DIVERSIFIED COMPOSITION OF SUNFLOWER (Helianthus annuus L.) SEEDS WITHIN CULTURAL PRACTICES AND GENOTYPES (HYBRIDS AND POPULATIONS)

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SUMMARY

Environmental factors, mainly temperature and water availability, play a major role with genotype factor in oil quality of sunflower. To manage seed composition through cultural practices and genotype choice under scarce water resources is one way to provide an added-value to crop yield and to diversify uses for edible oil or for non food applications in Mediterranean regions.

The study of interactions between crop management and genotypes for yield and also for major seed compounds (oil, protein and fatty acids) was conducted in the Rotation-Quality device in Toulouse-Auzeville (France) during three experimental years.

Two standard populations and four hybrids (two oleic and two standard), well adapted to cropping systems in Morocco and France, were investigated in conventional and late sowing dates associated with non-irrigated and irrigated treatments.

For all genotypes, delay of sowing improves protein content and seed weight but decreases yield, stronger for populations compared with hybrids whereas oil content is not depressed. Globally, in irrigated crop, only the percentage of protein in seed dry matter decreases.

The oleic and linoleic acids contents in standard hybrids seeds are sensitive to sowing date whereas oleic hybrids and populations' contents are relatively more stable. Indeed, oleic acid content in standard hybrid seeds increases with a concomitant reduction of linoleic acid content in the case of late sowing. Water regime does not affect the oleic and linoleic acids. The delay in sowing decreases stearic and palmitic acid contents in oleic hybrid seeds,

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and in standard hybrid seeds only palmitic acid content. These saturated fatty acids contents are not affected by irrigation treatment.

Close negative correlations between oleic and linoleic acids and between oleic and palmitic acids are present in all genotypes and treatments.

Our results suggest that there exist different genotype behaviors for fatty acids accumulation in response to crop management.

Key words: sunflower, genotype, fatty acids, oil quality, water regime, sowing date

INTRODUCTION

Sunflower (*Helianthus annuus L.*) occupies an important place among oil crops on the world market and its production multiplied by approximately 1.8 during last 20 years (Pouzet and Delplancke, 2000).

In Morocco, sunflower is traditionally cultivated in the spring, and it is exposed to severe climatic constraints due to the scarce water availability and high temperatures (El Asri *et al.*, 2000). Generally in Morocco, best yields are obtained with early sowing dates, from November to December, using late genotypes (Boujghargh, 1993; Gosset and Vear, 1995).

In France, this species is cultivated in semi-arid zones, in agreement with its capacity of adaptation to drastic climatic constraints (Hattendorf *et al.*, 1988; Merrien and Grandin, 1990). However, in some areas, competition with other oil crops limits its use, in particular for edible oil production. Moreover, since the new orientation of the Common Agricultural Policy and the modification of the fallow in 2000, sunflower undergoes a part of economic constraints. A broader diversification is looking for to lead to new industrial projects of valorization by using the diversity of seed fatty acids composition (Lacaze-Dufaure, 1998).

Thus, the production quality represents nowadays a major asset for various applications. In food industry, sunflower oil has the advantage of being stable, particularly in the case of oleic varieties which contain high levels of unsaturated fatty acids (Fuller *et al.*, 1967; Fitch Haumann, 1994). Unsaturated fatty acids are involved in the prevention of cardiovascular diseases (Kinter *et al.*, 1996; Jing *et al.*, 1997; Berry and Rivlin, 1997; Delplanque, 2000).

The diversity of fatty acids composition is also interesting for non-food industries such as the stearic acid for cosmetic industry and the linoleic and the oleic acids for paint or the lubricant industries (Lacaze-Dufaure, 1998).

Fatty acid composition is known to vary according to genotypes (standard, midoleic and oleic) and to environmental conditions (Connor and Sadras, 1992). Genotype and temperature can modify the oleic/linoleic ratio (Harris *et al.*, 1978; Champolivier and Merrien, 1996; Lagravère *et al.*, 2000), whereas, the effect of nitrogen supply is limited and depends more on the stage of application (Steer and Seiler, 1990). For natural field conditions, the effect of temperature through the delay of sowing has not been extensively studied. Connor and Sadras (1992) report that sowing date influences the fatty acid composition by modifying the ontogenesis. For standard hybrids, delay of sowing involves a reduction of oleic acid content and an increase of linoleic acid content (Jones, 1984; Unger, 1986). The same variation is observed for oleic hybrids (Flagella *et al.*, 2002).

The effect of irrigation is poorly documented and data are generally contrasting. Some authors pointed out that if a water stress occurs during the grain filling period, it generates an increase in oleic acid content and a concomitant reduction of linoleic acid content (Talha and Osman, 1974). Contrary to that, Unger (1982) reports that irrigation has no effect on this ratio, whereas Baldini *et al.* (2000) and Flagella *et al.* (2002) underline a clear rise of this ratio in irrigated conditions.

In order to highlight the accessible benefit margin through managing seed composition by technical practices and genotypes potentialities, our study proposes to specify the effect of delay of sowing and the contribution of irrigation in post-flowering on the variation of seed fatty acid composition in hybrids grown in France and in populations varieties representative of sunflower crops in Morocco.

Thus, long-term experiments, conducted on a range of productive genotypes with different qualitative characteristics (oleic and standard), allowed to analyze the variation of the principal saturated (palmitic and stearic) and unsaturated (oleic and linoleic) fatty acids in relation to the productivity (yield, oil and protein contents).

MATERIAL AND METHODS

Experiments were carried out for three years (1999, 2000 and 2002) in southwestern France (43.551°N latitude) on a deep calcareous clayey silt soil with high water-holding capacity (Cabelguenne *et al.*, 1999). Different genotypes were investigated in Toulouse-Auzeville on Rotation-Quality device at the "Institut National de la Recherche Agronomique" (INRA), France:

- 1. two populations (cv. Karima and Salima, commonly used in Morocco, provided by INRA Maroc and registered in Official Catalogue in 1990),
- 2. two standard hybrids [Santiago II and Aranda, provided by North Company Krups (now Syngenta group) in 1999 and 1995, respectively] and
- 3. two oleic hybrids (Proleic 204 from Rustica (now Euralis Soltis) in 1993, and Trisun from Mycogen Verneuil in 1994).

Treatments

The treatments and genotypes used are mentioned in Table 1. Experiments were conducted by cultural practices commonly used in southwestern France. Sunflower was sown in mid-April (conventional sowing date) and one month later for the late sowing date (mid-May).

Year	Treat	ments		Genotypes	
real	Sowing dates	Water regimes	Populations	Standard hybrids	Oleic hybrids
1	Conventional	Non irrigated		Santiago II	Proleic 204
I	Late		-	Aranda	Trisun
	Conventional	Non irrigated		Santiago II	Proleic 204
П		Irrigated	-	Aranda	Trisun
	Conventional	Non irrigated	Karima	Santiago II	Proleic 204
	Late	Irrigated	Salima	Saniiayon	FIDIEIC 204

Table 1: Cultural practices and genotypes tested along the three years

Years I, II and III correspond to 1999, 2000 and 2002 respectively

Two contrasted water regimes were applied corresponding to:

- 1. a non-irrigated treatment where the crop received only the rainfall, and
- 2. an irrigated treatment. Irrigation was applied after flowering during the seed ripening period in order to satisfy nearly 70% of the entire sunflower water requirement. Irrigation amount was determinate via meteorological approach considering variations of cultural coefficients with sunflower phenological stages (Picq, 1989) based on data collected from the meteorological station of INRA.

A randomized blocks device was adopted in year I, with three replicates. In years II and III, experiments were conducted in a split-plot device with three and four replicates, respectively, in which large plots were associated with water regimes.

Plants were sown by a pneumatic seeder 3 m wide, at 71 000 plants/ha and 3-4 cm depth. At harvest, yield was estimated at 67.5 m_c in years I and II and at 270 m_c in year III. Seed weight was established on weight of 1000 seeds evaluated by a seed counter (Chopin Numigral). Seeds were dried in ventilated vacuum oven during 48 hours at 80°C in order to obtain 0% of moisture.

Climatic sequences characterization

The temperature and rainfall variations recorded from January to September during the three experimental years are gathered in Figure 1.

According to the total rainfall amount, year I was relatively dry, particularly along the growth cycle of the second sowing date (76 mm of water deficit as compared with conventional sowing date). During year I, the difference in water supply between the two sowing dates was more accentuated during the vegetative phase. However, during flowering period, the situation was reversed for the conventional sowing date: precipitation was poor during the growth cycle for the conventional sowing date compared with that observed in the second date (37 mm deviation). On the contrary, in year III, cumulated precipitations were almost similar for the crops grown in the two sowing dates during all the cycles. In year II, during the vegetative

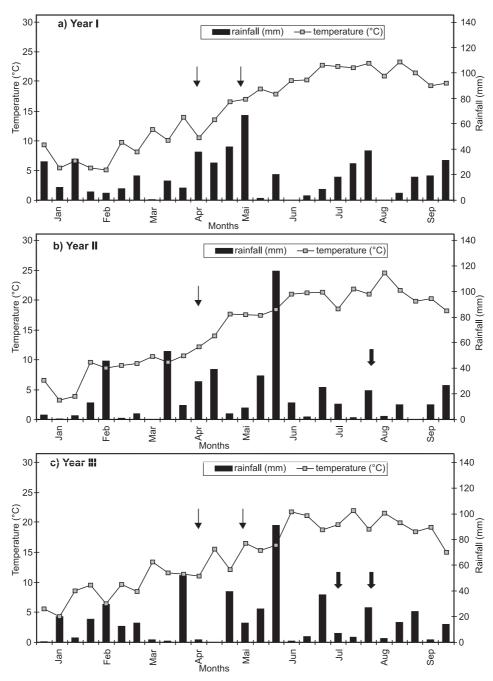


Figure 1: Rainfall and temperature mean values in: a) year I (1999), b) year II (2000) and c) year III (2002). Sowing dates are indicated by a thin arrow (↓). The amount of irrigation applied in post-flowering period are indicated by a full arrow (↓).

		equirements of sunflower	growin				T · ·
Year	Sowing	Climatic parameters			ogical sta		Total
	dates		S-F1	F1-MO	MO-M2	M2-Maturity	
		Rainfall (mm)	183.0	8.5	23.5	68.0	281.0
	SD1	Mean temperature (°C)	17.0	22.6	22.2	22.4	
		Temperature sum (°Cd)	772.4	249.1	321.5	581.3	1874.2
Year I		WD Nirr (mm)		-71.4	-59.0	-30.8	
rearr		Rainfall (mm)	104.0	41.5	35.5	23.5	205.0
	SD2	Mean temperature (°C)	19.7	22.5	22.2	22.3	
		Temperature sum (°Cd)	800.1	280.2	184.9	463.6	1729.3
		WD Nirr (mm)		-45.7	-17.3	-47.9	
		Rainfall (mm)	239.0	31.5	24.0	14.0	308.5
	SD1	Mean temperature (°C)	18.0	18.5	21.1	22.2	
Year II		Temperature sum (°Cd)	870.6	207.1	322.3	451.5	1751.5
		WD Nirr (mm)		-1.9	-67.3	-59.3	
		WD Irr (mm)		-1.9	-27.3	-59.3	
		Rainfall (mm)	191.0	31.0	33.0	40.5	295.5
	SD1	Mean temperature (°C)	16.7	20.4	20.6	18.6	
		Temperature sum (°Cd)	845.9	242.7	406.6	280.6	1775.8
		WD Nirr (mm)		-52.1	-55.6	-14.1	
Veer III		WD Irr (mm)		-12.1	-55.6	-14.1	
Year III		Rainfall (mm)	184.5	31.5	40.5	15.0	271.5
	SD2	Mean temperature (°C)	19.1	20.1	18.6	18.3	
		Temperature sum (°Cd)	791.3	291.0	301.9	152.2	1536.3
		WD Nirr (mm)		-51.8	-19.2	-11.0	
		WD Irr (mm)		-21.8	-19.2	-11.0	

phase (S-F1), a supply of around 50 mm compared with years I and III was observed in the case of the conventional sowing (Table 2).

Table 2: Rainfall, mean temperature, temperature sum and water deficit in the three experimental years at the different phenological stages. 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

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. Years I, II and III correspond to 1999, 2000 and 2002 respectively

In regard to mean temperature, year I was warmer compared with the other two years and it was characterized by constant and relatively high levels of temperature since the beginning of flowering period until the end of the cycle (approximately 22°C on the average); this happened in the case of both sowing dates. In year II, a remarkable increase of temperature occurred only from the beginning of seed filling period (M0) to maturity. Year III presented the coolest temperature conditions, particularly during the S-F1 period at conventional sowing date and towards the end of its cycle. Moreover, during this same year, a difference in water deficit was quite similar until the beginning of seed filling and was contrasted during MO-M2 period.

Consequently, in non water supplied treatment, water deficit has varied at flowering period and the end of the cycle by conventional sowing from -71 mm to -31mm in year I, from -2 mm to -60 mm in year II and from -52 mm to -14 mm in year III. For late sowing it varied from -46 mm to -48 mm in year I and from -22 to -11 mm in year III for the same periods. However, water supply during the M0-M2 period in year II and at flowering in year III has decreased the water deficit by 40 mm and 30 mm, respectively.

Biochemical analysis

Analyses were carried out on three samples/plot/genotype/treatments in the third year (12 replications) and on three replications/genotype/treatments in the other two years.

Oil and protein contents

Whole seeds were analyzed for oil content by Nuclear Magnetic Resonance (NMR Oxford-4000) in Morocco (INRA Meknčs). Nitrogen percentage was determined by a CHN analyzer (Leco® CHN-2000) in France (INRA Toulouse Centre) on 100 mg of dry matter of seed flour. Protein content was established according to the standard estimation ($6.25 \times N\%$).

Fatty acids contents

Fatty acids contents were analyzed in "Oléo-Lipo-Chimie" Laboratory in "INRA-Transformation des Végétaux" ("Ecole Nationale Supérieure des Ingénieurs en Arts Chimiques Et Technologiques", Toulouse, France).

Dried seed samples of 3 g were ground with a mixer (Krups type 203) for 30 sec. Total fatty acids contents were analyzed by Gas Chromatography (Varian GC-3800) on oil extracted by iso-hexane with Accelerated Solvent Extractor (Dionex ASE[®]-200) equipped with 11 ml cells, analytical balance and Dionex vials for collection of extract (40 ml, P/N 49465), in the third year, according to Matthaüs and Brühl (2001), and with Soxhlet type extractor (ISO 659: 1998) in both the first and the second year. Oil extract was evaporated in a rotary evaporator (Janke and Kunkel IKA-WERK) by distillation at reduced pressure and 40°C until the solvent was totally removed. Fatty acids were esterified according to ISO 5509: 1990 Norm. Comparing time retention of FAME Mix rapeseed oil (SUPELCO, USA) identified integration peaks.

Statistical analysis

ANOVA procedure was performed on SigmaStat statistical package (version 2.0, USA) to analyze the collected data. Within year, the different genotypes, sowing dates and/or water regime treatments and their interactions on yield, seed weight, oil and protein contents, major fatty acids contents were analyzed separately. Differences between mean values were obtained using Student-Newman-Keuls test. Correlations were calculated by the Spearman test.

RESULTS

Genotypes characterization for yield and seed composition

The genotype variability of cultivars used in this study (populations, standard and oleic hybrids) shows different behaviors concerning the levels of production (yield, oil and protein contents), seed weight and major saturated (palmitic and stearic) and unsaturated (oleic and linoleic) fatty acids. The others fatty acids detected (myristic, arachidic, linolenic, behenic) did not exceed 2% of total fatty acids and showed modest variations.

Table 3: Effects of genotype (G), sowing dates (SD) and interaction G*SD within year (I and III, non irrigated treatment) on oil and protein contents, yield, seed weight, palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids contents

Variable	Year		Genotype	Sowing date	G*SD	Residus
		MS	32.5	0.002	0.5	1.0
Oil content	I	Р	***	NS	NS	
(% SDM)		MS	221.2	2.07	13.0	6.4
	III	Р	***	NS	NS	
		MS	31.4	3.9	0.8	0.3
Protein content	I	Р	***	**	NS	
(% SDM)		MS	26.5	113.7	18.1	1.6
	III	Р	***	***	***	
		MS	162.6	11.6	6.9	2.7
V:= - (+/)	I	Р	***	NS	NS	
Yield (t/ha) -		MS	1358.5	1318.4	26.0	18.7
	III	Р	***	***	NS	
		MS	309.7	178.8	35.0	7.8
o	I	Р	***	***	*	
Seed weight (mg) -		MS	693.0	483.5	148.1	26.4
	111	Р	***	***	***	
		MS	12.2	1.1	0.1	0.02
010 0 (0()	I	Р	***	***	***	
C16:0 (%) -		MS	19.6	1.1	0.5	0.02
	III	Р	***	***	***	
		MS	1.2	0.2	0.01	0.01
010 0 (0()	I	Р	***	***	NS	
C18:0 (%) -		MS	10.1	1.9	0.6	0.04
	III	Р	***	***	***	
		MS	7244.9	66.7	20.9	1.6
010 1 (0()	I	Р	***	***	***	
C18:1 (%) -		MS	15992.2	56.4	214.6	7.7
	III	Р	***	**	***	
		MS	6752.3	43.2	19.8	1.5
C10-0 (0()	I	Р	***	***	***	
C18:2 (%) -		MS	14276.7	30.4	188.1	6.9
	III	Р	***	*	***	

*: significant for p<00.5, **: significant for p<0.01, ***: significant for p<0.001, NS: non significant MS: Mean Square, p: probability value, SDM: seed dry matter

Yield, seed weight, oil and protein contents

Variance analysis showed a very highly significant effect of genotype for all variables in the three experimental years (Tables 3 and 4).

Table 4: Effects of genotype (G), water regimes (WR) and interaction G*WR within year (II and
III, conventional sowing date) on oil and protein contents, yield, seed weight,
palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids contents

Variable	Year		Genotype	Water regime	G*WR	Residus
		MS	18.4	0.06	1.01	1.26
Oil content	II	Р	***	NS	NS	
% SDM) -		MS	342.0	243.4	32.2	5.02
	III	Р	***	***	**	
		MS	19.8	1.0	0.9	0.4
Protein content	II	Р	***	NS	NS	
(% SDM) -		MS	8.5	74.4	5.3	3.8
	111	Р	NS	***	NS	
		MS	153.5	50.5	0.4	5.5
	II	Р	***	**	NS	
rield (t/ha) -		MS	1751.9	2.4	58.1	14.6
	III	Р	***	NS	**	
		MS	170.7	7.5	6.5	3.7
	II	Р	***	NS	NS	
Seed Weight (mg) -		MS	341.9	19.3	74.1	31.5
	III	Р	***	NS	NS	
		MS	12.6	0.05	0.007	0.005
	II	Р	***	**	NS	
C16:0 (%) -		MS	25.6	0.2	0.2	0.03
	III	Р	***	*	***	
		MS	5.2	0.004	0.09	0.03
	II	Р	***	NS	NS	
C18:0 (%) -		MS	11.5	1.5	0.04	0.07
	III	Р	***	***	NS	
		MS	7779.8	0.5	0.7	1.1
	II	Р	***	NS	NS	
C18:1 (%) -		MS	18393.1	5.9	45.6	7.7
	III	Р	***	NS	***	
		MS	7017.8	0.4	0.7	1.0
	II	Р	***	NS	NS	
C18:2 (%) -		MS	16400.0	10.2	39.3	7.5
	III	Р	***	NS	**	

* : significant for p<00.5, ** : significant for p<0.01, *** : significant for p<0.001, NS : non significant MS : Mean Square, p : probability value SDM : seed dry matter

Generally, standard hybrids produced more seeds and oil than oleic hybrids (Table 5). This increase was +0.7t/ha or +0.3t/ha for yield and +3.87% or +2.21% to for oil content in years I and III, respectively. Populations had lowest yield and oil content levels. However, populations had the highest seed weight (58 to 64 mg), followed by standard hybrids (43 to 61.5 mg) and then by oleic hybrids (41 to

Vicriphico		Year I	ar I			Year II	ir II			Year II	_	
variables	Trisun	Proléic 204	Santiago II	Aranda	Trisun	Proléic 204	Santiago II	Aranda	Santiago I	Trisun Proléic 204 Santiago II Aranda Trisun Proléic 204 Santiago II Aranda Santiago II Proléic 204 Karima Salima	. Karima	Salima
Oil content (% SDM)	46.52 c	46.52 c 48.12 b	51.15 a	51.22 a	50.69 b	51.15 a 51.22 a 50.69 b 49.98 b	53.36 a	53.29 a	53.36 a 53.29 a 51.67 a	49.46 b	49.46 b 44.08 c 44.76 c	44.76 c
Protein content (% SDM)	I) 15.33 c	19.97 a	17.93 b	15.18 c	15.18 c 18.97 b	21.66 a	21.80 a	18.27 b	19.81 c	20.85 b	21.55 ab 22.10 a	22.10 a
Yield (t/ha)	2.58 b	1.82 c	2.84 a	2.99 a	2.99 a 2.51 b	1.81 c	2.59 b	3.03 a	3.44 a	3.13 b	1.84 c	1.82 c
Seed weight (mg)	45.30 c	47.75 bc	61.53 a	50.03 b	50.03 b 43.73 bc	40.79 c	52.84 a	42.87 c	55.21 c	50.50 d	58.44 b	64.04 a
C16:0 (%)	3.07 d	3.37 c	5.03 b	6.08 a	6.08 a 2.79 d	3.31 c	4.92 b	5.94 a	5.23 а	3.35 b	5.22 a	5.23 a
C18:0 (%)	3.32 b	2.80 c	3.72 a	2.78 c	2.78 c 3.93 b	3.23 d	5.36 a	3.64 c	4.37 a	2.93 c	4.29 a	4.08 b
C18:1 (%)	90.72 a	87.32 b	33.50 c	24.60 d	24.60 d 85.47 a	80.66 b	22.30 c	19.35 d	29.53 c	81.88 a	28.20 c	31.38 b
C18:2 (%)	1.38 d	4.70 c	56.32 b 65.20 a 3.92 d	65.20 a	3.92 d	8.85 c	64.03 b 66.94 a	66.94 a	59.13 a	9.69 c	60.38 a	60.38 a 57.35 b
SDM: seed dry matter				:								

Table 5: Effects of genotype on oil and protein contents, yield, seed weight, pamitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic

For each year. mean values with same letter are not significantly different at the threshold of 5 %

Average Santiaç	Santiago II Proléic 204	Karima	Salima Average
I I			
	52.01 a 48.75 a	46.04 a	45.11a 47.98a
49.26 a 50.74	50.74 a 49.86 a	44.23 a	45.92 a 47.69 a
16.70 b 19.98	19.98 c 21.84 b	20.39 c	20.88 b 20.77 b
17.51 a 20.65	20.65 c 22.51 b	24.13 a	24.47 а 22.94 а
2.49 a 3.58	3.58 a 3.37 a	2.30 a 2.	2.21 a 2.87 a
2.63 a 2.72 a	72.a 2.91.a	1.38 a 1.	1.50 a 2.13 b
48.43 b 53.50 cd	50 cd 51.51 d	57.70 c	57.47 c 55.05 b
53.88 a 57.02	57.02 c 49.95 d	63.16 b	67.92 a 59.51 a
4.60 a 5.54 a	54.a 3.35.e	5.13 c 5.	5.30 b 4.83 a
4.18 b 4.93	4.93 d 3.40 e	5.02 cd 5.	5.12 c 4.62 b
3.23 a 4.50 a	50a 3.18b	4.46 a 4.	4.58 a 4.18 a
3.08 b 4.35 a	35a 2.98 c	4.43 a 3.	3.82 b 3.89 b
57.29 b 24.74	24.74 e 82.80 a	31.00 d	29.59 d 42.03 b
60.63 a 34.15	34.19 c 80.20 b	27.45 e	32.42 c 43.57 a
33.24 a 63.44 a	.44 a 8.56 d	57.59 b 58	58.72 b 47.08 a
30.56 h 54.77 h	77 h 11 31 c	61.05 a	56.65 b 45.95 b
08 b 29 b 63 a 24 a	35 a .74 e .19 c .44 a	2.98 c 82.80 a 80.20 b 8.56 d	2 4.43 a a 31.00 d b 27.45 e d 57.59 b

HELIA, 27, Nr. 40, p.p. 73-98, (2004)

50.5 mg). Thus, seed weight levels were similar in the oleic hybrids and significantly different for the standard hybrids (+10 mg for Santiago II). Concerning the protein content, a significant difference was noted between oleic hybrids on one hand and standard hybrids on the other. Higher contents were observed for population cultivars and for Proléic 204 and Santiago II (21.7% on the average) compared with Trisun and Aranda (16.9% on the average).

Fatty acid contents

The factor genotype had a very highly significant effect on all fatty acid contents in the three experimental years (Tables 3 and 4).

The oleic hybrids had lowest palmitic and linoleic acid percentages compared with the standard hybrids and populations. Consequently, palmitic acid content varied from $3.22\% \pm 0.17$ for oleic hybrids to $5.43\% \pm 0.2$ for standard hybrids and populations. There was a range of variation of oleic acid content in the oleic hybrids from 85 to 90% and 81 to 87% in Trisun and Proléic 204, respectively, and from 22% to 34% and 19% to 24% in the standard hybrids Santiago II and Aranda, respectively. Population cultivars reached about 30% of oleic rate. Concomitant variations were observed concerning the linoleic acid content. Stearic acid content was contrasted within each genotype, with the highest contents for Santiago II and Karima and the weakest for Proléic 204 and Aranda.

Effects of sowing date and its interaction with genotype on yield and major seed compounds

Sowing date

Variance analysis showed very highly significant effects of sowing date on seed weight, protein and fatty acids contents, whereas oil content was not affected in years I and III (table 3). Generally, delay of sowing was associated with the improvement of protein content (+4.9% in year I and +9.5% in year III) and seed weight (+11.3% and +7.5%). Globally, a reduction of saturated fatty acids (between 4 to 9% and between 5 to 7% for palmitic and stearic acid contents, respectively) and linoleic acid contents (from 2.5 to 8%) occurred with late sowing. Also, a slight increase of oleic acid content (from 3.5 to 6%) was observed.

Sowing date \times genotype interaction

Sowing date affected differently the crop parameters and seed composition within genotype (Table 6). Seed weight and protein content increased significantly with the delay of sowing for populations (+13.7% and 9.6%, respectively) whereas no modification appeared in the case of hybrids. For seed weight as well as for protein content, the interaction sowing date×genotype depended on the year (Table 3). Oil content was stable regardless of the genotype, whereas, generally, yield was depressed when late sowing date was applied.

Effect of delayed sowing was clearly expressed in the variance analysis that showed very highly significant effects of sowing date×genotype interaction on fatty acid contents in both, year I and III (Table 3). The percentage of stearic acid content decreased with delaying sowing in seeds of oleic hybrids (-6.6%) and in those of the variety Salima (-16.6%). In seeds of the standard genotypes (Santiago II, Aranda and Karima), stearic percentage did not fluctuate. Globally, palmitic acid percentage in total fatty acids was lowered with delay of sowing in all genotypes and years.

Concerning the unsaturated fatty acids, the oleic hybrids remained generally stable for oleic and linoleic acid contents with delay of sowing. Conversely, for standard hybrids and Salima, the oleic acid content increased ($26.6\% \pm 3.0$ in conventional sowing date to $32.6\% \pm 5.8$ in late sowing date) concomitantly with the reduction of linoleic acid content under the effect of sowing date.

Variation of yields and major seed compounds with water regime and interaction with the genotype

Water regime

In the experimental conditions presented here, water supply applied after flowering did not have a significant effect on seed weight and on oleic and linoleic acids contents. Significant variation for yield, oil and protein contents depended on the year (Table 4). However, palmitic acid content varied with water regime significantly in both yeard II and III. In year II, stearic acid, protein and oil contents also varied very significantly with water availability (-6.3%, -7.8% and +6.6%, respectively, in irrigated treatment) (Table 7).

Water regime×genotype interactions

According to the genotype, the response to water management was variable considering the different parameters. Thus, irrigated conditions×genotype interaction has no effect in year II on all crop parameters and seed composition. It was also the case for seed weight, protein and stearic acid contents in year III. However, during this last year a significant effect of water availability×genotype interaction was noticed on yield, oil, palmitic and unsaturated fatty acids contents. With regard to hybrids and populations, stability of oil content and yield was recorded except for the standard hybrid Santiago II whose seeds were slightly penalized in oil content but improved in yield by water supplies. In the case of hybrids, protein content presented a drop with water supply (-11.4%) compared with protein contents in population seeds.

A strong stability of unsaturated fatty acid contents under the two water managements was noticed for hybrids tested (standard and oleic) as well as for one variety in the population pool (Salima). The palmitic acid content stayed unchanged under irrigated treatment for all genotypes whereas this component was enhanced in Karima seeds under the same conditions. The stearic acid content in seeds of the population varieties decrease non-significantly with water supply (-6%). In addition, the content of this acid for the standard hybrid seeds was more stable compared with the oleic hybrid.

	Conchrace			Year II					Year III		
Variables	dellotypes	Trisun	Proléic 204	Proléic 204 Santiago II	Aranda	Average	Santiago II	Santiago II Proléic 204	Karima	Salima	Average
	Water regimes										
Oil content	Nirr	51.28 ab	50.06 b	53.26 a	52.89 a	51.88 a	52.01 a	48.75 b	46.31 d	45.11 d	48.05 a
WDS (%)	Irr	50.10 b	49.88 b	53.45 a	53.67 a	51.78 a	49.70 b	47.89 b	41.08 d	40.78 d	44.86 b
Protein content Nirr	Nirr	18.88 bc	22.44 a	21.81 a	18.39 bc	20.38 a	19.98 a	21.84 a	20.47 a	20.88 a	20.79 a
(WDS %)	Irr	19.06 c	20.88 b	21.79 ab	18.15 c	19.97 a	18.29 b	18.77 b	19.06 ab	20.01 ab	19.03 b
Yield	Nirr	2.37 b	1.68 c	2.46 b	2.85 ab	2.34 b	3.58 b	3.37 b	2.31 c	2.21 c	2.87 a
(t/ha)	Irr	2.66 b	1.93 c	2.72 b	3.21 a	2.63 a	4.03 a	3.45 b	2.09 c	2.02 c	2.90 a
Seed weight	Nirr	43.14 c	41.51 cd	51.01 b	42.33 c	44.50 a	53.50 b	51.51 b	57.03 b	57.47 b	54.88 a
(bm)	Irr	44.32 c	40.07 d	54.67 a	43.41 c	45.62 a	53.51 b	51.76 b	54.53 b	63.30 a	55.78 a
C16.0 /0/)	Nirr	2.80 e	3.37 d	5.01 c	5.96 a	4.28 a	5.54 a	3.35 d	5.13 c	5.30 b	4.83 b
	Irr	2.78 e	3.26 d	4.84 b	5.91 a	4.20 b	5.57 a	3.33 d	5.53 a	5.29 b	4.93 a
/0/ 0.010	Nirr	3.78 bc	3.27 d	5.37 a	3.78 с	4.05 a	4.50 a	3.18 b	4.46 a	4.58 a	4.18 a
0.0.0	Irr	4.08 b	3.18 d	5.36 a	3.49 c	4.03 a	4.36 ab	2.86 c	4.20 b	4.29 b	3.93 b
()0/ TOFU	Nirr	85.31 a	80.35 b	21.83 c	19.69 d	51.80 a	24.74 c	82.80 a	31.00 b	29.59 b	42.03 a
CIO.I (%)	Irr	85.63 a	80.97 b	22.76 c	19.02 d	52.09 a	25.42 c	83.57 a	26.38 c	30.78 b	41.54 a
()0/ C-0FU	Nirr	4.25 d	9.05 c	64.37 b	66.54 a	36.05 a	63.44 a	8.56 c	57.59 b	58.72 b	47.08 a
(o/) 7:01 O	Irr	3.58 d	8.64 c	63.69 b	67.33 a	35.81 a	63.06 a	8.08 c	62.06 a	57.72 b	47.73 a

86

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DISCUSSION

Data are discussed within differences in climatic sequences in the experimental years (water deficit and temperature) occurring during plant growth and their impact according to critical stages of the crop.

In the experimental conditions tested in this study, the crop was grown under contrasted climate sequences in the different years and, during each year, between the two sowing dates. This fact has generated variable responses according to environmental conditions (precipitations and temperature), and according to the genotypes, in particular with regard to the productivity (yield, oil and protein contents) and the fatty acids composition of sunflower seeds.

Impact of delay of sowing

Yield and major seed compounds

Total rainfall from sowing to flowering (S-F1 period) were similar in years I and III in conventional sowing. The production levels were higher in year III compared with year I which may be explained by the relatively low water deficit due to large precipitations during F1-M0 and M0-M2 in year III (Table 2). However, in year III, conventional sowing was favorable for yield compared with the late sowing. The data are in agreement with those of D'amato and Giordano (1992), Sarno et al. (1992), Flagella et al. (2002) who specified that yield and oil content are improved in the case of early sowing. The benefit may be explained by a longer duration of the crop cycle which varies according to the genotypes and the phenological stages. Indeed, with quite similar precipitations in phenological phases between the two sowing dates, the crop cycle was reduced in late sowing (particularly between MO-M2) and it varied from 190°Cd to 275°Cd according to genotypes. In year I, similar reductions in the crop cycle duration were observed associated with a variation of almost 80 mm between conventional and late sowing. Nevertheless, the scarcity of rainfall between F1 and M0 was stronger at conventional sowing (WD=-71 mm against -46 mm in late sowing) and can explain the comparable yield levels at harvest registered for the two sowing dates. Sunflower has a great sensitivity to severe water stress applied from beginning of flowering until seed filling (Hall et al., 1985; Flénet et al., 1996). Moreover, Bouniols et al. (2000) specified that the variations of vield and oil content particularly for standard genotypes were associated with a delay of sowing; while oleic genotypes were generally more stable.

The yield can be mainly interpreted through the total number of grains and, even more, through the rate of abortion, which was not taken into account in this study. In fact, except for Santiago II where a stability of seed weight was observed, for others genotypes the delay of sowing generated a significant increase in this yield component with an advance for populations varieties followed by hybrids standard and, finally, oleic hybrids. In these experiments, great stability of oil content was recorded for all genotypes and years. In the literature, many studies associated the increase in oil content with the decrease of temperature. For example Triboï-Blondel *et al.* (2000) specified that an increase of 1°C during flowering induces a reduction of 1% of oil content and an increase of 0.7% of protein content. The stability of oil content could be explained by the temperature levels, which were relatively high, during the reproductive phase. From flowering to maturity, the mean temperature reached about 22°C on the average in year I and it varied from 20°C to 18.3°C in year III.

For proteins, the other major seed compound, the content varied little, except for a notable increase in population seeds with delayed sowing.

The well-known inverse relation between oil and protein contents and the positive relation between yield and oil content (Piva *et al.*, 2000; El Asri *et al.*, 2000) were still established for several crop conditions except for delay of sowing which was the aim of this present study.

A negative correlation between oil and protein contents present was more conspicuous in the case of late sowing date. A gain of 10 points for oil content corresponded to a loss of 4 and 2.5 points of protein percentage for late and conventional sowing dates, respectively (Figure 2a).

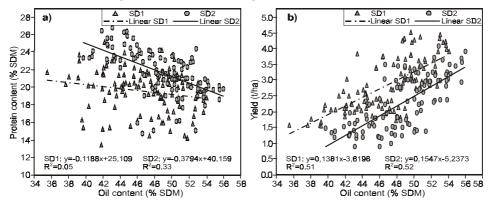


Figure 2: Relationships in conventional (SD1) and late (SD2) sowing dates between: a) oil and protein contents and b) oil content and yield

On the other hand, a significant positive correlation was observed between yield and oil content regardless of sowing date. When oil content was enhanced by 10 points, it generated a parallel increase of yield by about 1.3 t/ha regardless of the treatment and genotype. So, for a similar level of productivity, the oil percentage was higher in the case of late sowing (Figure 2b). Moreover, the translation of the curve related yield and oil content under the effect of sowing date was due to a higher increase of oil content that is associated with an increase of yield which was more accentuated under conventional sowing date.

Fatty acids contents

The study of the influence of sowing date on unsaturated fatty acid contents demonstrated that the percentages were relatively stable in the oleic hybrids. These data are in agreement with the observations of Lagravère et al. (2000). In the standard hybrids seeds, the contents were more variable. It is well-known that oleic hybrids are generally less affected by environmental conditions (Fernandez-Martinez et al., 1986; Garcčs et al., 1989; Triboi-Blondel et al., 2000). In thestandard hybrids, the increase of oleic acid seed content was associated with a reduction of linoleic acid content in the case of delayed sowing. Garcès and Mancha (1991) pointed out that a rise of temperature (more than 20°C) leads to an inhibition of the enzyme Δ -12 desaturase which is responsible of the conversion of oleic acid into linoleic (Garcès et al., 1989; Heppard et al., 1996). The activity of this enzyme is situated between F1 and M0 stages (Garcès and Mancha, 1991). Under the studied experimental conditions, average temperatures during F1-M0 period were similar in conventional and late sowing. However, the temperature sum for F1-M0 period in late sowing date compared with conventional sowing revealed a difference from 29 to 143°Cd according to genotype. Consequently, the temperature played a major role on the oleic/linoleic ratio depending on the enzyme-sensitive phase duration. For the populations, the delay of sowing induced contrasted effects depending on the variety (a reduction of C18:1 and an improvement of C18:2 contents for Karima; an increase of C18:1 and no change in C18:2 contents for Salima). A relative stability characterized the saturated fatty acid palmitic and stearic contents in Karima seeds while a slight, but significant, reduction of these fatty acids contents was noticed in Salima seeds in the case of delayed sowing. It was also the case in oleic hybrids seeds whereas in the standard hybrids only the palmitic acid content became modified.

Concerning the ratio of palmitic to oleic acid, a negative correlation was observed with the oleic and standard genotypes which is in agreement with data of Champolivier and Merrien (1996) collected for oleic hybrids. However, this negative correlation was stronger under late sowing date (SD2) for the oleic hybrids ($r^2=0.71$ against 0.22 in SD1) and under conventional sowing date (SD1) for the standard hybrids and populations ($r^2=0.63$ against 0.38 in SD2) (Figures 3a and 3b).

For both sowing dates, all genotypes (standard and oleic hybrids, populations) presented a very close negative relation ($r^2=1$) between oleic and linoleic acid contents (Figure 3c). Several authors point out the same relation for other oil crops: for example, for soybean (Bouniols *et al.*, 2000) and for various genotypes of sunflower (Champolivier and Merrien, 1996; Izquierdo *et al.*, 2002 in oleic and standard sunflower hybrids; Seiler *et al.*, 1985, 1994, 1999 in wild sunflower populations).

Impact of water availability

The profit observed in year II for yield confirmed the positive effect of irrigation applied between M0 and M2. Irrigation decreased water deficit to -27.3 mm against -67.3 mm in rainfall treatment. During F1 to M0 period, water deficit was very weak and comparable in the two treatments (-2 mm).

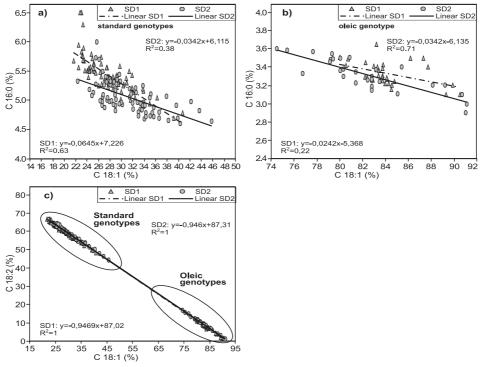


Figure 3: Relationships in conventional (SD1) and late (SD2) sowing dates between: a) palmitic and oleic acids contents for standard (hybrids and populations) b) palmitic and oleic acids contents for oleic hybrids c) oleic and linoleic acids contents for standard and oleic hybrids

In year III, water deficit was stronger during M0 to M2 period (-55.6 mm against -27.3 in year II) in both irrigated and non-irrigated conditions. Water supplied between F1 to M0 period reduced the water deficit to -12 mm against -52 mm under rainfall conditions but did not have any influence on yield. It indicates that the seed biomass accumulation is dependent on the intensity of water stress applied essentially between M0 and M2 stages. Moreover, the higher precipitation level during the vegetative phase (S to F1) in year II allowed probably an exuberant canopy which depressed yield at harvest compared with year III. The adjustment of leaf area to water availability calls for a control of sunflower water consumption (Connor *et al.*, 1985; Ordonez and Company, 1990).

Oil content did not vary significantly under irrigated conditions except for Santiago II. Piva *et al.* (2000) specified that strong water deficits during M0-M2 period (levels comparable with those in the present experiments) induced a significant reduction of oil content. The observed stability of oil content could be due to strong water deficits both in irrigated and non-irrigated treatments, but not enough contrasted between the two treatments during the sensitive phase of seed oil synthesis (between 20 and 40 days after flowering; Champolivier and Merrien, 1996). The same assumptions seem to explain the weak variation of protein content. It appears that the seed composition of oil and protein is sensitive to moderate water deficits during M0-M2; maximum variation is reached as soon as the water deficit becomes stronger.

Oil and protein contents exhibited a negative correlation in non-irrigated conditions ($r^2=0.3$) whereas in irrigated conditions no evident changes in protein content correspond to the significant variations in oil content. A gain of 10 points for oil content resulted in protein content losses of 5 and 1 points, respectively, in non irrigated and irrigated treatments (Figure 4a).

For all treatments, there was a positive and significant correlation between yield and oil percentage (Figure 4b).

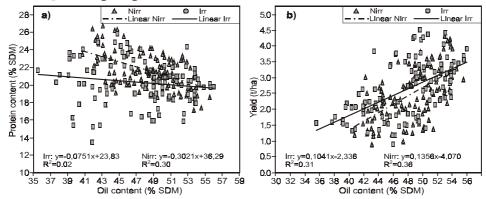
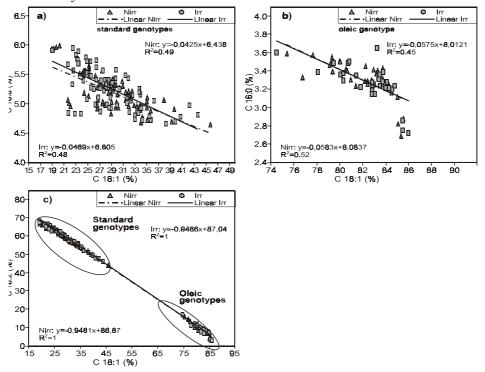


Figure 4: Relationships in non irrigated (Nirr) and irrigated (Irr) treatments between: a) oil and protein contents and b) oil content and yield

Concerning oil quality, no effect was observed on unsaturated fatty acids composition (oleic and linoleic acid contents). These results appear to be in opposition with those of Flagella *et al* (2002) who observed an increase of linoleic acid and concomitantly a decrease of oleic content under irrigated treatment for oleic genotypes. Talha and Osman (1974) have suggested similar variations for standard hybrids. However, Unger (1982) and Salera and Baldini (1998) found also few modifications in these contents for the two types of hybrids. The divergences are probably due to the difficulty to characterize water deficit in the different experiments.

Conversely, the saturated fatty acids contents (palmitic and stearic acids) were modified under irrigated conditions, particularly stearic acid content for which water supply generated a significant reduction in year III. These data are in agreement with those reported by Flagella *et al.* (2002). This behavior was accentuated



for the populations and standard hybrids whereas the oleic hybrids showed a relative stability of the saturated acids content in seed.

Figure 5: Relationships in non irrigated (Nirr) and irrigated (Irr) treatments between: a) palmitic and oleic acids contents for standard (hybrids and populations) b) palmitic and oleic acids contents for oleic hybrids c) oleic and linoleic acids contents for standard and oleic hybrids

The negative correlation between palmitic and oleic acid contents appeared in the oleic and also in the standard genotypes. A similar correlation was observed under water treatment (r^2 varied from 0.49 in non-irrigated treatment to 0.46 in irrigated treatment for the standard hybrids and populations, and from 0.52 to 0.45 for the oleic hybrids for non-irrigated and irrigated treatments, respectively) (Figure 5a and 5b). Each type of cultivars showed a very close negative relation ($r^2=1$) between oleic and linoleic acid contents under different water regimes as well as in the case of delayed sowing (Figure 5c).

CONCLUSION

This work is a part of studies within a bilateral co-operation framework between France and Morocco. It aims to study the effects of delayed sowing and water availability on the diversification of qualitative composition (oil, proteins, major fatty acid contents) of sunflower seeds which could be accessible by cultural practices and choice of genotypes.

This study was conducted for several years and it dealt with the analysis behavior of genotypes bred for quality difference and productivity characters (non-oleic and oleic hybrids, and populations) which are able to provide various profitable uses (edible oil, sunflower street food and non-food products).

In our experiments, the greatest variability of seeds fatty acid contents was induced by the effect of the sowing date. The irrigated treatment affected seed composition very slightly. This was probably due to the weak contrasts between the climates in the test years. However, in the case of the population variety Karima, irrigated conditions were associated with increases in the ratio of saturated fatty acids (stearic/palmitic) and in the ratio of unsaturated fatty acids (oleic/linoleic).

Depending on the genotype, the delay of sowing corresponded to different behaviors of major fatty acids accumulation in seeds. Regardless of the date of sowing, the oleic hybrids maintained the same levels of unsaturated fatty acid in spite of a reduction of the saturated fatty acid part. The standard hybrids presented the opposite variation whereas the increase of oleic acid content (and the concomitant reduction of the linoleic acid content) resulted from the depletion of palmitic acid proportion. Conversely, the stearic acid was considered as a final product which could explain why its content was unchanging.

It should be noticed that the populations, largely used in Morocco, showed a contrasted behavior under the effect of the delayed sowing as compared with the hybrids. The saturated fatty acid contents of the variety Karima were stable and the unsaturated fatty acids balance was modified in favor of the linoleic acid. The other variety population, Salima, behaved like the oleic hybrids, particularly for the linoleic acid.

This study highlights the genotype variability which appears at the productivity level (seeds yield and oil yield) according to the genotypes with a superiority of the standard hybrids, followed by the oleic hybrids and populations. However, the populations had highest protein content and were associated with larger seeds (higher seed weight) when compared with the hybrids. The latter characteristic may arouse a special interest in confectionery use of sunflower seeds in Morocco. Experiments are in due process in order to validate these effects on other agronomic situations and on a wider genetic pool.

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VARIACIONES EN LA COMPOSICIÓN DE GRANOS DE GIRASOL (Helianthus annuus L.) EN FUNCIÓN DEL GENOTIPO (HÍBRIDOS Y POBLACIONES)Y DEL MANEJO DEL CULTIVO

RESUMEN

Los factores del medio ambiente, principalmente temperatura y disponibilidad de agua, juegan, junto con el genotipo, un rol esencial en la calidad del aceite de girasol. Bajo las condiciones de déficit hídrico de la región Mediterránea, manejar la composición del grano con prácticas culturales y varietales ofrece a la producción del cultivo un valor agregado y permite diversificar el uso del aceite para consumo humano u otros consumos no alimenticios.

Las interacciones entre genotipos y el manejo del cultivo sobre la producción y la composición del grano (aceite, proteína y ácidos grasos) han sido estudiadas durante tres años en el dispositivo Rotación-Calidad del INRA de Toulouse-Auzeville (Francia).

Dos poblaciones clàssico y cuatro híbridos (dos oleicos y dos clàssico) bien adaptados a los sistemas de cultivo en Marruecos y Francia, fueron estudiados en siembras convencionales y tardías bajo condiciones de secano y regadío.

En todos los genotipos, un atraso en la fecha de siembra mejora el contenido protéico y la masa del grano, pero disminuye el rendimiento, principalmente en las poblaciones, mientras que el contenido de aceite no es modificado. En cultivos bajo regadío solo decrece el porcentaje de proteína del grano seco.

Los contenidos de ácidos oleicos y linoleicos de las semillas de híbridos clàssico, son sensibles a la fecha de siembra, mientras que ellos son estables en híbridos oleicos y poblaciones. En efecto, en el caso de siembras tardías el contenido de ácido oleico de semillas de híbridos clàssico incrementa con una reducción concomitante del contenido de ácido linoleico. Los contenidos de ácido oleico no son afectados por el régimen hídrico.

El atraso en la siembra disminuye los contenidos de ácidos esteárico y palmítico en granos de híbridos oleicos, mientras que en los granos de híbridos clàssico solo aumenta el contenido de ácido palmítico. Estos contenidos de ácidos grasos saturados no son afectados por el regadío. Las tendencias son variables según las poblaciones.

Una fuerte correlación negativa entre ácidos oleico y linoleico, y entre ácidos oleico y palmítico se observan en todos los genotipos y todos los tratamientos.

Nuestros resultados sugieren que existen diferentes comportamientos genotípicos por la acumulación de ácidos grasos en respuesta al manejo del cultivo.

DIVERSIFICATION DE LA COMPOSITION DES GRAINES DE TOURNESOL (Helianthus annuus L.) SELON LES CONDUITES CULTURALES ET LES GÉNOTYPES (HYBRIDES ET POPULATIONS)

RÉSUMÉ

Les facteurs environnementaux, principalement la température et la disponibilité hydrique, et les génotypes, jouent un rôle majeur sur la qualité de l'huile de tournesol. La maîtrise de la composition des graines par les conduites culturales et le choix des génotypes sous des disponibilités hydriques limitantes est une voie de diversification des utilisations (alimentaires et non alimentaires) permettant d'apporter une plus-value à la production.

Les interactions de la conduite culturale et des génotypes sur le rendement et les principaux composés de la graine (huile, protéines et acides gras) a été étudiée sur le dispositif de Rotation-Qualité à Toulouse-Auzeville (France) pendant trois années expérimentales. Deux populations standards et quatre hybrides (deux oléiques et deux standards) bien adaptés aux systèmes de culture au Maroc et en France, sont cultivés sous deux dates de semis, conventionnelle et tardive, associées aux régimes hydriques pluvial et irrigué.

Pour tous les génotypes, le retard de semis améliore les teneurs en protéines et le poids de graines mais décroît le rendement grain davantage chez les populations par rapport aux hybrides tandis que la teneur en huile n'est pas modifiée. Globalement, sous l'effet d'apport d'irrigation, seule la teneur en protéines des graines diminue.

Les teneurs en acides oléique et linoléique chez les hybrides sont sensibles au décalage de la date de semis tandis que les populations et les hybrides oléiques se montrent plus stables. Le retard du semis induit une augmentation de la teneur en acide oléique dans les graines des hybrides standard avec une réduction concomitante de la teneur en acide linoléique. Le traitement irrigué n'affecte pas les teneurs de ces deux acides. Le retard du semis décroît les teneurs en acides palmitique et stéarique dans les graines d'hybrides oléiques, et seulement la teneur en acide palmitique pour les hybrides standard. Le pourcentage de ces acides n'est pas modifié en conditions irriguées.

L'étroite corrélation négative entre les teneurs en acide oléique et linoléique, et entre celles des acides oléique et palmitique sont dégagées pour tous les génotypes et tous les traitements.

Nos résultats suggèrent qu'il existe différents comportements de génotype pour l'élaboration et la composition d'acides gras en réponse à la conduite culturale.

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