data (Table 1) for the experimental period were recorded at a weather station (Udine – S. Osvaldo Station; Osmer- FVG Region Meteorological Service) located 200 m away from the field site.

Sampling

Harvesting was done when all plants in a given treatment reached physiological maturity (PM), R9 phase (Schneiter and Miller, 1981). Seeds from outer rings

Table 1: Meteorological data for the field site during sunflower growth in 2009–2011.

	2009			2010			2011		
	T_{min} (°C)	T_{max} (°C)	Rainfall (mm)	T_{min} (°C)	T_{max} (°C)	Rainfall (mm)	T_{min} (°C)	T_{max} (°C)	Rainfall (mm)
April	9.2	20.6	131.5	6.7	19.6	75.1	7.9	22.1	18.1
May	13.1	26.1	28.0	11.7	21.6	230.2	11.5	26.3	85.2
June	15.2	26.8	104.2	15.5	27.1	68.7	15.6	26.7	185.1
July	17.1	29.7	104.5	18.1	30.6	143.7	15.8	28.1	148.4
August	18.2	31.8	66.2	16.2	28.0	122.1	16.9	31.3	23.3
September	14.5	26.9	145.6	12.2	23.4	264.8	14.6	29.3	83.9
Mean	14.5	27.0		13.4	25.1		13.7	27.3	
Total			580.0			904.6			544.0

were separated for fatty acid and oil content determination and analyzed. Seeds were dried in an oven at 60°C for 48 h.

To find the real PM, and thus to investigate the relationship between fatty acids contents and temperature, three plants per plots were sampled twice per week over a period of 5 weeks, commencing 10 or 13 days after flowering (daf) for a total of 10 samplings. At each sampling ten seeds per plant from each of three plants were taken from the outer region of the capitulum (first six rings). Seeds were dried in an oven at 60°C for 48 h. Plants reached true PM, when the seed weight became constant after three successive samplings.

Fatty acid analysis

Five plants per plot and 100 seeds per plant from the outer rings (1–6) were collected and dehulled. Kernels were ground to a fine powder using a coffee grinder. Two hundred milligrams of kernel powder was weighed to perform fatty acid analysis. Lipids were extracted in *n*-hexane. Fatty acids were converted in Fatty Acid Methyl Esters (FAMEs) by transesterification with a methanolic potassium hydroxide solution (2 N). FAMEs composition was determined by gas chromatography, and every fatty acid was expressed as a percentage of the total fatty acids detected in the oil. The gas chromatograph, equipped with a Flame Ionization Detector (FID) and a split–splitless injector, was fitted with a 60 m HP-88 capillary column (Agilent Technologies, USA). Helium was used as carrier gas, and the injector, detector and oven temperatures were 280, 250 and 200°C, respectively. Five microliters of sample was injected in split mode. Different FAMEs were identified by comparison with known standards.

Oil content analysis

Whole seeds, 10 g, before fatty acid determination, were analyzed for oil content by Nuclear Magnetic Resonance (NMR Oxford Instruments –4000; FGIS-USDA, 2009).

Growing degree-days accumulation

To study the environmental effects on fatty acids and to compare our results with previous works, data from years and date of sowing were divided according to GDD accumulated. The accumulation of GDD, above a base temperature of 6°C (T_b), was calculated using the following formula:

$$GDD = \Sigma[(T_{max} + T_{min})/2] - T_b$$

where T_{max} and T_{min} are the daily maximum and minimum temperatures, respectively, in °C. GPM is calculated from last flowering to PM and G25 from last flowering to 25 daf. Two GPM groups were created: A and B. Group A contained inbred lines at 500 GGD and the hybrid at 600 GGD. Group B was formed by inbred lines at 600 GGD and the hybrid at 700 GGD. G25 was also separated into two groups: the first one was constituted by plants that had accumulated 400 GGD and the second by plants that had accumulated 500 GGD.

Statistical analysis

Statistical analysis was performed using R version 2.15.0 (R Development Core Team, 2012). Shapiro–Wilk normality test was performed to test normality condition. A two-way ANalysis Of VAriance (ANOVA) was performed as fixed-effect model with GDD and genotypes. Significance of each source of variation was evaluated by *F*-test. When the *F*-ratio revealed significant differences, means were compared by the least significant difference (LSD) at $p \leq 0.05$.

Results

Duration of the growing period (emergence – PM) and relative phases are reported in Table 2. PM, expressed as daf when seed weight was constant, was reached some days before R9 phase (Schneiter and Miller, 1981). In inbred lines the difference between PM and R9 was smaller (about 4 days before than R9

Table 2: Date of sowing, cycle duration and emergence to flowering, and end of flowering (F)-PM phase duration for each genotype.

Genotype	Year	Date of sowing	Cycle duration (dd)	Emergence-flowering (dd)	F-PM – R9[a] (dd)	F-PM[b] (dd)
342mt	2009	6th May	106	68	31	31
		28th May	110	71	35	34
	2010	19th April	111	66	34	32
		1st June	91	52	33	32
	2011	18th April	110	62	41	35
		30th May	101	59	37	35
R978	2009	6th May	110	71	33	32
		28th May	115	74	34	34
	2010	19th April	119	73	35	32
		1st June	95	57	32	30
	2011	18th April	113	67	40	38
		30th May	101	60	37	33
Hybrid	2009	6th May	113	69	47	37
		28th May	113	72	46	36
	2010	19th April	124	64	53	42
		1st June	98	49	45	38
	2011	18th April	116	60	45	40
		30th May	106	56	40	38

Notes: [a]PM – R9 according to Schneiter and Miller (1981); [b]PM as seed constant weight.

phase) than in the hybrid (about 10 days before than R9 phase). Thus, the duration of end of flowering to PM phase was calculated on true PM expressed as daf when seed weight became constant.

The GDD accumulated from last flowering to PM (GPM) were 618 ± 49 (standard deviation) for the year 2009, 540 ± 49 and 630 ± 38 GDD for the years 2010 and 2011, respectively. Average GPM accumulated by inbred lines was 567°C days $\pm$ 67 while the hybrid accumulated 652°C days $\pm$ 53. The GDD accumulated from last flowering to 25 daf (G25) was 439 ± 33, 426 ± 22 and 405 ± 23 G25 for 2009, 2010 and 2011, respectively. Average G25 accumulated by inbred line 342mt was 436 ± 34, 420 ± 28 by inbred line R978 and 414 ± 23 by the hybrid. All tested genotypes accumulated the same GPM and G25 on average.

Oil and stearic acid contents were influenced by the interaction genotype $\times$ GPM accumulated from flowering to PM (Tables 3 and 4). The composition of seed fatty acids (palmitic acid and unsaturated oleic and linoleic acids) was influenced by G25 accumulated (Table 5) from last flowering to 25 daf. Line 342mt and hybrid showed a different fatty acid composition at

Table 3: ANOVA (mean square) for the main fatty acids and seed oil content.

Source of variation	DF	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Oil
GPM	1	2.80ns	0.03ns	0.15ns	3.57ns	18.02ns
Genotype (G)	2	5.21**	1.87ns	146.44***	73.35***	204.19***
GPM × G	2	1.17ns	3.53*	11.48ns	7.99ns	94.19**
Residuals	48	0.70	0.69	4.96	4.34	13.52

Notes: GPM are the GDD accumulated from end of flowering to PM (daf when seed weight becomes constant). *, ** and *** = Significant at the $p < 0.05$, 0.01 and 0.001 levels, respectively. ns = not significant.

Table 4: Stearic acid and oil content as affected by GPM (GDD accumulated from last flowering to PM) accumulated in the tested genotypes.

Genotype	GPM	Stearic acid %	Oil %
342mt	500	2.7ab	44.6bc
	600	3.3a	40.6c
R978	500	2.7ab	42.2c
	600	2.1b	47.9ab
Hybrid	500	2.0b	51.2a
	600	3.0a	48.1ab

Note: Means followed by the same letter are not significantly different (LSD at the 5% level).

Table 5: ANOVA (mean square) for the main fatty acids and seed oil content.

Source of variation	DF	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Oil
G25	1	12.60***	0.00ns	6.07ns	35.94***	0.39ns
Genotype (G)	2	2.86**	2.03ns	169.71***	98.87***	192.42***
G25 × G	2	5.03***	1.60ns	19.34**	9.37*	50.21ns
Residuals	48	0.43	0.77	3.54	2.55	16.21

Notes: G25 are the GDD accumulated from end of flowering to 25 daf. *, ** and *** = Significant at the $p < 0.05$, 0.01 and 0.001 levels, respectively; ns = not significant.

different levels of G25 while line R978 had the same fatty acid composition at every level of G25. Thus, temperature did not modify fatty acid composition in line R978, but it depended only on genotype.

Palmitic acid content was 4.20% on average, and it was the main saturated fatty acid in tested HO genotypes. The two high oleic inbred lines tested and

Table 6: Effects of interaction genotype × G25 (GDD accumulated from last flowering to 25 daf) on palmitic, oleic and linoleic acid contents.

Genotype	G25	Palmitic acid %	Oleic acid %	Linoleic acid %
342mt	400	3.8bc	84.8c	8.1a
	500	5.4a	88.2b	3.8b
R978	400	3.3c	92.0a	2.5bc
	500	4.9ab	90.8ab	1.4c
Hybrid	400	4.0c	89.9b	3.5b
	500	3.6bc	92.9a	1.2c

Note: Means followed by the same letter are not significantly different (LSD at the 5% level).

their hybrid had different palmitic acid contents (Table 6). The seeds of inbred lines had an increase in palmitic acid content with G25B. The highest value was reached by line 342mt with G25B (5.37%), and the lowest by R978 inbred line with G25A (3.28%). We reported a positive correlation between mean daily temperature of the F-PM phase and palmitic acid content only in inbred line 342mt (Figure 1), while there was no any correlation between temperature and palmitic acid content in inbred line R978. The palmitic acid content in the hybrid was within the parental range, and it did not show any variation linked to environmental condition.

The stearic acid content of the inbred lines was unaffected by heat summation, whereas it increased with GPM2 in the hybrid. The hybrid had a stearic

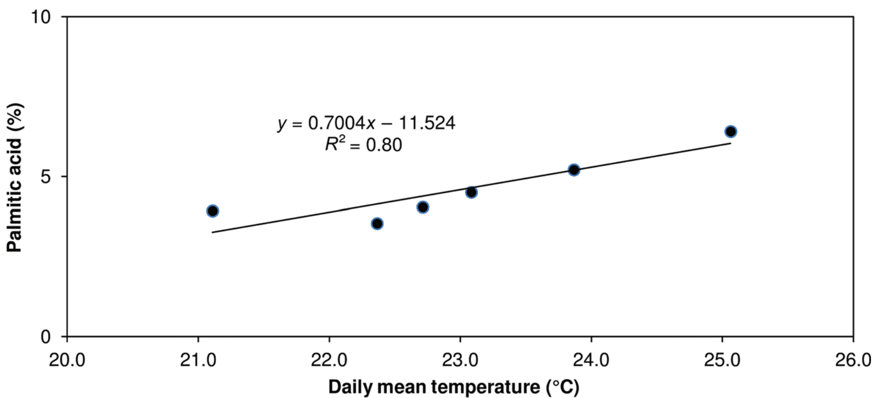

Figure 1: Correlation between mean air temperature and seed palmitic acid content in inbred line 342mt during F-PM phase (*p*-value 0.015).

acid content within the parental range and showed the same interaction genotype × GPM as its female parental line. The hybrid showed an increase of about 1% in stearic acid content when GPM increased by 100°C days. Inbred lines had the same stearic acid content through the GPM levels. At the level A of GPM accumulated, there was no significant difference among tested genotypes. At the level B of accumulated, inbred line R978 had the lowest stearic acid content (2.0%).

Small but significant variations in oleic acid content were manifested with different levels of G25 and with a difference among genotypes. ANOVA showed that there was a relative prominence of genotype effects compared to G25 (Table 5). The two high oleic inbred lines tested differed about their oleic acid content. Inbred line 342mt showed a lower oleic acid content on average than R978 inbred line and their hybrid (Table 6).

Oleic and linoleic acids were negatively correlated. There was a difference between high oleic inbred lines (Figure 2). Line 342mt showed a strong negative oleic–linoleic relationship (p-value < 0.001), indicating that increasing one point in oleic corresponded to a decrease of one point in linoleic acid. The correlation coefficient between oleic and linoleic acids was not significant in R978 (Table 7). The hybrid showed a strong negative oleic–linoleic relationship and had an average value of the regression parameter among parental lines (Figure 2). This suggests an additive effect of modifier genes on oleic acid content.

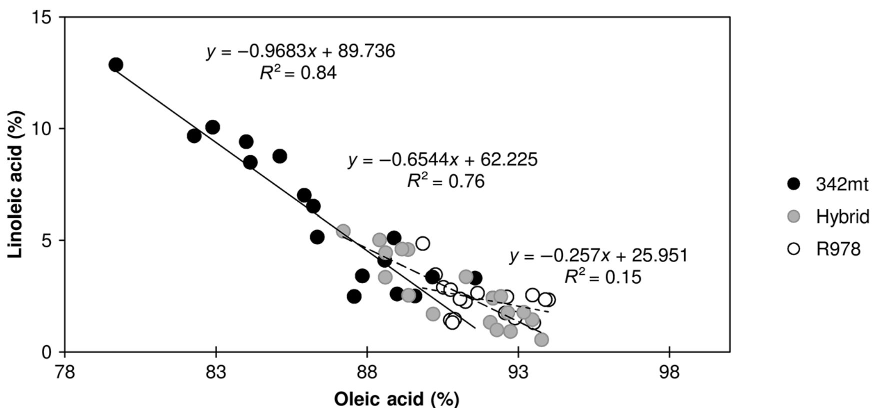

Figure 2: Relationship between oleic and linoleic acids in inbred lines and in the hybrid.

Linoleic acid content in seeds was significantly affected by G25 only in two genotypes: 342mt and the hybrid (Table 6). As for oleic acid content, linoleic acid content in inbred line R978 did not show any response to the G25 tested.

Table 7: Correlation coefficients between seed oil content and concentration of fatty acids in the three high oleic genotypes tested ($n = 18$).

Genotypes		Palmitic	Stearic	Oleic	Linoleic	Oil
342mt	Palmitic	1.00				
	Stearic	−0.27	1.00			
	Oleic	0.31	−0.45	1.00		
	Linoleic	−0.55*	0.21	−0.92***	1.00	
	Oil	0.18	−0.81***	0.32	−0.12	1.00
R978	Palmitic	1.00				
	Stearic	0.15	1.00			
	Oleic	−0.65**	−0.53*	1.00		
	Linoleic	−0.10	−0.32	−0.39	1.00	
	Oil	0.57*	−0.41	0.00	−0.13	1.00
Hybrid	Palmitic	1.00				
	Stearic	0.15	1.00			
	Oleic	−0.49*	−0.55*	1.00		
	Linoleic	0.22	0.15	−0.87***	1.00	
	Oil	−0.39	−0.29	−0.19	0.54*	1.00

Notes: *, ** and *** = Significant at the $p <$ 0.05, 0.01 and 0.001 levels, respectively.

Line 342mt and the hybrid showed a reduction in linoleic acid when heat summation dropped of 100°C days.

Oil content varied from 40 to 51%. ANOVA showed that oil content was influenced by genotype effects and the interaction genotype × GPM (Table 3). The hybrid showed a higher seed oil content than its parental lines. 342mt and the hybrid did not show any significant variation with the GPM tested while R978 showed an increase in seed oil content when GPM increases from 500 to 600°C days. There was no significant relationship between seed oil content and oleic acid content in any of the genotypes (Table 7). In inbred line 342mt, oil content was negatively correlated with stearic acid content while oil concentration in the hybrid was positively correlated with linoleic acid concentration.

Discussion

The GDD summation (flowering – PM) reported in this study compared with 699–836 (mid-flowering – PM) reported by Robertson and Green (1981), when these data are converted to a T_b of 6°C, and with the GDD summation (449–948°C days; R6–R9 phase) reported by Roche *et al.* (2006). Different sunflower genotypes

grown in diverse environments required a similar cumulative heat summation from flowering through PM.

Fatty acids composition was affected by the cumulative heat summation between flowering and 25 daf (G25). This result is in agreement with Izquierdo *et al*. (2002) who reported that the sensitive period for modifications in fatty acid composition was 0–400°C daf.

Effect of GDD on saturated fatty acids content

The oleic genotypes had lower palmitic acid percentages compared with the standard sunflower types as reported in other works (Lajara *et al*., 1990; Roche *et al*., 2004; Anastasi *et al*., 2010). High oleic genotypes with high oleic acid potentials had the lowest palmitic acid contents. Thus, increasing in oleic acid content to select high-HO genotypes could be achieved only through a reduction in palmitic acid content. High levels of saturated fat consumption are correlated with increased risk of coronary heart disease. Despite sunflower oil already having a high level of unsaturated fatty acids, it is still desirable to reduce its saturated fatty acid level for producing healthier oil.

Palmitic acid concentration increases with G25B only in the inbred lines. Several environmental factors like water availability (Roche *et al*., 2006; Jalilian *et al*., 2011), temperature (Rondanini *et al*., 2003; Izquierdo and Aguirrezábal, 2008) and nitrogen (Zheljazkov *et al*., 2009) could alter saturated fatty acid content in sunflower. In our experiment, temperature was the most variable environmental factor. The temperature modified the palmitic acid concentration of the oil in some high oleic hybrids (Izquierdo and Aguirrezabal, 2008). We found a positive correlation between mean daily temperature of the F-PM phase and palmitic acid content only in inbred line 342mt (Figure 1) while there was no any correlation between temperature and palmitic acid concentration in inbred line R978. Interestingly, oil seed percentage and palmitic acid were positively correlated in inbred line R978 (Table 7). The seed oil content increased with GPM2 (Table 6). The rise in palmitic acid content was related to an increase in seed oil content and not to a rise in air temperature *per se*. Thus, heat summation did not modify palmitic acid percentage in this line, but oil content.

Stearic acid concentration was reduced in tested HO genotypes with respect to LO sunflower. Stearic acid concentration was associated with an increase in heat sum only in the hybrid whereas the concentration was unaffected in the inbred lines. The difference could be associated with genotype (e.g. Roche *et al*., 2006).

As hypothesis, differences among genotypes could be related to isozymes of stearate desaturase (Fernandez-Moya *et al.*, 2003). About the isozymes, at GPM_A, mean air temperature was lower than at GPM_B (21.9°C and 23.6°C, respectively). In soybean, Byfield and Upchurch (2007) found that decreased SAD transcript accumulation at warmer temperature was positively associated with a significantly increased level of stearic acid but only in a high stearic mutant line. Conversely, in a soybean genotype, the stearic acid percentage was negatively related to daily mean temperature during grain-filling period (Zuil *et al.*, 2012). The effect of temperature on stearic acid content is unclear and seems to be genotype-specific. Considering that variation in stearic acid amount was small, the response to GPM could be related to a "long-term" effect of temperature on stearic acid content.

Interestingly, the two saturated fatty acids displayed an opposite interaction with heat summation between parental lines and hybrid. Palmitic acid concentration was affected by temperature in inbred lines and not in the hybrid while these genotypes showed an opposite behavior on stearic acid concentration. The inheritance and the environmental effects on saturated fatty acids content are composite. Palmitic and stearic acids are both quantitatively inherited. Genetic background and environment also play significant roles in palmitic and stearic acid inheritance (Roche *et al.*, 2006). In the literature, there are only a few works on inheritance of saturated fatty acids content, and the trait appears to be complex and multigenic (Vick *et al.*, 2004). Further studies are needed on inheritance of saturated fatty acids in HO genotypes.

Effect of GDD on C18 unsaturated fatty acids content

Oleic acid contents were relatively stable in the high oleic genotypes tested. These data are in agreement with Lagravére *et al.* (2004) and Roche *et al.* (2004). Oleic acid content was insensitive to the heat sum in the line R978. On the other hand, line 342mt and the hybrid, that they had a different oleic acid content in their seeds (86.5 and 91.4%, respectively), showed the same interaction genotype × G25 with an increase of 3% in oleic acid content as heat summation increased from 400 to 500°C day. This indicates the presence of a genotype × environment interaction on oleic acid concentration. Flagella *et al.* (2002) reported a similar variation in oleic acid content (about 2%) in other high oleic sunflower hybrids as affected by date of sowing. Oleic acid content is modified by temperature in some genotypes, thus high oleic inbred lines with different origins are not equivalent for high oleic trait. The basis for differences between high oleic and normal sunflower genotypes is a differential activity of the

enzyme Δ12-desaturase, which catalyzes the desaturation of oleic acid to linoleic acid (Garcés and Mancha, 1991; Kabbaj *et al.*, 1996). High oleic mutants had substantially lower FAD2-1 desaturase gene transcript accumulation than sunflower standard type (Kabbaj *et al.*, 1996; Hongtrakul *et al.*, 1998). The high oleic trait was controlled by at least three loci: oleHL, a suppressor locus and modifier loci (Lacombe *et al.*, 2004). We suppose that the inbred lines and thus the hybrid tested were homozygous at the Pervernets locus. Thus, observed differences can be related only to modifier genes (Miller *et al.*, 1987; Velasco *et al.*, 2000; Varès *et al.*, 2002; Lacombe *et al.*, 2004). The genotype × environment interaction was caused by modifier genes. The observed response to temperature in inbred line 342mt and the hybrid could be related to a residual activity of the Δ12-desaturase (mediated by modifier) or to some genes that have no phenotypic expression at higher temperatures (Velasco *et al.*, 2000). From a plant breeder's point of view, knowledge of the G × E interaction facilitates the efficient use of appropriate breeding and selection procedures. A hybrid that is stable in different growing conditions is preferred. The difference among genotypes in their linoleic acid content was related to modifier genes, as for oleic acid, because there is a close relationship between oleic and linoleic acid synthesis.

Seed oil content

Seed oil concentration in this study was generally high and similar to previous reports (Roche *et al.*, 2004; Anastasi *et al.*, 2010).

Results suggest that sunflower seed oil concentration depends on genotype, but it may be expressed differentially under different environmental conditions because seed oil concentration of sunflower is sensitive to environmental conditions during the grain-filling period (Connor and Hall, 1997). No relationship was found between seed oil content and temperature or rainfall. Seed oil content may therefore be affected by other variables not studied in this work. Differences among genotypes in response to GDD could be related to a different oil accumulation pattern (Mantese *et al.*, 2006) or due to the fact that R978 is a fully branched type. Thus, it would be possible to suppose a different interaction between seed characteristic (embryo oil concentration, pericarp/kernel ratio, etc.) and environment.

Seed oil content and fatty acid relationship

In high oleic hybrids a negative correlation between stearic acid and oil content was reported by Van der Merwe *et al.* (2012). In inbred line R978, oil content was

positively correlated with palmitic acid content. This result is not in agreement with Velasco *et al.* (2007) who reported a negative correlation between oil and palmitic acid contents. In the hybrid, oil content was positively correlated with linoleic acid. Further studies are needed on oil content and its relationship with fatty acids in high oleic mutants.

With regard to the ratio of palmitic to oleic acid, a negative and significant correlation was observed in line R978 and in the hybrid, and these results are in agreement with data by Champolivier and Merrien (1996), Roche *et al.* (2004) and Izquierdo *et al.* (2006), collected for oleic hybrids and for high stearic mutants (Velasco *et al.*, 2007). Flagella *et al.* (2002) showed that an increase in palmitic acid in sunflower is accompanied by a decrease in both oleic and stearic acids. Studies on soybean (Rebetzke *et al.*, 1996), peanut (Andersen and Gorbet, 2002), sesame (Were *et al.*, 2006) and winter oilseed rape (Möllers and Schierholt, 2002) also revealed strong inverse relationships between palmitic and oleic acids. Line 342mt did not show any significant correlation between palmitic and oleic acids, but we found an inverse significant correlation between palmitic and linoleic acids. This could be associated with the relationship between palmitic acid and temperature in this genotype.

Oleic and linoleic acids were negatively correlated. There was a difference between high oleic inbred lines (Figure 2). Line 342mt showed a strong negative oleic–linoleic relationship (p-value < 0.001), indicating that increasing one point in oleic corresponded to a decrease of one point in linoleic acid. The correlation coefficient between oleic and linoleic acids was not significant in R978 (Table 7). The hybrid showed a strong negative oleic–linoleic relationship and had an average value of the regression parameter between parental lines (Figure 2). This suggests an additive effect of modifier genes on oleic acid content.

We suppose that the linoleic acid level reached by R978 was a physiological threshold for this genotype. We speculate that in R978 only the constitutive desaturase system (FAD2-2 and FAD2-3) present in the whole plant (Martìnez-Rivas *et al.*, 2001) could be active and responsible for low linoleic acid synthesis (physiological threshold) while seed-specific desaturase is fully inactive. The total elimination of linoleic acid from the seed oil by conventional breeding is probably impossible. The negative relationship between oleic acid content and saturated fatty acids suggested that increasing the oleic acid content over 93–95% is possible only with a reduction in saturated fatty acids content. In line 342mt and in the hybrid, linoleic acid synthesis is probably due to a residual activity of the achene-specific desaturase system (Lagravére *et al.*, 2004). This residual activity is regulated by environmental condition.

Conclusions

Fatty acid composition in high oleic sunflower depended mainly on genotype, but environmental conditions can affect the 90% threshold of oleic acid content. Seed fatty acid composition was mainly influenced by GDD accumulated from end of flowering to 25 daf (G25). At the highest level of G25 accumulated, there was an increase in palmitic and oleic acid contents, whereas linoleic acid content decreased. Oleic acid content was affected by temperature in two of three high oleic genotypes. This result suggests that temperature could be acting on different biological processes in high oleic genotypes. Further studies are needed.

Saturated fatty acids content was affected by GDD with a different response among genotypes. To obtain hybrids with an oleic acid content higher than 93–95% it is necessary to select inbred lines against saturated fatty acid content because the total elimination of linoleic acid from the seed oil by conventional breeding is probably impossible. Further studies are therefore needed on saturated fatty acids content in high oleic genotypes.

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