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Effect of growth temperature on the high stearic and high stearic-high oleic sunflower traits

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Abstract. We investigated variability in the response of oil fatty acid composition to temperature among high stearic and high stearic-high oleic sunflower (*Helianthus annuus* L.) genotypes. Two experiments were conducted with high stearic (including the *CAS-3* mutation) and high stearic-high oleic inbred lines (including both the *CAS-3* and the high oleic *Soldatov* mutations). Plants were cultivated in pots with soil, irrigated, and fertilised. Plants were exposed to different day/night temperatures during grain filling: 16/16°C, 26/16°C, 26/26°C, and 32/26°C. Oil fatty acid composition was determined by gas–liquid chromatography in seeds harvested after physiological maturity. Higher temperature during grain filling increased palmitic and oleic acid percentages and reduced stearic and linoleic acid percentages, suggesting some modifications on enzymatic activities. When the high oleic mutation was included, the variation in stearic and oleic acid percentages in response to temperature was reduced but not the variation in palmitic acid concentration. Variations in fatty acid composition in high stearic genotypes were mainly associated with night temperature as reported previously for traditional and high oleic hybrids. Knowing the effect of temperature on oil fatty acid composition in traditional and mutated genotypes is useful for selecting the environment in which to produce grains with the desired oil quality.

Additional keywords: grain filling, mutations, night temperature, oil quality, oleic acid, stearic acid.

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Introduction

Oil stability, plasticity, and nutritional quality are mainly determined by fatty acid composition. For example, oil stability increases with saturated fatty acid content and decreases with polyunsaturated fatty acid content. In addition, oleic acid is desired because it reduces blood cholesterol. In some cases, oils are transformed to modify fatty acid composition in order to obtain the best properties required for their final use. For example, margarines or shortenings require oils with high plasticity and stability, characteristics favoured by saturated fatty acids such as palmitic or stearic. As traditional sunflower oil presents a very low concentration of saturated fatty acids, it is often hydrogenated to improve its plasticity and stability. Hydrogenation increases the concentration of saturated fatty acids but also produces *trans* fatty acids, which are associated with the development of coronary pathologies, the prevalence of allergies, and the risk of cancer among other pathologies (Willett and Ascherio 1994; Hu *et al*. 1997; Eckel *et al*. 2007). Because *trans* fatty acids are not healthy, many countries have established regulations to label the presence of *trans* fatty acids in foods (e.g. Eller *et al*. 2005).

Genotypes with increased saturated fatty acids have been developed as an alternative to hydrogenated oils in, for example, sunflower (Osorio *et al*. 1995; Fernández-Moya *et al*. 2002), soybean (Boersma *et al*. 2012; Rahman *et al*. 2003), and brassica seeds (Knutzon *et al*. 1992). In sunflower, the high stearic CAS-3 mutant and derivatives produce >20% of saturated fatty acids *v.* <10% in traditional cultivars. For human consumption, high stearic oils are preferred because this fatty acid does not increase blood cholesterol as other saturated fatty acids do (i.e. palmitic or myristic acids; Crupkin and Zambelli 2008). Genetic characterisation of the high stearic trait has demonstrated that, in all cases, at least two genes are necessary for the trait to be maximised (Pérez-Vich *et al*. 1999). Biochemical and molecular characterisation of high stearic lines have shown a reduction in stearoyl-ACP desaturase activity and an acyl-ACP thioesterase with higher

activity of stearoyl-ACP compared with traditional sunflower cultivars (Cantisán *et al*. 2000). Genotypes carrying two traits, the high stearic mutation (i.e. the *SAD* mutation) and a high oleic mutation (i.e. the *OLD* mutation, which reduces the activity of the oleoyl-PC desaturase enzyme), have been obtained, producing high stearic-high oleic oils (Fernández-Moya *et al*. 2005; Serrano-Vega *et al*. 2005; Pleite *et al*. 2006) with high thermo-oxidative stability and plasticity (Márquez-Ruiz *et al*. 1999; Martínez-Force *et al*. 2000).

Hybrids with high environmental stability of their fatty acid composition are usually preferred so they can be sown in a wide range of environmental conditions while maintaining oil quality. Producing specific oil, such as high stearic, requires detailed knowledge of the effects of environmental conditions, crop management, and their interaction with genotype on this trait. It is well known that, in sunflower, temperature during grain filling can greatly affect oil fatty acid composition, mainly by modifying the oleic/linoleic acid ratio. This effect can vary according to genotype. For example, oleic acid percentage in a traditional sunflower cultivar increased from 17% to 59% when plants were exposed to increased night temperatures during grain filling (Izquierdo *et al*. 2006). Cultivars carrying the *OLD* mutation also showed increased oleic acid percentage when temperature increased, but the variation was lower than that observed in traditional genotypes (e.g. <7 *v*. almost 40 percentage points, respectively, of oleic acid; Izquierdo and Aguirrezábal 2008). The increase in oleic acid percentage is mainly associated with a reduction in linoleic acid percentage, but reductions in total saturated fatty acids have also been observed (Izquierdo and Aguirrezábal 2008).

Variability in the response of oil fatty acid composition to temperature was observed not only among genotypes carrying different mutations but also within each type of genotype (e.g. traditional, high oleic). For example, for the same temperature range, increments in oleic acid percentage from 16 to almost 40 percentage points were observed among seven traditional genotypes (Izquierdo and Aguirrezábal 2008). The response of fatty acid composition to temperature was also different among high oleic genotypes carrying the same *OLD* mutation (Triboï-Blondel *et al*. 2000). This variability has been attributed to the presence of modifier genes in the genetic background which affect the stability of fatty acid composition. It is unknown if the response of oil fatty acid composition to temperature also varies among high stearic lines and if the *OLD* mutation stabilises oil fatty acid composition when temperature changes during grain filling. In high stearic and high stearic-high oleic mutants with reduced stearoyl-ACP desaturase activity and with an acyl-ACP thioesterase with higher activity of stearoyl-ACP, the quantity of substrate for oleic acid synthesis and OLD enzyme is lower than in genotypes without the *SAD* mutation, so a lesser effect of temperature on fatty acid composition is expected.

The objective of this work was to investigate variability in the response of oil fatty acid composition to temperature among high-stearic and high stearic-high oleic sunflower (*Helianthus annuus* L.) genotypes. Also, the effect of night temperature on fatty acid composition, reported by Izquierdo *et al*. (2006) for traditional and high oleic genotypes, is tested in genotypes carrying the *SAD* mutation.

Materials and methods

Plant material and growth conditions

Two experiments were performed with sunflower inbred lines with different oil fatty acid composition: high stearic (HSHL) and high stearic-high oleic (HSHO). The 'high stearic' and 'high oleic' traits were obtained from the *CAS-3* (Osorio *et al*. 1995) and the *OLD* (Soldatov 1976) mutations. A traditional (T) and a high oleic (HO) line were also included as controls. In Expt 1, lines HA89B (T), RHA345 (HO), CAS-3 (HSHL), and ADV-3807 (HSHO) were evaluated. In Expt 2, HSHL lines ADV-2504 and ADV-3512, HSHO lines ADV-2803 and ADV-3816, and the lines included in Expt 1 were evaluated.

In both experiments, plants were sown in 10-L pots filled with soil. After seedling emergence, plants were thinned to one per pot. Soil was fertilised according to Izquierdo *et al*. (2002) and periodically irrigated to avoid water deficit. Phenology was registered according to Schneiter and Miller (1981). Capitula were covered with pollination bags and self-pollinated. Plants were kept under natural conditions until flowering, when treatments were applied.

Plants were exposed to different day/night temperatures during grain filling (from 5 days after flowering to physiological maturity). In Expt 1, the day/night temperatures were 16/16°C and 26/26°C. Treatments applied in Expt 2 were 16/16°C, 26/16°C, 26/26°C, and 32/26°C. In both experiments, temperature regimes were randomised in four growth chambers of dimensions 2.0 m by 2.7 m by 2.4 m (Refrimax S.R.L., Mar del Plata, Argentina). Three plants per genotype and chamber were used and the average of two fatty acid analyses per plant was calculated. Chambers were calibrated with 12-h photoperiod and incident photosynthetically active radiation at the top of the plants of 690 ± 75 mmol m^{-2} s^{-1}. Air humidity in chambers was measured with humidity sensors (SHW00P0420, CAREL SRL, Brugine, Italy) and chambers were calibrated to obtain the same vapour pressure deficit during the light period (15 hPa VPD), resulting in relative humidity of 62%, 80%, and 86% for 16°C, 26°C, and 32°C, respectively. Shorter plants were raised in order to receive the same incident radiation. Air temperature of the growth chambers was registered every 60 s and hourly averages were recorded with dataloggers (Cavadevices, Buenos Aires). Physiological maturity was estimated visually from the hard yellow colour of the capitulum back face and from the brown colour of its bracts (Farizo *et al*. 1982). Twenty seeds per plant were harvested after physiological maturity for lipid analysis.

Lipid analysis

Oil extraction and methylation were performed following the technique proposed by Ruiz-López *et al*. (2003). Oil fatty acid composition from each grain was determined by gas–liquid chromatography an Agilent 6890 gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA). The column used was a Supelco SP-2380 fused silica capillary column (30 m length, 0.25 mm i.d., 0.20 µm film thickness: Bellefonte, PA, USA), and hydrogen was used as the carrier gas at 28 cm s^{-1}. The detector and injector temperature was 200°C, and the oven temperature was kept at 170°C. Each fatty acid (palmitic, stearic, oleic, linoleic, and arachidic) was expressed as

percentage of the total fatty acids identified in the oil. Arachidic acid presented very low concentrations (≤1.8%) and in general it was not affected by the treatments (data not shown).

Data analyses

Statistical data analysis was performed using the R software (R Development Core Team 2010). For both experiments and every fatty acid, a statistical model with fixed effects of genotype, temperature, and genotype × temperature interaction was considered. A heteroscedastic model for the error variance was adjusted depending on the type of line (T, HO, HSHL, HSHO). When the interaction was significant ($\alpha = 0.05$), the analysis continued studying the temperature effect within every genotype. To further analyse the effect of temperature on fatty acids concentrations, the following set of orthogonal contrasting hypotheses was defined to be tested for each genotype in Expt 2: (*i*) 16/16°C *v.* 26/16°C to measure a daily temperature effect with night temperature fixed at 16°C; (*ii*) 26/26°C *v.* 32/26°C to evaluate a daily temperature effect fixing night temperature at 26°C; and (*iii*) 16/16°C, 26/16°C *v.* 26/26°C, 32/26°C, to compare treatments with low/high night temperature.

Results

Experiment 1

Temperature during grain filling modified oil fatty acid composition in all inbred lines (Table 1). Increases in temperature from 16/16°C to 26/26°C resulted in higher palmitic and oleic acid concentrations ($P < 0.0001$ and $P = 0.0003$, respectively), and lower linoleic acid concentration ($P < 0.01$) in all inbred lines. A temperature × genotype interaction was observed only for stearic acid concentration ($P < 0.0004$). Stearic acid content was higher for 16/16°C than 26/26°C in the high stearic line CAS-3. In general, the highest variation in fatty acid composition was observed in the HSHL genotype. For example, variation in oleic acid concentration between treatments was 8.1, 5.8, 18.6, and 5.7 percentage points for the traditional, HO, HSHL, and HSHO genotypes, respectively.

Table 1. Oil fatty acid composition (mol%) of sunflower inbred lines for different day/night temperature treatments applied during the grain filling period in Experiment 1

Genotypes: T, traditional; HO, high oleic; HSHL, high stearic; HSHO, high stearic-high oleic. Data are mean ± standard error. Within a row, means followed by the same letter are not significantly different (l.s.d. at $P = 0.05$). Mean differences between temperature treatments were significant for palmitic, oleic, and linoleic acid concentrations. A significant genotype × treatment interaction was found only for stearic acid concentration

Fatty acid	Genotype		Temperature	
			16−16°C	26−26°C
Palmitic	T	HA89B	5.1 ± 0.42	5.1 ± 0.35
	HO	RHA345	3.3 ± 0.04	3.9 ± 0.03
	HSHL	CAS-3	5.3 ± 0.19	6.2 ± 0.19
	HSHO	ADV-3807	4.5 ± 0.06	5.4 ± 0.06
	All		4.5 ± 0.11a	5.2 ± 0.10b
Stearic	T	HA89B	3.9 ± 0.38a	4.4 ± 0.31a
	HO	RHA345	3.7 ± 0.16a	2.9 ± 0.13a
	HSHL	CAS-3	26.2 ± 1.23a	18.4 ± 1.23b
	HSHO	ADV-3807	20.9 ± 0.65a	20.4 ± 0.65a
Oleic	T	HA89B	49.8 ± 8.21	57.9 ± 6.70
	HO	RHA345	85.3 ± 1.01	91.2 ± 0.83
	HSHL	CAS-3	14.9 ± 2.72	33.5 ± 2.72
	HSHO	ADV-3807	63.7 ± 0.67	69.4 ± 0.67
	All		53.4 ± 2.18a	62.9 ± 1.83b
Linoleic	T	HA89B	41.0 ± 8.06	32.3 ± 6.58
	HO	RHA345	7.4 ± 0.88	1.7 ± 0.72
	HSHL	CAS-3	52.1 ± 3.95	40.6 ± 3.95
	HSHO	ADV-3807	9.3 ± 0.75	3.0 ± 0.75
	All		27.4 ± 2.26a	19.4 ± 1.93b

Experiment 2

Temperature treatments applied in Expt 2 also modified the oil fatty acid composition of all genotypes (Table 2). A temperature × genotype interaction was observed for all of the analysed fatty acid concentrations ($P \leq 0.0001$); the lowest palmitic acid concentration was observed under the lowest temperature conditions (16/16°C) ($P < 0.01$) except for line ADV-2803 ($P = 0.08$). The treatment 32/26°C increased the concentration of this fatty acid compared with the other treatments in lines HA89B, ADV-2504, and ADV-3816. In the other genotypes, a similar concentration of palmitic acid was observed for treatments 26/16, 26/26, and 32/26°C. Variations in palmitic acid percentage as a consequence of temperature treatments were similar in HSHL or HSHO genotypes (mean variation was 2.3 and 2.0 percentage points for HSHL and HSHO lines, respectively; Fig. 1).

In general, the stearic acid concentration was reduced when temperature increased. This effect was observed in HSHL and HSHO lines (except ADV-2504 and ADV-3816, in which no effect of treatments on stearic acid concentration was observed). Reductions of almost 10 percentage points in the concentration of this fatty acid were observed between extreme treatments. Although the HSHL line ADV-2504 presented a lower variation in stearic acid concentration than the HSHO line ADV-2803, the mean variation of HSHL lines was 3 points higher than the variation of HSHO genotypes (Fig. 1).

Oleic acid concentration increased when temperature increased in traditional, HSHL, and HSHO genotypes. In most cases, the greatest effect was observed between treatments 26/16°C and 26/26°C and not between treatments with similar night temperature (i.e. 16/16°C *v.* 26/16°C and 26/26°C *v.* 32/26°C). The magnitude of variation in oleic acid percentage among treatments depended on the genotype. In general, HSHL lines presented greater variations than HSHO lines, and the traditional genotype was intermediate. Mean variations for this fatty acid were 24.3 and 9.5 percentage points for HSHL and HSHO lines, respectively (Fig. 1). When the variations in stearic or oleic acids of each line were normalised by their respective mean fatty acid concentration, HSHO lines also presented a lower relative variation than HSHL lines (Fig. 1). The relative variation in oleic acid concentration of HSHL and HSHO lines was higher than the relative variation observed for HO genotype (116% and 14% *v.* 6%).

The linoleic acid concentration varied among treatments. Increasing temperatures resulted in lower linoleic acid

Table 2. Oil fatty acid composition (mol%) of sunflower inbred lines for different day/night temperature treatments applied during the grain filling period in Experiment 2

Genotypes: T, traditional; HO, high oleic; HSHL, high stearic; HSHO, high stearic-high oleic. Data are mean ± standard error. Within a row, means followed by the same letter are not significantly different (l.s.d. at $P = 0.05$)

Fatty acid	Genotype		Temperature			
			16−16°C	26−16°C	26−26°C	32−26°C
Palmitic	T	HA89B	4.1 ± 0.20a	5.7 ± 0.13c	4.9 ± 0.18b	5.6 ± 0.19c
	HO	RHA345	2.9[A]	4.0	4.2 ± 0.06	4.5 ± 0.01
	HSHL	CAS-3	5.0 ± 0.03a	6.5 ± 0.25b	6.6 ± 0.17b	6.7 ± 0.04b
	HSHL	ADV-2504	6.0 ± 0.35a	9.6 ± 0.18c	8.7 ± 0.09b	10.3 ± 0.08c
	HSHL	ADV-3512	5.1 ± 0.03a	6.0 ± 0.08b	5.7 ± 0.20ab	5.8 ± 0.15b
	HSHO	ADV-3807	4.9 ± 0.41a	6.6 ± 0.34b	6.5 ± 0.10b	6.8 ± 0.04b
	HSHO	ADV-2803	5.2 ± 0.08a	5.8 ± 0.05ab	6.4 ± 0.55b	6.4 ± 0.08b
	HSHO	ADV-3816	5.1 ± 0.06a	6.5 ± 0.34b	6.9 ± 0.09b	7.8 ± 0.24c
Stearic	T	HA89B	4.6 ± 0.15a	6.7 ± 0.39b	4.0 ± 0.38a	4.7 ± 0.65b
	HO	RHA345	3.1	4.3	3.0 ± 0.06	2.4 ± 0.30
	HSHL	CAS-3	25.8 ± 0.84b	25.0 ± 2.37b	16.4 ± 1.75a	16.2 ± 0.10a
	HSHL	ADV-2504	23.7 ± 1.54a	25.1 ± 1.98a	20.5 ± 0.36a	21.5 ± 0.85a
	HSHL	ADV-3512	28.1 ± 1.23b	26.9 ± 0.42b	21.1 ± 1.16a	19.7 ± 0.64a
	HSHO	ADV-3807	22.8 ± 1.94b	19.6 ± 0.06ab	20.9 ± 0.37ab	17.3 ± 1.33a
	HSHO	ADV-2803	23.6 ± 1.57b	19.2 ± 0.46a	18.0 ± 0.12a	16.9 ± 0.66a
	HSHO	ADV-3816	22.6 ± 1.34a	20.8 ± 0.61a	22.6 ± 0.74a	21.8 ± 0.59a
Oleic	T	HA89B	55.0 ± 3.13a	49.9 ± 0.81a	71.1 ± 3.45b	58.8 ± 4.36ab
	HO	RHA345	90.5	85.8	87.3 ± 2.97	90.7 ± 1.19
	HSHL	CAS-3	13.9 ± 0.56a	11.2 ± 0.80a	18.9 ± 0.19b	25.9 ± 1.17c
	HSHL	ADV-2504	9.0 ± 0.36a	8.0 ± 0.57a	37.0 ± 2.14b	36.6 ± 0.55b
	HSHL	ADV-3512	8.8 ± 0.37a	8.7 ± 0.33a	35.6 ± 1.16b	37.8 ± 1.44b
	HSHO	ADV-3807	59.3 ± 2.72a	65.6 ± 1.63ab	68.4 ± 0.48b	71.4 ± 1.36b
	HSHO	ADV-2803	61.7 ± 0.02a	69.2 ± 0.56b	69.6 ± 1.04b	72.2 ± 0.50c
	HSHO	ADV-3816	60.1 ± 0.51a	65.8 ± 0.37b	65.6 ± 1.36b	66.0 ± 0.32b
Linoleic	T	HA89B	36.0 ± 2.79b	37.3 ± 1.34b	19.6 ± 3.69a	30.4 ± 3.36ab
	HO	RHA345	3.3	5.5	5.3 ± 2.99	2.1 ± 0.89
	HSHL	CAS-3	54.2 ± 1.49a	56.4 ± 2.99a	57.2 ± 1.80a	50.4 ± 1.21a
	HSHL	ADV-2504	60.3 ± 0.95b	56.1 ± 2.81b	32.7 ± 1.64a	30.5 ± 1.32a
	HSHL	ADV-3512	56.7 ± 0.95b	57.2 ± 0.70b	36.3 ± 1.59a	35.5 ± 1.34a
	HSHO	ADV-3807	11.5 ± 0.44c	6.9 ± 1.37b	2.8 ± 0.03a	3.1 ± 0.17a
	HSHO	ADV-2803	8.4 ± 1.48b	4.7 ± 0.11a	4.8 ± 0.60a	3.4 ± 0.11a
	HSHO	ADV-3816	10.8 ± 1.00c	5.7 ± 0.08b	3.4 ± 0.64ab	2.8 ± 0.09a

[A]Due to the low number of plants obtained in this genotype the treatments with high temperature were kept and only 1 plant per treatment was studied in 16–16 and 26−16°C.

concentration, except in CAS-3, in which no effects of treatments were observed (Table 2). Although the relative variation was higher in HSHO than in HSHL lines because of the low concentration of linoleic acid, absolute variations were higher in HSHL than in HSHO genotypes, as observed for stearic and oleic acid concentrations. The mean variations of linoleic acid concentration were 19.4 and 7.2 percentage points for HSHL and HSHO lines, respectively (Fig. 1).

When orthogonal contrasts analysis was performed for data from Expt 2, an effect of night temperature on oleic acid concentration was observed in all genotypes except for the high oleic RHA345, which presented the lowest variability in this fatty acid. An effect of night temperature was also observed for palmitic, stearic, and linoleic acid concentrations (Table 3). In general, no effect of daily temperature was observed on the concentration of fatty acids. Palmitic acid concentration was associated with daily temperature, but only when night temperature was 16°C (comparison 16–16 *v.* 26−16°C).

Discussion

Temperature during grain filling modified the oil fatty acid composition of high stearic and high stearic-high oleic sunflower lines. In all genotypes tested in this work, increasing temperature increased oleic acid percentage and reduced linoleic acid percentage as observed in traditional genotypes of the same species (Lájara *et al*. 1990; Izquierdo *et al*. 2002, 2006; Rondanini *et al*. 2003) and other crop species (Canvin 1965; Izquierdo *et al*. 2009). The quantitative magnitude of the variation was different among genotypes and it was mainly associated with the presence or absence of the *OLD* mutation.

The *OLD* mutation increases the stability of oil fatty acid composition independently of the presence of other mutations that modify fatty acid synthesis. In accordance with the literature (Triboï-Blondel *et al*. 2000; Izquierdo *et al*. 2002; Izquierdo and Aguirrezábal 2008), the oleic/linoleic acid ratio was in general more susceptible to temperature in the traditional genotype than in the high oleic genotype with the *OLD* mutation. The same was observed among genotypes with the *SAD* mutation,

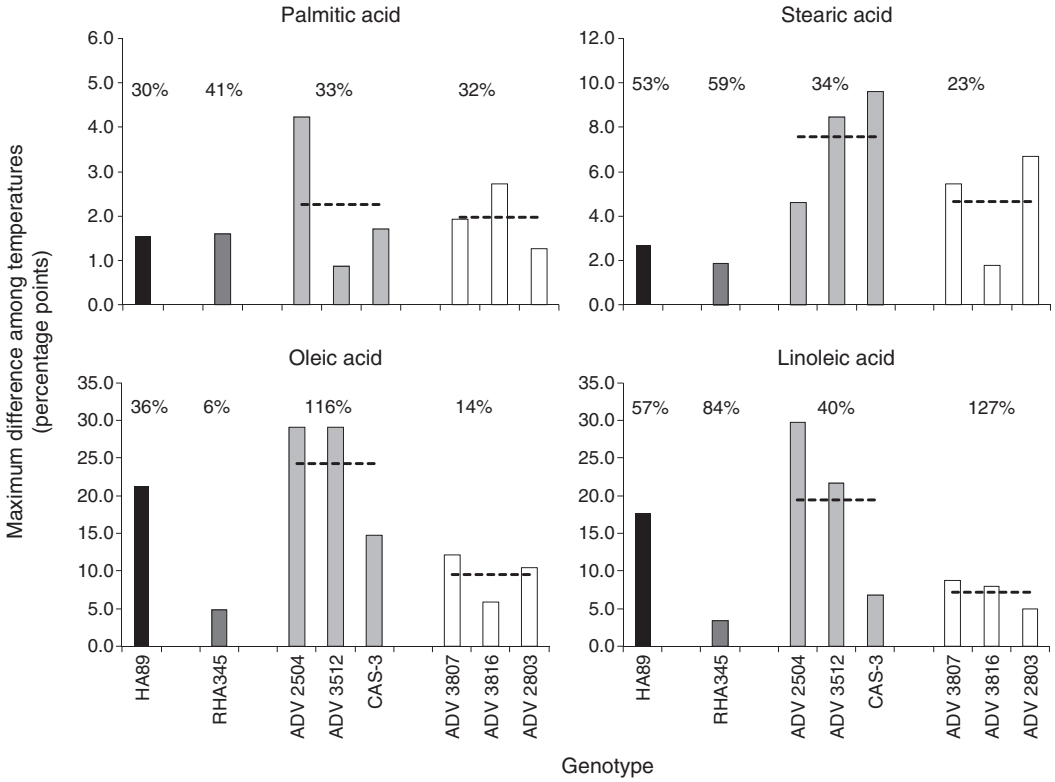

Fig. 1. Maximum difference in fatty acid concentrations among temperature treatments for traditional (black bar), high oleic (HO, dark grey bar), high stearic (HSHL, light grey bars), and high stearic-high oleic (HSHO, white bars) lines from Experiment 2. For HSHL and HSHO, dotted lines represent the average difference of each group of genotypes. The percentage value above each group of genotypes corresponds to the mean relative variation of each fatty acid (calculated as maximum difference among treatments divided by the mean fatty acid concentration of each line).

since high stearic genotypes presented more variability in oleic/linoleic acid ratio than high stearic-high oleic genotypes. The variations in oleic/linoleic acid ratio are explained by direct effects of temperature on the oleoyl-PC desaturase enzyme (Garcés and Mancha 1991; Garcés *et al*. 1992). Increasing temperature reduces the total activity of this enzyme and the oleic acid is stored in the triacylglycerols instead of being desaturated to linoleic acid. The high oleic genotypes, with the mutation that reduces oleoyl-PC desaturase activity, are also affected by temperature but to a lesser extent than traditional genotypes. This would be a consequence of a lower amount of OLD enzyme in its seeds (Garcés *et al*. 1992). The same would occur in HSHO genotypes compared with HSHL, since the former presented less variation in oleic/linoleic acid ratio compared with HSHL genotypes. The variations in oleic and linoleic acids concentrations of genotypes carrying the *SAD* mutation, independently of the presence of the *OLD* mutation, were higher than in the HO genotypes, indicating that HSHL and HSHO genotypes would present higher amount of OLD enzyme than high oleic genotypes.

The concentration of saturated fatty acids of high stearic lines was also affected by temperature during grain filling. The stearic acid percentage was reduced and the palmitic acid percentage was increased when temperature increased. A reduction in stearic acid percentage when temperature increased was also observed in traditional and high oleic sunflower genotypes (Izquierdo and Aguirrezábal 2008) and in other genotypes with increased stearic acid concentration, such as CAS-4 and CAS-8 (Martínez-Force *et al*. 1998). In this work, the high stearic lines carrying the CAS-3 mutation showed reduced stearic acid concentration when temperature increased, indicating that high temperature increase the stearoyl-ACP desaturase activity.

The presence of the *OLD* mutation also modified the magnitude of variation of stearic acid percentage. As observed for unsaturated fatty acids, the variation in stearic acid percentage of HSHL lines was higher than that of HSHO lines. In traditional sunflower genotypes, increasing temperature increased oleic/linoleic acid ratio and also the sum of oleic + linoleic acids, reducing the concentration of saturated fatty acids (Izquierdo and Aguirrezábal 2008). Although both saturated fatty acids changed with temperature, the *OLD* mutation only affected the response of stearic acid concentration, since variations in palmitic acid were similar in genotypes with and without this mutation.

The effect of night temperature on oleic and linoleic acid percentages reported for traditional and high oleic sunflower genotypes (Izquierdo *et al*. 2006; Izquierdo and Aguirrezábal 2008) was also observed in the HSHL and HSHO inbred lines. This observation confirms previous results obtained only with

Table 3. Mean difference in the concentration of fatty acids estimated for the orthogonal contrast 16/16 *v.* 26/16°C, 26/26 *v.* 32/26°C, and night temperature 16°C *v.* night temperature 26°C

Genotypes: T, traditional; HO, high oleic; HSHL, high stearic; HSHO, high stearic-high oleic. *$P < 0.05$ for difference from 0

Test	Genotype		Palmitic	Stearic	Oleic	Linoleic
16/16 *v.* 26/16°C	T	HA89B	–1.5*	–2.1	5.1*	–1.3
	HO	RHA345	–1.1*	–1.2	4.6	–2.2
	HSHL	CAS-3	–1.4*	0.8	2.7	–2.2
	HSHL	ADV-2504	–3.6*	–1.4	1.0	4.2
	HSHL	ADV-3512	–0.9*	1.2	0.0	–0.5
	HSHO	ADV-3807	–1.7*	3.2	–6.3*	4.6
	HSHO	ADV-2803	–0.6*	4.3*	–7.5*	3.7
	HSHO	ADV-3816	–1.4*	1.7	–5.7*	5.1
26/26 *v.* 32/26°C	T	HA89B	–0.7*	–0.7	12.3*	–10.8*
	HO	RHA345	–0.3	0.6	–3.4	3.2
	HSHL	CAS-3	–0.1	0.2	–7.0*	6.8*
	HSHL	ADV-2504	–1.5*	–1.0	0.5	2.2
	HSHL	ADV-3512	–0.2	1.4	–2.2	0.8
	HSHO	ADV-3807	–0.3	3.6*	–3.0	–0.3
	HSHO	ADV-2803	0.0	1.1	–2.5	1.4
	HSHO	ADV-3816	–1.0*	0.8	–0.4	0.6
Night 16°C *v.* night 26°C	T	HA89B	–0.3	1.3	–12.5*	11.6*
	HO	RHA345	–0.9*	1.0	–0.8	0.7
	HSHL	CAS-3	–0.9*	9.1*	–9.9*	1.5
	HSHL	ADV-2504	–1.7*	3.4*	–28.3*	26.6*
	HSHL	ADV-3512	–0.2	7.1*	–27.9*	21.0*
	HSHO	ADV-3807	–0.9*	2.1*	–7.5*	6.3*
	HSHO	ADV-2803	–0.9*	4.0*	–5.5*	2.4
	HSHO	ADV-3816	–1.5*	–0.5	–2.8*	5.1*

traditional and high oleic genotypes. The 'night' effect seems to be related to a higher desaturation from oleic to linoleic acid during the dark period of the daily cycle (Pleite *et al*. 2008). Such a mechanism is then conserved in sunflower genotypes carrying other mutations highly modifying the mode of fatty acid synthesis. Also, the stearic acid concentration was better accounted for by night temperature than by other temperatures. However, it is not known whether this association with night temperature results from a direct effect of night temperature on stearoyl-ACP desaturase alone or whether it is a consequence of combined effects also on other enzymes (e.g. oleayl desaturase, β-ketoacyl-ACP synthase I or II).

The knowledge that night temperature affects oil fatty acid composition is important for selecting the environment to produce grains with the desired oil fatty acid composition. For high stearic-high oleic genotypes, the environment should be selected according to the most critical fatty acid, since high temperature increases oleic but reduces stearic acid concentration. Also, the effects of temperature on oil yield components of these mutated genotypes and its interaction with fatty acid composition should be considered for the selection of genotypes (e.g. Velasco *et al*. 2007).

The effects of temperature on fatty acid composition reported here were investigated by applying different temperature treatments in growth chambers. As a general rule, caution must be taken when results from pot-grown plants under controlled conditions are extrapolated to field conditions (Passioura 2012). Temperature is, however, a main factor determining fatty acid composition in several species and genotypes within species (e.g. Izquierdo *et al*. 2002, 2006; Sobrino *et al*. 2003). Diverse environmental factors such as solar radiation, rainfall, nitrogen availability, soil salinity, and crop health (e.g. Irving *et al*. 1988; Pritchard *et al*. 2000; Izquierdo *et al*. 2009; Echarte *et al*. 2010) could also affect fatty acid composition. However, in sunflower these factors produce lower variations in fatty acid compositions than those expected via changes in temperature. This smaller effect of other factors probably explains why results obtained by studying the effect of temperature on fatty acid composition of sunflower plants grown in pots under controlled conditions have well predicted the behaviour of field-grown plants (Izquierdo and Aguirrezábal 2008; Echarte *et al*. 2010).

It is known that variations in fatty acid composition affect oil properties and oil quality. However, other traits also define oil quality, e.g. the triacylglyceride composition. The way the fatty acids are arranged in the triglyceride molecules influences physical, chemical, and nutritional properties. According to these results and the literature, we can state that the fatty acid compositions of high stearic and high stearic-high oleic sunflower genotypes are differently affected by temperature. Although the triacylglyceride composition of high-saturated sunflower lines has been already characterised (e.g. Fernández-Moya *et al*. 2000, 2005), it is not known if that composition is differently affected by temperature in genotypes with different mutations that modify the fatty acid synthesis. So, it would be interesting to know if temperature also affects the triglyceride composition, to further understand the variations in oil quality when temperature during grain filling changes.

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