Sterol content in sunflower seeds (Helianthus annuus L.) as affected by genotypes and environmental conditions

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Suggestion for an abbreviated running title: Genotypes and environment effects on sterol contents in sunflower seeds.

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In memory of Andrée Bouniols who left us in 2008

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Abstract

Phytosterols play essential roles in many plant cell mechanisms. They are of industrial interest since, as part of the diet, they can reduce Low Density Lipoprotein cholesterol. An increase in plant sterol contents by improved crop varieties or crop management could help to answer industrial demands and also to develop environmentally friendly extraction methods. The aim of this study was to evaluate genotypic variability for sterol content in cultivated sunflower and effects of sowing date, in particular sowing date. Results showed large variability among a collection of sixteen sunflower inbred lines and hybrids. Total sterols varied almost twofold between extreme genotypes. A delay of sowing, giving higher temperatures during seed formation, induced a general increase in total sterol concentration by up to 35 per cent, as well as sterol composition but this varied according to genotype. These results are discussed with the aim of improving sterol content by sunflower breeding programmes.

Key-words: sunflower seeds, phytosterols, genetic variability, genotype*environmental conditions interactions, fatty acid profiles

Introduction

Phytosterols are naturally present in plants, playing important roles in membrane fluidity and permeability (Schaller, 2003), embryogenesis (Clouse, 1996), and as precursors of brassinosteroid hormones involved in plant growth and development (Lindsey, Pullen, & Topping, 2003). During the last decade, there has been new interest in sterols due to their potential benefits for human health. Their role in reducing Low Density Lipoprotein cholesterol (LDL) (Ostlund, 2007) have been clearly established. Several studies have highlighted other interesting properties such as anti-cancer and anti-oxidation activities (Van Rensburget, Daniels, Van Zyl & Taljjard, 2000; Awad, Roy & Fink, 2003)

Consequently, these bioactive molecules are now used for various industrial applications, particularly in nutrition as functional food, for example in enriched margarines (Moreau Whitaker, & Hicks, 2002). By chemical modification, phytosterols could also be used as raw materials in pharmaceutical production as a source of steroids (Van Dansik, 2000) or in cosmetics (Folmer, 2003). This diversification in use of sterols has led to an increase in industrial demand.

Phytosterols are present in different plant parts, mostly in seeds. Their level depends on species and sunflower seeds contain a relatively high concentration (Mouloungui, Roche & Bouniols, 2006). However, sterols are present at low concentration rendering their extraction rather difficult. Improvement of phytosterol concentration in seed could increase their accessibility and thus help for development of soft methods of sterol extraction.

Adapted crop management could also improve phytosterol content, comparable with fatty acid composition where both environmental and genetic factors have been shown to be sources of considerable variability of oil quality in sunflower seed. Roche, Bouniols, Mouloungui, Barranco & Cerny (2006) demonstrated that the choice of genotype associated with adapted cultural practices could be used to orientate fatty acid biosynthesis. Genotypic

variability for sterol content has been reported in soybean (Yamaya, Endo, Fujimoto & Kitamur, 2007) and in cereals (Nurmi, Nyström, Edelman, Lampi & Piironen 2008; Alignan et al., 2009). Effects of genotype on sunflower seed oil and protein contents have been widely reported, but, so far, there have been no studies of genotypic variability of sterol content. No information is available on interactions between genetic factors and environmental conditions affecting sterol concentration and composition in sunflower seed. Knowledge of the genetic variability available is essential to make possible heredity and heritability studies and mapping of genes controlling sterol content. Although Roche et al. (2006) described the behaviour of genetic variability of total sterol contents, only 3 genotypes were analyzed in interaction with water stress. This work did not study the sterol composition. Considering the large genetic potentialities of sunflower, it therefore appears crucial to enlarge representative genetic backgrounds and to study genotype*environment interactions.

The aim of this study was to determine differences in sterol content and composition among sixteen sunflower lines and hybrids with different fatty acid profiles since this is the main quality trait currently important in breeding programmes.

Materials and methods

Plant material

Sterol contents were studied in 13 inbred lines and 3 hybrids listed in table 1, chosen for their variation in genetic origin and fatty acid profile (high oleic, low oleic, low stearic or low palmitic acid contents). The line HA300 was provided as having high sterol content. The line HA821 was a reselection from HA300. 59259 was a dwarf line. 83HR4 and 83HR4OL were near isogenic lines differing only in their fatty acid profile.

Trial environmental conditions

The trial was conducted in south-western France at the Regional Centre for Organic Agriculture Trials, at Auch (32) in 2006, on a deep limestone clay silt soil with high water-holding capacity (Table 2). Two sowing dates were used to generate temperature variations during seed development. The trial was sown at a density of $7.1x10^4$plants/ha on April, 26th for the conventional sowing date (CSD) and on June, 1st for the late sowing dates (LSD). The development of the crop was monitored weekly using the main phenological stages E, R1, R5.1, R6, R8 and R9 as described by Schneiter and Miller (1981). The trial was conducted as a complete randomised block design with 32 treatments (16 genotypes x 2 sowing dates) with 3 replications. Plot size was 16 m², 10 plants in each plot were bagged before flowering to obtain seed by self-pollination.

Extraction and determination of sterol contents

The small-scale sample extraction method optimized in our laboratory for reliable and economic analysis of sterols in sunflower seeds described in Roche, Alignan, Bouniols, Cerny, Mouloungui & Merah (in press, doi:10.1016/j.foodchem.2009.09.026) was used to extract and determine sterol content. Seed samples were bulks of all the bagged heads for

each treatment. Sterols analyzed were desmethylsterols (including β-sitosterol and campesterol), methylsterols and dimethylsterols.

Statistical data analysis

Phytosterol data are expressed as a weight percentage of seed dry matter (mg of sterol per 100g of seed dry matter). Statistical analyses were carried out on all the plots with 3 analytical replications (9 replications per treatment). Analyses of variance and Student-Newman & Keuls test were applied to experimental results to determine the significance between genotypes and sowing dates. The statistical analyses were performed using statistical package (Sigmastat version 2.0, USA). The different genotypes, sowing date treatment and their interaction on phytosterol contents were analyzed separately.

Results

Characterization of trial environment

Delaying sowing led to increased mean temperatures during the vegetative ([S-E] and [E-R5.1]) (+2.6 and 4 °C respectively) and the reproductive ([R5.1-R6] and [R6-R9]) phases (2.5 and 1.8 °C, respectively) compared with the conventional sowing date.

Late sowing received 70 mm less rainfall than the conventional sowing, with a pronounced drought period during ripening [R5.1-R6].

Effect of genotype

Significant differences were observed between genotypes for all variables (tables 3 and 4).

The inbred line XRQ showed the highest mean total sterol content followed by HA300 and HA821.LP1. The lowest sterol contents were recorded for the dwarf line 59259, the oleic hybrid Trisun and then the oleic inbred lines OF and OPA3.

The genotype XRQ also had the highest mean content of the main sterol category desmethylsterols including β-sitosterol and campesterol, and of the precursor category dimethylsterols but not the intermediate methylsterol category. HA 300 and HA 821.LP1 had slightly lower desmethylsterol contents than XRQ but higher methylsterol contents. The two hybrids Olbaril and Santiago II had similar and intermediate mean values for all sterol categories.

Total sterol mean value of the high oleic genotypes was slightly lower than that of standard genotypes (217.0 and 236.7 mg/100 g of SDM respectively) but the two comparable near-isogenic lines 83HR4 and 83HR4OL had similar values for total sterols and desmethylsterols, with a slightly higher values for the high oleic line (table 4). There were significant differences only for β-sitosterol and campesterol, again with the high oleic line having greater sterol contents.

Effect of sowing date

Delayed sowing had a significant effect on total sterols as consequence of its impact on desmethylsterols, the main category (tables 3 and 4). Except for dimethylsterol contents for which a significant decrease was observed (-8%), a delay of sowing was associated with an increased content of all sterol categories (+11%, +12%, +16% for total, desmethyl- and methylsterol contents).

To gain insight into the effects of temperature on sterol composition, the relation between two major pathway end-products was evaluated (figure 1). The ratio showed a positive and highly significant correlation (r^2=0.84) between β-sitosterol and campesterol for all the genotypes whatever the sowing date. This positive correlation was conserved across the two sowing dates and closer for LSD (r^2= 0.78, df = 30) than for CSD (r^2=0.56, df = 14). The slope of the linear regression of late sowing date (5.2) was almost 50% greater than for conventional

sowing date (3.8), indicating a tendency to produce more β-sitosterol (+ 12%) rather than more campesterol (+7%).

Sowing date*genotype interaction

Although there were strong main factors effects (genotype and sowing date), sowing date affected sterol contents differently according to genotype (table 4). Nine of the 16 genotypes showed significant increases in total sterol content after late sowing. The most striking increases were for XRQ (+148%), HA383LS21 (+138%) and for the hybrid Trisun, which had the lowest sterol content after conventional sowing and a moderate value after late sowing (+189%), whereas HA300 and HA821.LP1 had the highest sterol contents from conventional sowing date and lower values after late sowing (85-90%). The lines 83HR4 and 83HR4OL also followed this pattern as did OPA3. The general increase or decrease according to genotype was the same for all sterol categories, except for dimethylsterol contents in HA383.LS2.

Discussion

Sterols may act as protector agents of sunflower embryo against environmental stresses

Phytosterol contents varied according to sowing date. The highest levels of total sterols were obtained with delayed sowing giving the highest mean temperatures during flowering and seed ripening (+ 4 °C between seed emergence (E) and flowering (R5.1); and + 2.5 °C flowering and seed ripening onset (R6) compared to the conventional sowing date). This result confirms those obtained in our previous studies (Roche et al., 2006). The same increase in total sterol content were observed in soybean seeds submitted to increased temperatures in greenhouse (20/10 °C, 27/17 °C, 35/25 °C day/night) (Vlahakis & Hazebroek, 2000) and in the field (Yamaya et al., 2007). In fact, phytosterols may be involved in mechanisms of plant

adaptation to temperature related to their ability to resist to oxidation within the cellular membranes (Piironen, Lindsay, Miettinen, Toivo & Lampi, 2000). Due to their role in the regulation of membrane fluidity and permeability (Schaller, 2003), our results suggest that an increased accumulation of phytosterols may be a response of sunflower seed to high temperatures. This accumulation occurred mostly during the first stages of seed filling (until 40 % of SDW) (Roche et al., in press doi:10.1016/j.foodchem.2009.09.026). This is supported by higher temperatures recorded in LSD from R5.1 to R6 stages compared to CSD (table 2). These results reinforce the hypothesis that modification of sterol content in mature sunflower seeds may be an adaptive strategy to seed desiccation reducing membrane fluidity to limit the effects of critically high temperatures. Peng, Kawagoe, Hogan & Delmer (2002) suggest that, in cotton membranes, β-sitosterol may be involved in cellulose biosynthesis as a primer for glucan chain elongation that also contributes to provide strength and flexibility to plant tissues.

High temperature during grain filling modifies sterol composition in sunflower seeds

Higher temperatures during flowering modified the desmethylsterol biochemical composition of mature sunflower seeds, such as β-sitosterol/campesterol ratio. Although an increase of temperature induced an increase in both desmethylsterols components, the reduction of the ratio under high temperatures translates a more efficient biosynthesis of β-sitosterol to the expense of campesterol. These data are in disagreement with those reported by Yamaya et al. (2007) on soybean but in accordance with our previous results on several sunflower genotypes submitted to more severe weather conditions (Roche et al., 2006). The significant correlation may represent the biochemical balance between the sterol precursors of brassinosteroid biosynthesis through campesterol and their role as essential membrane components through β-sitosterol. This balance is regulated by sterol methyl transferases (SMT1 and SMT2) enzymes

involved in sterol metabolism. Previous genetic and biochemical studies on *Arabidopsis thaliana* mutants overexpressing the enzyme SMT2 responsible of gramisterol methylation, led to an over accumulation of β-sitosterol, suggesting its implication in the modification of the β-sitosterol/campesterol ratio (Lindsey et al., 2003). The new sterol content could affect the biosynthetic interface between sterols and brassinosteroids, known to be involved in regulation of growth and development and response to environmental stresses (Krishna, 2003). The changing ratio of the β-sitosterol/campesterol observed in our experimental conditions may be explained by a modification of SMT2 activity under higher temperatures. The amount of other sterols remained almost unchanged, particularly stigmasterol, which is thought to be the product of the desaturation at C22 of β-sitosterol (for review Benveniste, 1986), supporting our hypothesis. Although a sterol-22-desaturase in plants has not yet been characterized in terms of enzymatic activity, this metabolic step could be either considered as a rate-limiting enzymatic step in the pathway or a highly regulated point in the course of plant development for as yet unknown reasons. Whereas desmethyl- and methylsterol contents were higher when temperature increased (LSD), our results showed that dimethylsterol content was reduced by an increase of temperature. These results support the hypothesis that dimethylsterols as precursors of methyl- and desmethylsterols are regulated differently from the last two suggesting a different regulation of SMT1 which catalyses the conversion of cycloartenol into methylencycloartanol.

Genetic potentialities of sunflower seeds to produce sterols are affected by temperature

The 13 inbreds lines chosen as a first sample of the genetic variability for sterol contents present in modern inbred sunflower lines showed that there are highly significant differences. It appears likely that a wider study could reveal even greater differences. The present study revealed a large variation and highly significant genotypic differences for each category of

sterols. The largest variation reached more than twice as much (58.5, 30.7, 38.8% for desmethyl, methyl- and dimethylsterols respectively). Although the variability of sterol content differed according to sterol categories and genotypes, some genotypes harbored distinguishable sterol contents. For example, XRQ contained the highest desmethyl- and dimethylsterol contents, whereas Trisun and 59259 contained the lowest contents of desmethyl- and methylsterols and OPA3 contained the lowest dimethylsterol content. Despite theses values represent a mean between conventional and late sowing dates, it appears that, the genotypes containing the lowest content of desmethylsterols generally also possessed the lowest content of dimethylsterols and the same behaviour was observed for the genotypes containing the highest content of desmethyl- and dimethylsterols. The inbred HA300, suggested as having high sterol contents confirmed this character, especially after the conventional sowing date. The significant genetic effects were confirmed by the similar behaviours of HA300 and HA821 which are related, and of 83HR4 and 83HR4OL which are nearly isogenic. This result showed no direct relation with saturated fatty acids (palmitic and stearic acids). The line XRQ had not been studied before for its sterol content, but should constitute a useful source for breeding for high sterol contents if the results of the present experiment are confirmed. Why some lines show decreases in sterol content after late sowing as not yet explained. All the inbred lines had quite similar flowering dates, so the temperatures during their ripening periods should not have varied significantly. However, there were some variations in seed production between lines due to differences in self compatibility. Irregular seed size and development after self-pollination could be causes of different reactions to temperature.

Results from the literature on various species are contrasted. In soybean, only a weak genetic difference was found in sterol composition between Japanese and non Japanese seeds (Yamaya et al., 2007) whereas Nurmi et al. (2008) and Alignan et al. (2009) on wheat

reported a strong effect of genetic background. In the present study, the significant genetic variability of sterol contents showed that the different sterols were not accumulated at the same rate according to the genotypes and suggests different regulation related to genetic background. In addition, no difference was found between oleic and standard genotypes. The similarities between results for 83HR4 and 83HR4OL agree with the absence of any direct correlation between fatty acid and sterols biosynthesis although metabolic pathways of these two seed components are related through a common substrate: AcetylCoA.

Our results also revealed that the interaction between sowing date and genotypic potentialities could modify the stability of sterol content in sunflower seeds. The effect of high temperatures varies according to the genotype and the sterol category. Nevertheless, in more than 50% of the genotypes studied, there was an increase in total sterol, desmethylsterol and methylsterol contents after late sowing whereas the dimethylsterol content is lowered. This result supports the hypothesis that the character "high sterol content" may be under a genetic control (Alignan et al., 2008).

Conclusion

Choice of genotypes and effects of temperature could be used to orientate sterol biosynthesis to give sunflower seed production that would satisfy industrial requirements. The benefit of high crop temperature during seed filling on sterol concentrations could be used to increase sterol contents for industrial purposes through the delay of sowing in adapted zones. For example, the modification of the β-sitosterol/campesterol ratio under late sowing date could be a cultural practice useful to generate sunflower seeds enriched in campesterol as functional food ingredients. Moreover, our study highlights an effect of genetic variability on sterol content in the sunflower seeds. These results, in agreement with previous data on other

genotypes (Roche et al., 2006), suggest that sunflower genotypes should be screened to determine those that to fit industrial requirements. In food industries that require low concentrations of saturated fatty acids (C16:0 and C18:0) and a high concentration of oleic acid (C18:1) for healthy edible oils, a high concentration of sterols would provide increased functional properties. Our study suggests that sterol concentration in sunflower oil can be increased by integration of this new quality in breeding programmes.

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Figure caption

Figure 1. Significant correlation between β-sitosterol and campesterol in whole seeds of 16 sunflower genotypes in conventional (CSD) and late (LSD) sowing dates in 2006.

*** significant for p<000.1. CSD: conventional sowing date, LSD: late sowing date. SDM: Seed Dry Matter

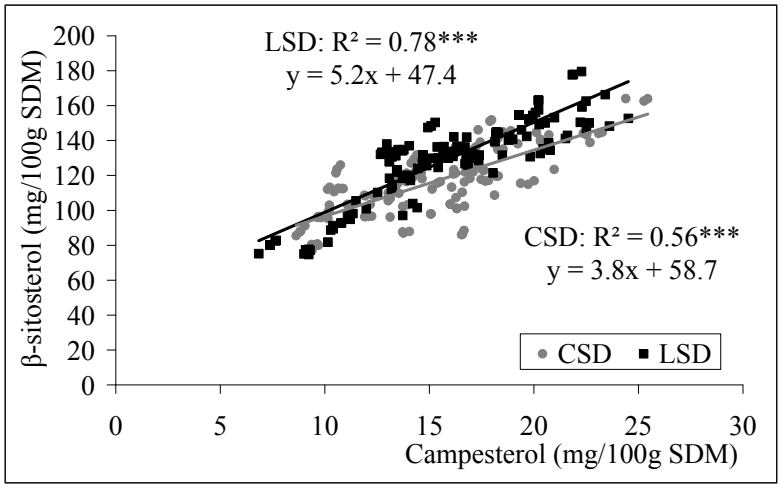

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Table 1. Characteristics of sixteen genotypes studied (Inbred lines and hybrids)

Genotype	Characteristics	Type	Origin	C16:0	C18:0	C18:1	C18:2
OF	High C18:1	LM	INRA, France	2.9	2.4	87.8	6.2
83HR4OL	High C18:1	LR	INRA, France	5.3	3.3	76.5	13.8
RHA345	High C18:1	LR	USDA, USA	3.7	4.3	84.9	6.0
OEG	High C18:1	LM	INRA, France	4.9	5.3	85.4	3.0
OPA3	High C18:1	LR	INRA, France	3.7	2.6	89.6	3.2
Trisun 870	High C18:1	H	Mycogen,USA	3.2	4.8	89.1	1.5
Olbaril	High C18:1	H	Pioneer Hi-Bred, USA	3.4	3.9	90.1	1.9
83HR4	Standard C18:1	LR	INRA, France	8.6	3.6	16.1	70.7
VHQ	Standard C18:1	LM	INRA, France	5.3	5.4	26.3	62.2
XRQ	Standard C18:1	LM	INRA, France	5.9	3.3	25.4	64.9
PSC8	Standard C18:1	LR	INRA, France	10.5	4.6	30.0	54.2
HA300	High sterol content, standard C18:1	LM	USDA, USA	5.4	4.2	47.2	42.4
HA821.LP1	Low C16:0, standard C18:1	LM	USDA, USA	5.4	6.4	22.0	65.6
HA382.LS2	Low C18:0, standard C18:1	LM	USDA, USA	6.9	4.8	24.7	63.1
59259	Dwarf, standard C18:1	LM	France	6.6	7.9	21.8	62.6
Santiago II	Standard C18:1	H	Syngenta, France	5.7	5.8	27.7	59.6

C16:0: palmitic acid content, C18:0: stearic acid content, C18:1: oleic acid content, C18:2: linoleic acid content expressed in % of total fatty acids

LM: Inbred line cytoplasmic male sterility Maintainer, LR: Inbred line cytoplasmic male sterility Restorer, H: Hybrid

Table 2. Rainfall and mean temperature during phenological periods of sunflower development in 2006

Climate parameters	Sowing date	Phenological phases			
		S-E	E-R5.1	R5.1-R6	R6-R9
Mean temperature (°C)	CSD	14.3	18.8	22.7	22.0
	LSD	16.9	22.8	25.2	20.2
Rainfall (mm)	CSD	34.4	96	3.8	133
	LSD	0.2	82.4	72.6	131.6

CSD: conventional sowing date, LSD: late sowing date

S: Sowing, E: emmergence, R5.1: beginning of flowering, R6: seed ripening onset, R8: end of seed ripening, and R9: physiological maturity, according to Schneiter and Miller (1981)

Table 3. Mean square values of the effects of genotype, sowing date and their interactions on total sterols, desmethylsterols, methylsterols and dimethylsterols contents in 2006.

Trait (mg/100gSDM)		Genotype (G)	Sowing date (SD)	G*SD	Residues
Total sterol content	MS	46846.7	17731.4	15050.0	656.4
	P	***	***	***	
Desmethylsterol	MS	42385.7	11730.8	10138.6	416.8
	P	***	***	***	
Methylsterol	MS	548.4	438.7	143.1	19.0
	P	***	***	***	
Dimethylsterol	MS	165.2	294.6	195.6	13.1
	P	***	***	***	
β-sitosterol	MS	4748.2	18753.0	4756.1	176.8
	P	***	***	***	
Campesterol	MS	137.6	119. 7	183.5	6.8
	P	***	***	***	

* : 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

Table 4. Effects of sowing date for each genotype on total sterol, desmethylsterols, methylsterols and dimethylsterols contents in 2006.

Traits	Sowing dates	Genotypes															**Mean**
		Standard									High oleic						
		XRQ	HA300	HA821.LP1	PSC8	VHQ	HA382.LS2	59259	*Santiago II*	83HR4	83HR4OL	*Olbaril*	*Trisun*	RHA345	OEG	OF	OPA3	
Total sterol content (mg/100g SDM)	CSD	244.5 b	280.5 a	277.8 a	200.8 b	212.3 b	188.6 b	195.6 a	*201.6 b*	217.3 a	237.0 a	*194.3 b*	*123.1 b*	226.9 b	171.3 b	244.3 a	220.2 a	**214.8 b**
	LSD	362.7 a	253.9 b	244.7 a	256.3 a	241.1 a	259.4 a	161.1 b	*240.7 a*	221.8 a	223.9 a	*254.3 a*	*232.7 a*	261.8 a	270.2 a	171.5 b	188.1 b	**240.3 a**
	Mean	**303.6 a**	**267.2 b**	**261.3 b**	**228.5 d**	**226.7 d**	**224.0 d**	**178.4 e**	***221.1 d***	**219.5 cd**	**230.5 d**	***224.3 d***	***177.9 e***	**244.4 c**	**220.7 d**	**207.9 d**	**204.2 d**	
Desmethylsterol (mg/100g SDM)	CSD	210.4 b	225.2 a	227.6 a	173.8 b	176.0 b	159.3 b	168.4 a	*172.4 b*	179.0 a	204.9 a	*166.7 b*	*107.0 b*	194.3 b	145.7 b	205.0 a	195.0 a	**181.9 b**
	LSD	312.5 a	214.4 b	205.3 a	217.7 a	206.5 a	221.8 a	144.1 b	*207.2 a*	188.9 a	199.3 a	*216.3 a*	*198.9 a*	220.6 a	231.8 a	142.1 b	170.2 b	**206.2 a**
	Mean	**261.4 a**	**219.8 b**	**216.4 bc**	**195.7 c**	**191.3 c**	**190.6 c**	**156.2 e**	***189.8 c***	**184.0 d**	**202.1 cd**	***191.5 c***	***152.9 e***	**207.4 bc**	**188.8 c**	**174.2 d**	**182.6 d**	
Methylsterol (mg/100g SDM)	CSD	11.5 b	28.1 a	29.1 a	13.3 b	16.6 a	13.9 b	10.4 a	*12.4 b*	19.5 a	17.0 a	*9.9 b*	*4.6 b*	12.6 b	11.8 b	15.4 a	11.3 a	**14.8 b**
	LSD	20.3 a	18.3 b	26.8 a	19.2 a	18.4 a	24.9 a	6.8 b	*15.8 a*	21.6 a	14.0 b	*17.2 a*	*14.0 a*	20.7 a	15.8 a	16.3 a	11.3 a	**17.6 a**
	Mean	**15.9 c**	**23.2 b**	**28.0 a**	**16.3 c**	**17.5 bc**	**19.4 c**	**8.6 e**	***14.1 d***	**20.6 bc**	**15.8 c**	***13.6 d***	***9.3 e***	**16.7 c**	**13.8 d**	**15.8 c**	**11.3 ed**	
Dimethylsterol (mg/100g SDM)	CSD	22.6 b	27.3 a	21.2 a	13.8 a	19.7 a	15.4 a	16.9 a	*16.7 a*	18.7 a	15.1 a	*17.7 b*	*11.5 b*	20.0 a	13.7 b	23.9 a	13.9 a	**18.0 a**
	LSD	30.0 a	21.2 b	12.6 b	19.3 a	16.2 a	12.7 b	10.2 b	*17.6 a*	11.2 b	10.6 b	*20.7 a*	*19.9 a*	20.5 a	22.6 a	12.1 b	6.6 b	**16.5 b**
	Mean	**26.3 a**	**24.2 a**	**16.9 b**	**16.5 b**	**18.0 b**	**14.0 bc**	**13.6 bc**	***17.2 b***	**15.0 bc**	**12.9 c**	***19.2 b***	***15.7 bc***	**20.3 b**	**18.2 b**	**18.0 b**	**10.2 d**	
β-sitosterol (mg/100g SDM)	CSD	135.9 b	136.5 b	141.2 a	108.4 b	113.4 b	99.2 b	100.9 a	*113.4 b*	111.9 a	130.0 a	*106.3 b*	*65.1 b*	118.4 b	88.2 b	126.6 a	130.4 a	**114.1 b**
	LSD	190.3 a	142.4 a	125.8 b	140.1 a	128.6 a	133.7 a	92.9 a	*138.1 a*	112.6 a	125.3 b	*145.9 a*	*129.2 a*	140.0 a	156.6 a	83.5 b	99.0 b	**130.3 a**
	Mean	**163.1 a**	**139.5 b**	**133.5 c**	**124.3 f**	**121.0 g**	116.5 h	**96.9 k**	***125.8 f***	**112.3 i**	**127.7 e**	***126.1 de***	***97.2 k***	**129.2 d**	**122.4 fg**	**105.1 j**	**114.7 hi**	
Campesterol (mg/100g SDM)	CSD	17.5 b	16.5 a	20.5 a	14.9 b	13.4 a	10.6 b	15.8 a	*14.2 b*	10.7 a	16.9 a	*14.0 b*	*10.4 b*	17.5 a	9.4 b	**20.5 a**	18.0 a	**15.1 b**
	LSD	28.5 a	16.7 a	16.1 b	17.9 a	15.5 a	13.4 a	10.8 b	*18.8 a*	11.2 a	14.7 a	*19.1 a*	*20.5 a*	18.4 a	19.8 a	**8.4 b**	11.5 b	**16.3 a**
	Mean	**23.0 a**	**16.6 c**	**18.5 b**	**16.4 c**	**14.5 e**	**12.0 f**	**13.3 ef**	***16.5 c***	**11.0 f**	**15.8 d**	***16.6 c***	***15.5 d***	**18.0 b**	**14.6 e**	**14.5 e**	**14.8 e**	

Hybrids are represented in italic. SDM: seed dry matter. CSD: Conventional sowing date. LSD: Late sowing date

CSD and LSD values with same letter are not significantly different at the threshold of 5 %. Mean values of each sowing date and of each genotype with same letter are not significantly different at the threshold of 5 %.