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RESEARCH ARTICLE OPEN ACCESS

Genotypic and environmental variability of tocopherols and phytosterols in linseed (*Linum usitatissimum* L.) oil☆

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Abstract – Linseed, *Linum usitatissimum* L., oil is highly valued for its nutritional and industrial properties, which is due to its unique profile in fatty acids and of minor components such as tocopherols and phytosterols. In this study, we investigated the effects of genotype, growing year, and location on the content and composition of these compounds in *L. usitatissimum* oil, with a focus on understanding their variability under field conditions. Ten genotypes were cultivated over three growing seasons (2013–2015) in one southern (Lavaur) and two northern (Airaines and Poix-de-Picardie) locations in France. Oil was extracted using a modified protocol and analyzed via high-performance liquid chromatography for tocopherols and gas chromatography-mass spectrometry for phytosterols. Our results revealed a mean total tocopherol content of 403.7 mg/kg oil, with γ −tocopherol as the dominant isoform (397.4 mg/kg oil), and a mean total phytosterol content of 396.7 mg/100 g oil. Notably, genotype accounted for 49% of tocopherol variability, whereas environmental factors, especially location and year, contributed significantly to phytosterol variation (up to 44% variance). The adverse effects of the hotter, drier 2015 season on both compound classes underscore the impact of abiotic stress on oil quality. These findings provide a framework for breeding programs aimed at optimizing *L. usitatissimum* oil for both nutritional enhancement and industrial applications.

Keywords: climatic conditions / minor components / tocopherol / phytosterol / *Linum usitatissimum* L

Résumé – Variabilité génotypique et environnementale des tocophérols et des phytostérols dans l'huile de lin (*Linum usitatissimum* L.). L'huile de lin, *Linum usitatissimum* L., est très appréciée pour ses propriétés nutritionnelles et industrielles, ce qui est dû à son profil unique en acides gras et en composants mineurs tels que les tocophérols et les phytostérols. Dans cette étude, nous avons étudié les effets du génotype, de l'année de culture et du lieu de culture sur la teneur et la composition de ces composés dans l'huile de *L. usitatissimum*, en nous concentrant sur la compréhension de leur variabilité. Dix génotypes ont été cultivés pendant trois années culturales (2013-2015) dans un site au sud (Lavaur) et deux sites au nord (Airaines et Poix-de-Picardie) de la France. L'huile a été extraite selon un protocole modifié et analysée par chromatographie liquide haute performance pour les tocophérols et par chromatographie gazeuse couplée à la spectrométrie de masse pour les phytostérols. Nos résultats ont révélé une teneur totale moyenne en tocophérols de 403,7 mg/kg d'huile, le γ −tocophérol étant l'isoforme dominante (397,4 mg/kg d'huile), et une teneur totale moyenne en phytostérols de 396,7 mg/100 g d'huile. Le génotype représentait 49 % de la variabilité du tocophérol, tandis que les facteurs environnementaux, en particulier le lieu et l'année, contribuaient de manière significative à la variation des phytostérols (jusqu'à 44 % de la variance). Les effets négatifs de la saison 2015, plus chaude et plus sèche, sur les deux familles de composés soulignent l'impact

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du stress abiotique sur la qualité de l'huile. Ces résultats fournissent un cadre pour les programmes de sélection visant à optimiser l'huile de *L. usitatissimum* à des fins d'amélioration de la qualité nutritionnelle et des applications industrielles.

Mots-clés : conditions climatiques / composés mineurs / tocophérols / phytostérols / *Linum usitatissimum* L

Highlights

- Linseed oil is highly valued for its nutritional properties.
- The growing location had a significant impact on the total tocopherol content.
- Phytosterol content showed significant variation across locations and genotypes.
- Abiotic stress controlled total tocopherol and phytosterol levels.

1 Introduction

Linseed, *Linum usitatissimum* L., oil is well known because of its high content of the essential oil alpha-linolenic acid (ω3) and the health benefits its intake may provide (Basch *et al.*, 2007; Shim *et al.*, 2014). Asia is the world's largest continent producing linseed oil with 0.32 Mt produced in 2020, followed by Europe with 0.27 Mt (FAOSTAT, 2023). In Europe, Belgium has the largest linseed oil production with 0.12 Mt in 2020. In France, linseed oil area of production reached 26 898 ha t in 2023 (Agreste, 2024). The crop is primarily cultivated in the North of France for fiber production, while oil production is extended from the north to the center of the country. The flax varieties used for fiber production have been bred specifically for their fiber quality; although their seeds are partially recovered, they are not used for oil extraction. On the contrary, linseed varieties are selected for their oil quality, but their fiber is not used as it does not meet the required quality standards. The *L. usitatissimum* oil contains a high content of bioactive minor components with interest for health or non-food uses. It contains high levels of phospholipids (Herchi *et al.*, 2011), polyphenols (El-Beltagi *et al.*, 2011), flavonols (El-Beltagi *et al.*, 2011), tocopherols (Khattab and Zeitoun, 2013; Marquard *et al.*, 1977; Oomah *et al.*, 1997), and phytosterols (Herchi *et al.*, 2009; Teneva *et al.*, 2014).

Tocopherols represent a family of compounds, known as Vitamin E, with well-documented antioxidant properties. They play a crucial role in protecting against oxidative stress, with proven benefits in reducing the risk of cardiovascular diseases and exhibiting potential anti-cancer effects (Järvinen and Erkkilä, 2016; Shahidi and De Camargo, 2016). Total tocopherol content of *L. usitatissimum* oil ranged from 395 to 500 mg/kg based on varietal and location effects (Khattab and Zeitoun, 2013; Marquard *et al.*, 1977). Moreover, Oomah *et al.* (Oomah *et al.*, 1997) found that the average values for α, γ, δ, and total tocopherols in eight linseed cultivars were 1.5, 215.0, 5.6, and 221.9 mg/kg oil, respectively. In linseed, γ-tocopherols represent up to 97% of the total tocopherol content (Oomah *et al.*, 1997), which corresponds to 90.4 mg/kg of seed, based on the average of seven cultivars. Studies on tocopherol content in linseed oils show significant variation, with values ranging from 153.5 mg/kg of oil (Choo *et al.*, 2007) to 538 mg/kg of oil (Matthäus and Özcan, 2017), including plastochromanol-8 as part of the total tocopherol content. To our knowledge, no other study has been done to evaluate the variability in tocopherol content and composition across different genotypes and growing years in linseed.

The sterols are an important part of the unsaponifiable fraction in many plant oils. Several studies have proved that an intake of 2 g of sterols per day can lower plasma total and low-density lipoprotein (LDL) cholesterol levels (Musa-Veloso *et al.*, 2011; Ras *et al.*, 2013), which may help in reducing the coronary heart disease risk. Phytosterol contents and compositions are affected by species, genotypes, and growing conditions, especially during grain filling (Alignan *et al.*, 2009; Amar *et al.*, 2009; Ayerdi Gotor *et al.*, 2015; Hamama *et al.*, 2003). In linseed, the six major sterols present in the oil were β-sitosterol, cycloartenol, campesterol, 24-methylencycloartanol, Δ5-avenasterol, and stigmasterol that represent 96% of the total sterol content (Schwartz *et al.*, 2008). However, little is known about the variability of these sterols within different genotypes or across growing years. This study compared several genotypes of *L. usitatissimum* over three years, grown in one southern (Lavaur) and two northern (Airaines and Poix-de-Picardie) locations in France.

The aim of this study was to evaluate both the content and composition of tocopherols and phytosterols, as well as to explore the effects of genotype, growing year, and location on their variation. Understanding these factors is essential for optimizing linseed oil quality for both food and non-food applications. This work is complementary to our previous analysis made on the impact of environmental factors and genotype on linseed composition and subsequent oil expression (Savoire *et al.*, 2015).

2 Materials and methods

2.1 Chemicals

All reagents and standards used were of analytical grade from Sigma-Aldrich, St. Quentin Fallavier, France.

2.2 Samples collection

Samples were collected in two different locations in the North of France: Airaines (49° 57′ 57″ N, 1° 56′ 35″ E) and Poix-de-Picardie (49° 46′ 36″ N, 1° 59′ 07″ E) situated in the main linseed production area. The third location was in Lavaur (43° 41′ 59″ N, 1° 49′ 11″ E) in the South of France (Fig. 1). Experimental field trials were conducted using randomized plots with two replicates, following conventional agronomic practices. Sowing was carried out in the North between September 15th and September 25th, and in the South from October 1st till the 20th, depending on annual weather

	YEAR		
	2013	2014	2015
Genotypes tested	6	10	6
Number of locations	2	3	1

Fig. 1. Localization of trials, and main linseed production areas in France (in Green).

conditions. Fertilization included 80 kg of Nitrogen (N), 40 kg of phosphorus (P_2O_5), and 30 kg of potassium (K_2O) per hectare, with a sowing density of 400 grains/m^2. Experimentations in the North were conducted in deep silty clay soils, while those in the South were on clay-limestone soils with lower water retention capacity. All sites applied at least one herbicide to reduce the weeds presence. Up to ten winter linseed genotypes were studied during three growing seasons from 2013 to 2015. These numbered lines, in the final stage of the breeding process, were candidates for official seed catalogue registration within the next two to three years. Although no selection was made based on minor nutrient composition, these genotypes were selected for their high yield potential and overall disease resistance. This research was included in a larger project willing to determine the variability on fatty acids composition (Savoire *et al.*, 2015). Grains were harvested at maturity, then stored in the dark at room temperature before oil extraction within two months after harvest.

2.3 Oil extraction

Seed oil extraction was performed using our previously developed method with some modifications (Savoire *et al.*, 2015). A hundred of seeds were oven dried at 110 °C for 24 h. Ten seeds were then transferred to a 1.5 mL screw tube and ball-milled three times for 1 min in 0.5 mL of isooctane using the FastPrep®-24 instrument (MP Biomedicals, Illkirch-Graffenstaden, France). Samples were centrifuged at 10 000 g for 5 min at room temperature. The supernatant containing solvent extraction and oil matter were recovered. Three supernatants of each sample were pooled into a vial and then evaporated under nitrogen to recover oil. The analyses below were carried out on freshly extracted oils. Oil extraction was made in triplicate.

2.4 Tocopherol analysis

Tocopherol analyses were performed according to our previous protocols, with a few minor modifications (Ayerdi Gotor *et al.*, 2006; Rhazi *et al.*, 2022). About 40 mg of oil sample were dissolved into 1 mL of isooctane. The high-performance liquid chromatography (HPLC) apparatus consisted of a surveyor system coupled to fluorometer detector (Thermo Fisher Scientific Corporation, Courtaboeuf, France). Tocopherols were separated using an Acclaim™ C30 analytical column, 150 × 4.6 mm, particle size 5 µm (Thermo Fisher Scientific Corporation, Courtaboeuf, France) connected in series with a pre-column with the same characteristics. The mobile phase was composed of methanol, acetonitrile, and tert-Butyl methyl ether HPLC-grade. The gradient profile was as described previously (Rhazi *et al.*, 2022). The eluant was monitored with a fluorescence detector, and chromatograms were recorded at emission wavelength of 344 nm after excitation at excitation wavelength of 298 nm. The column was maintained at 30 °C during the run and the autosampler temperature was set at 10 °C to preserve tocopherols. The flow rate was 1.0 mL/min throughout the run. The injection volume was 30 µL. The identification was based on retention time, and the quantification of tocopherols was carried out using external calibration with standards. The peak areas of the individual tocopherols were plotted against the corresponding concentrations to construct the calibration graphs. Results were expressed as mg of tocopherols per kg oil.

2.5 Phytosterol analysis

Sterols extraction and gas chromatography-mass spectrometry (GC-MS) analysis were conducted as described previously (Ayerdi Gotor *et al.*, 2007; Rhazi *et al.*, 2022).

	YEAR		
	2013	2014	2015
Genotypes tested	6	10	6
Number of locations	2	3	1

Fig. 2. Meteorological data from trail sites in northern France (Airianes and Poix-de-Picardie; A, labelled N) and southern France (Lavaur; B, labelled S) during the 2013–2015 linseed growing seasons. Solid lines represent maximum temperatures (TM), and dotted lines denote minimum temperatures (Tm). Vertical bars indicate cumulative rainfall. Blue corresponds to northern France (2013–2015), and orange to southern France.

Sterol quantification was carried out according to official procedure (ISO12228-1, 2014). About 40 mg of freshly obtained oil, and 200 μL of internal standard, were submitted to saponification reaction by adding 1 mL of ethanolic KOH (0.5 M) followed by incubation at 100 °C for 15 min. The solution of internal standard was prepared immediately before use by solubilizing 1 mg of betulin in 1 mL of acetone. Saponification was stopped by adding 1 mL of ethanol. The resulting solution was introduced into a glass column containing approximately 2 g of aluminum oxide powder soaked with ethanol. The unsaponifiable molecules were recovered into a new balloon by washing the column with 5 mL of ethanol and 30 mL of diethyl ether. Samples were concentrated using a rotavapor system and recovered with 2 mL of pyridine. Unsaponifiable matter (900 μL) was derivatized with 100 μL of N-methyl-N (trimethylsilyl)-heptafluorobutyramide (5:95 v/v) for 15 min at 105 °C in an oil bath. The trimethylsilyl derivatives of the phytosterols were determined by GC-MS using the Thermo Scientific GC-MS benchtop system that combines a Trace 1310 GC with an ISQ 7000 Single Quadrupole mass spectrometer (Thermo Fisher Scientific Corporation, Courtaboeuf, France). Separations were achieved employing Phenomenex fused silica capillary ZB-5 inferno column (30 m × 0.25 mm × 0.25 μm, Paris, France). The injector temperature was set at 320 °C and the oven was programmed as follows: initial temperature 240 °C, hold time 0 min, rate 4 °C/min, to 320 °C, and held for 10 min until it decreased to initial conditions. The mass spectrometer was used in electron impact mode (electron energy 70 eV). The transfer tube and source temperature were set at 250 °C and 200 °C, respectively. Helium carrier gas flow was set at 0.7 mL/min. Splitless injections were performed with 1 μL sample volume. The split valve was opened 3 min after injection. Identification of trimethylsilyl derivatives was based on their MS spectrum and retention time of standards and with the relative times to betulin given in the norm (ISO12228-1, 2014). The amount of phytosterol molecules was determined, based on the corresponding calibration curves. Analysis was realized in triplicates.

2.6 Weather

Weather parameters, namely: mean maximum temperature (TM, °C), mean minimum temperature (Tm, °C), and monthly rainfall during the critical growth period of linseed varieties (between March 1st to July 31st over the three years of the study), were recovered from the MétéoCiel database (https://www.meteociel.fr). Data for the Nord climatic conditions (N) were collected from a weather station located between Airianes and Poix-de-Picardie, while data for the South climatic conditions (S) were obtained from the Lavaur weather station.

2.7 Statistical analysis

After testing the homogeneity of variances and normality of obtained data using Levene and Shapiro-Wilk tests, respectively, analysis of variance (ANOVA) was used to evaluate the effects of variety and year and their interaction on tocopherols and phytosterols content and composition. The least significant difference (LSD) at the 0.05 significance level was used to distinguish differences among the mean values. All statistical analyses were performed using SPPS version 22.1.

3 Results

3.1 Environment and genotype effects

The meteorological dataset shows that 2015 was the hottest and driest year in both northern and southern France, although

Table 1. Mean values of tocopherol content and isoform composition for 10 linseed genotypes, with location- and year-specific averages.

Factor		Concentration (mg/kg oil)		
		δ −tocopherol	γ −tocopherol	Total tocopherols
Genotype	Var 01	6.0	392.3	398.3
	Var 02	6.7	379.4	386.1
	Var 03	5.6	365.7	371.2
	Var 04	6.0	388.0	393.9
	Var 05	4.5	347.0	351.4
	Var 06	7.6	424.3	431.9
	Var 07	6.0	426.4	432.4
	Var 08	4.7	384.9	389.7
	Var 09	6.9	404.10	410.9
	Var 10	6.1	415.9	422.0
	Mean	6.0	392.8	398.8
	SD	0.9	25.6	26.2
	CV (%)	15.9	6.5	6.6
Year	2013	6.4	408.1	414.6
	2014	6.4	411.6	418.1
	2015	2.0	362.6	364.6
	Mean	5.0	394.1	399.1
	SD	2.0	21.0	23.0
	CV (%)	39.5	5.3	5.8
Location	Lavaur	3.9	386.2	390.1
	Airaines	6.7	412.2	418.9
	Poix-de-Picardie	6.8	408.8	415.7
	Mean	5.8	402.4	408.2
	SD	1.3	10.8	12.1
	CV (%)	21.8	2.8	3.0

CV: Variation coefficient; SD: Standard deviation.

it was not exceptionally dry during the linseed growing period (Fig. 2). The southern regions were always hotter than the northern regions, with 2013 being the coldest year. In contrast, 2014 was the rainiest year. These significant variations in the weather during the growing period of linseed were probably responsible for the modifications in the content and composition of minor components, independent of genotype.

3.2 Oil content

The oil content was consistent across cultivars but lower at the southern site (Lavaur). Grain samples from 2015 presented a reduced oil content of 46%, compared to 48 and 49% in samples from 2013 and 2014, respectively (data not shown).

3.3 Tocopherols

The linseed oils had a mean total tocopherol content of 403.7 ± 37.0 mg/kg of oil (Tab. 1), with γ-tocopherol being the dominant isoform at 397.4 ± 39.2 mg/kg of oil. The mean total phytosterol content was 396.7± 89.4 mg/100 g of oil. Variety 07 had the highest tocopherol content with a total of 432.4 mg/kg, while variety 05 had the lowest at 351.4 mg/kg (Tab. 1). The two northern sites (Airanes and Poix-de-Picardie) presented significantly higher tocopherol content compared to the southern site (Lavaur). In 2015, the concentration of tocopherols in linseed oil was significantly lower than in 2013 and 2014.

The 2014 data were analyzed using ANOVA with a random model to highlight the contribution of each variance component: genotype (G), year of harvest (Y), and location (L) (Tab. 2) because it presented the larger number of the same cultivars cultivated in the three locations. The analysis showed that genotype and genotype × location were the main factors influencing total tocopherol, explaining 49 and 44% of the total variance, respectively. This variability presents an opportunity to increase the content on these two families of minor components through breeding, potentially leading to varieties with improved nutritional profiles and better oil quality preservation over a longer period.

The growing location had a significant impact on the total tocopherol content but not on the individual tocopherols, including γ- and δ-tocopherol (Tab. 2). Genotypes cultivated in Lavaur and Airaines showed a significantly lower content of total tocopherols (399.2 mg/kg of oil) compared to those grown in Poix-de-Picardie (412.7 mg/kg of oil) in 2014. The year 2014 had a significantly higher content of δ-tocopherol ($p < 0.01$) and total tocopherols ($p < 0.001$) compared to 2013 and 2015 (Tab. 2).

Table 2. Sum of squares from a combined analysis of variance for **γ**-tocopherol, **δ**-tocopherol and total tocopherol content in linseed for 10 varieties cultivated during the 2014 growing season across three locations (Airaines, Poix-de-Picardie, and Lavaur).

	n	δ −tocopherol	γ −tocopherol	Total tocopherol
Genotype (G)	10	4818***	53847***	2830***
Environment (E)	3	741***	55683***	4845***
G x E	30	3688***	33872***	3040***

*** Significant at $p < 0.001$.

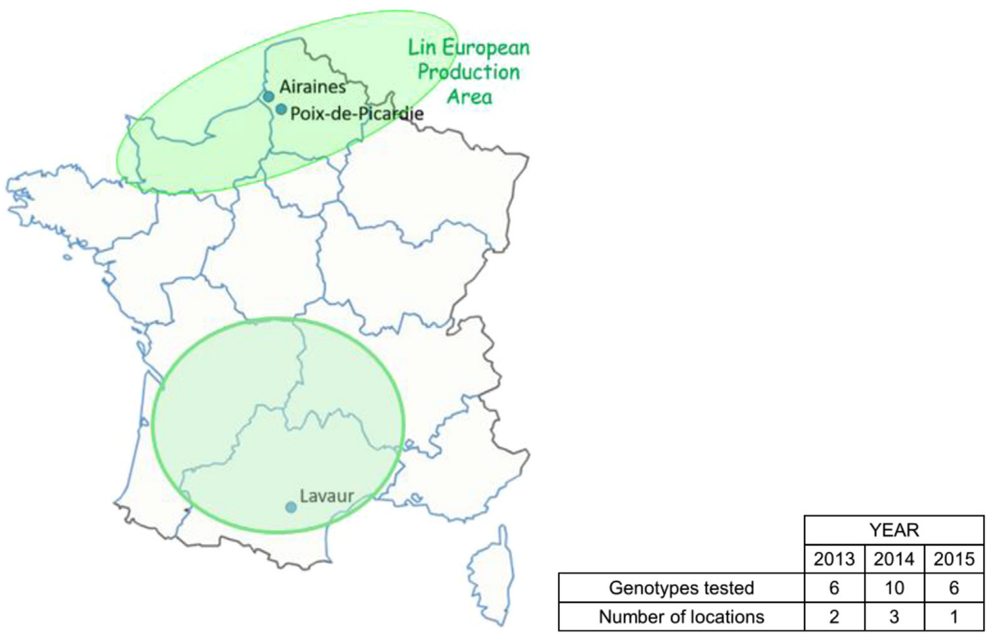

Fig. 3. The upper figure (A) represents the GC chromatogram of silylated linseed oil sterols : 1: brassicasterol; 2: 24-methylene campesterol; 3: campesterol; 4: stigmasterol; 5: β-sitosterol; 6: Δ5-avenasterol; 7: cycloartenol; 8: Δ7-stigmasterol; 9: Δ7-avenasterol; and 10: betulin (internal standard); the B part mass spectrum of the cycloartenol at the retention time (RT) 12.58.

There were also significant differences between genotypes at the same location in 2014, with total tocopherol content ranging from 331.7 to 473.7 mg/kg of oil and γ-tocopherol content varying from 329.6 to 466.5 mg/kg of oil.

3.4 Phytosterols

Linseed oil contained six major phytosterols: campesterol, stigmasterol, β-sitosterol, Δ5-avenasterol, cycloratenol and Δ7-stigmasterol. Among these, β-sitosterol was the most important, comprising 36 ± 3% of the total phytosterol content, which corresponds to 146 ± 41 mg/100 g of oil, followed by cycloartenol at 22 ± 3%. A chromatogram of the phytosterol profile is presented in Figure 3.

Table 3 presents the content and composition of sterols in different linseed genotypes. Variety 06 had the highest total phytosterol content, with 501.34 mg/100 g of oil, while Variety 02 presented the lowest content, with 315.36 mg/100 g of oil. Significant differences in total phytosterol content were observed across years; 2015 had the lowest concentration, with a mean of 231.78 mg/100 g of oil, while 2013 presented the highest content, with 443.94 mg/100 g of oil. The highest phytosterol content was observed at the Poix-de-Picardie and Lavaur locations.

Analysis of variance showed significant differences in the content of the seven phytosterols, as well as in total phytosterols, total tocopherols, and α-tocopherol content across genotypes, locations, and years. Significant differences were also observed for the genotype × year interaction, except for β-sitosterol, avenasterol, and cycloartenol content, as well as for the genotype × location interaction with respect to Δ7-stigmasterol content.

Phytosterol content showed significant variation across sites and genotypes (Tab. 4). In the southern regions, there was a significant reduction in each individual phytosterol, as well as in total phytosterols. The total phytosterol content was largely influenced by location (44%). The Poix-de-Picardie location always presented the highest levels.

In 2014, the total phytosterol content was significantly higher ($p < 0.01$) compared to 2013 and 2015, with β-sitosterol also showing a significant increase ($p < 0.001$) (Tab. 4). This trend was observed for all phytosterols except Δ7-Stigmasterol.

There were also significative differences between genotypes at the same location in 2014, with total phytosterol

Table 3. Mean values of phytosterol content and isoform composition of 10 linseed genotypes, with location- and year-specific averages.

		Phytosterol concentration (mg/100 g oil)						
Factor		Campesterol	Stigmasterol	β-sitosterol	Δ5, Avenasterol	Cycloartenol	Δ7, Stigmasterol	Total
Genotype	Var 01	82.41	22.72	148.03	30.81	82.35	19.52	385.83
	Var 02	57.86	16.61	121.45	27.93	74.93	16.57	315.36
	Var 03	58.03	19.45	133.01	22.83	85.63	15.98	334.92
	Var 04	66.60	27.84	121.78	30.44	89.77	20.90	357.34
	Var 05	91.15	43.35	153.18	39.63	72.47	22.65	422.44
	Var 06	100.01	35.34	213.64	43.52	82.24	26.58	501.34
	Var 07	74.43	24.90	126.02	35.86	92.83	17.03	371.06
	Var 08	72.14	28.62	116.77	29.36	86.73	18.83	352.46
	Var 09	88.71	32.18	166.42	39.98	97.87	26.29	451.46
	Var 10	76.57	32.69	126.18	34.85	81.37	17.49	369.15
	Mean	76.79	28.37	142.65	33.52	84.62	20.18	386.13
	SD	13.99	7.95	29.70	6.38	7.73	3.88	56.73
	CV (%)	18.21	28.02	20.82	19.02	9.13	19.20	14.69
Year	2013	77.80	30.28	153.06	38.43	111.53	26.93	443.94
	2014	75.49	28.54	140.07	33.18	97.28	21.65	396.22
	2015	36.85	11.85	84.02	22.17	67.45	9.43	231.78
	Mean	63.38	23.55	125.72	31.26	92.09	19.34	357.31
	SD	23.00	10.18	36.69	8.30	22.49	8.98	111.30
	CV (%)	36.29	43.21	29.18	26.54	24.43	46.41	31.15
Location	Lavaur	67.46	27.28	121.47	25.77	81.25	15.46	338.69
	Airaines	74.94	26.65	136.72	34.04	84.41	17.14	373.90
	Poix-de-Picardie	93.54	33.03	180.18	43.72	97.63	29.39	477.49
	Mean	78.65	28.99	146.13	34.51	87.76	20.66	396.69
	SD	13.43	3.51	30.46	8.99	8.69	7.60	72.15
	CV (%)	17.08	12.12	20.85	26.04	9.90	36.80	18.19

CV: Variation coefficient; SD: Standard deviation.

Table 4. Sum of squares of combined analysis of variance for individual phytosterols and total phytosterol content in linseed for 10 varieties cultivated during the 2014 growing season across three locations (Airaines, Poix-de-Picardie, and Lavaur).

	Campesterol	Stigmasterol	β-sitosterol	Avenasterol	Cycloartenol	Δ7-Stigmasterol	Total phytosterols
Genotype (G)	13075***	4818***	53847***	2830***	11364***	1179	206115***
Environment (E)	10824***	741***	55683***	4845***	4528***	3469***	312359***
G x E	13086***	3688***	33872***	3040***	12770***	2170	154026***

*** Significant at $p = 0.001$.

content ranging from 192.3 to 519.6 mg/100 g of oil, β-sitosterol from 67.0 to 95.7 mg/kg of oil, and cycloartenol from 36.9 to 154.4 mg/kg of oil.

4 Discussion

Previously, we have studied the effect of environmental factors and cultivar on linseed composition and subsequent oil expression (Savoire *et al.*, 2015). Linseed oil content ranged between 30 and 50% of dry weight and contained 40 to 66% C18:3. The cultivar factor had a significant influence on oil and C18:3 content, while the growing location only affected lipid content. In addition, annual effects were noted on oil and C18:3 content. The observed increases in oil content can be explained by the increased degree day after flowering and sunshine duration while the significant decreases in C18:3 content were probably related to lower rainfall and a slightly lower temperature during seed filling.

This current study elucidates the intricate interplay of genetic, environmental, and G×E interactions that govern tocopherol and phytosterol variability in linseed oil. By assessing these compounds under realistic field conditions, our findings not only extend previous insights into oilseed variability but also provide a valuable framework for breeding and agronomic strategies aimed at sustaining oil quality amidst climate change.

Our study identifies γ-tocopherol as the predominant isoform in linseed oil (396.7 ± 39 mg/kg oil), as previous findings in other *Linum* varieties where γ-tocopherol typically constitutes 96–98% of total tocopherols (Gandova *et al.*, 2023; Nykter *et al.*, 2006; Oomah *et al.*, 1997). These results also align with those reported by Marquard *et al.* (1977) and more recently by Dąbrowski *et al.* (2025), who observed tocopherol contents in linseed oil ranging from 395 to 500 mg/kg. The substantial reductions in tocopherol (365 mg/kg oil) and phytosterol (232 mg/100 g oil) content observed during the 2015 growing season underscore the sensitivity of these minor compounds to abiotic stress. The 2015 season experienced the more stressful weather conditions during pre-flowering, flowering and grain filling stages in both North and South locations, characterized by high temperatures and low precipitation. These factors significantly reduced total tocopherol levels compared to 2014 and 2013. In the South location, where temperatures were significantly higher than in the North locations, tocopherol content was further reduced. Previous studies have demonstrated that tocopherol levels fluctuate under heat stress, reflecting their dual role as antioxidants and stress indicators (Ali *et al.*, 2022; Ayerdi Gotor *et al.*, 2015). While elevated temperatures can enhance the activity of tocopherol biosynthetic enzymes such as homogentisate phytyltransferase (VTE2) and p-hydroxyphenylpyruvate dioxygenase (HPPD) (Marquard, 1990; Obranović *et al.*, 2015), they can simultaneously impair chloroplast function, leading to excessive reactive oxygen species (ROS) accumulation, lipid peroxidation, and subsequent tocopherol depletion (Štolfa Čamagajevac *et al.*, 2018). This phenomenon is well-documented in other oilseed crops, such as rapeseed, *Brassica napus* L., where heat stress during flowering exacerbates oxidative damage and reduces tocopherol content (Gawrysiak-Witulska *et al.*, 2011), as well as in sunflower (Ayerdi Gotor *et al.*, 2015).

Drought stress further alters plant metabolism by down-regulating the mevalonate pathway, a crucial biosynthetic route for phytosterol production, as nicotinamide adenine dinucleotide phosphate (NADPH) is redirected toward essential stress responses such as glutathione synthesis (Zhang *et al.*, 2023). This metabolic shift, evident in drought-induced lipid remodeling in soybean, *Glycine max* L. Merr. (Shahriari *et al.*, 2022), highlights the importance of phytosterols as indicators of plant stress adaptation. While the suppression of the mevalonate pathway is a common stress response, some plants exhibit an upregulation of phytosterol biosynthesis under specific conditions, revealing a complex regulatory network that warrants further investigation (Zhang *et al.*, 2020).

Beyond abiotic factors, the rhizosphere microbiome plays a pivotal role in modulating lipid metabolism through biotic interactions. Root exudates, including lipids, recruit beneficial microbes that enhance plant resilience by influencing lipid biosynthesis (Korenblum *et al.*, 2022). Microbial metabolites can trigger systemic metabolic changes, leading to increased protective lipid production (Zhuang *et al.*, 2024). Additionally, diverse microbial communities contribute to nutrient solubilization and hormone biosynthesis, further regulating lipid metabolism and overall plant health (Bharti *et al.*, 2021). Despite these insights, the potential of microbial interactions in modulating linseed oil quality remains largely unexplored and represents a promising avenue for future research.

Phytosterol contents observed in this study are consistent with values reported in the literature, ranging from 315 to 508 mg/100mg of oil (Dąbrowski *et al.* (2025); Matthäus and Özcan, 2017). Shifts in phytosterol composition, particularly the balance between β-sitosterol and cycloartenol, reflect adaptive strategies for membrane stability and stress signaling. Sterols function as plant hormones and play an important role in protecting plants against environmental stress, as well as regulating growth and development. β-Sitosterol enhances membrane rigidity under abiotic stress (Rossi and Huang, 2022), while cycloartenol modulates stress-responsive pathways (Aboobucker and Suza, 2019; Du *et al.*, 2022). Similar trends in quinoa (Schlag *et al.*, 2022; Stoleru *et al.*, 2022) and linseed (Choo *et al.*, 2007; Herchi *et al.*, 2009) underscore the ecological plasticity of oilseed crops, enabling them to fine-tune their lipid profiles for resilience across diverse climatic conditions. This plasticity highlights the potential for breeding strategies aimed at enhancing resilience to climate fluctuations (Donohue, 2016), although challenges persist in mitigating the adverse effects of G×E interactions, such as reduced oil yield under extreme environmental conditions.

The observed variation in phytosterol content across different locations, with notably high levels in Poix-de-Picardie (477 mg/100 g oil), underscores the potential of agroecological zoning to optimize crop value. In temperate regions, linseed presents a significant opportunity for the functional food market, particularly in cholesterol-lowering products (Čeh *et al.*, 2020). However, the heightened susceptibility of linseed grown in southern locations to heat stress necessitates the development of heat-tolerant cultivars. Advances in gene editing, such as clustered regularly interspaced short palindromic repeats (CRISPR)-mediated modifications of thermotolerance genes, hold promise for maintaining oil quality under increasing temperatures (Clemis *et al.*, 2023) and aligning with EU Farm-to-Fork and Green Deal objectives (Stavropoulos *et al.*, 2023). For example, Saha *et al.* (Saha *et al.*, 2019) identified 34 putative heat shock factor (HSF) genes in the *L. usitatissimum* genome and designed guide ribonucleic acid (RNA) sequences for precise gene editing with minimal off-target effects. Editing these HSF genes could lead to heat-tolerant linseed cultivars capable of maintaining oil quality under elevated temperatures. Similarly, overexpression of the drought-responsive element binding protein 2A (DREB2A) gene has been shown to confer drought tolerance in a transgenic line of linseed *cv. Blanka* (Tawfik *et al.*, 2016). While selective breeding, leveraging traits with high heritability such as flowering time (Toor *et al.*, 2024), offers immediate solutions, gene-editing technologies provide long-term resilience against climate uncertainties. These findings emphasize the importance of genetic diversity in linseed breeding programs aimed at improving oil quality and stability (Kaur *et al.*, 2024; Shankar *et al.*, 2024). While δ-tocopherol offers clear advantages in temperate climates, the potential benefits of γ-tocopherol in arid regions warrant further exploration, particularly in the context of shifting agricultural zones due to climate change.

Comparative studies across oilseed species underscore the need for crop-specific metabolic models to refine breeding strategies. The role of tocopherols, particularly γ-tocopherol and α-tocopherol, in enhancing the oxidative stability and stress resilience of *G. max* and other oilseed crops is well-documented. Genetic modifications and breeding strategies can optimize tocopherol profiles, improving nutritional value and shelf life across species. In *G. max*, γ-tocopherol is the predominant form, contributing to oxidative stability under environmental stress (Tavva *et al.*, 2007). Genetic studies have identified quantitative trait loci (QTLs) linked to tocopherol synthesis, particularly those affecting the conversion of γ-tocopherol to α-tocopherol, which improves oil quality (Park *et al.*, 2023). Similarly, in *B. napus*, genetic modifications targeting the γ-tocopherol methyltransferase (VTE4) gene have successfully increased α-tocopherol content, enhancing the oil's nutritional profile (Wang *et al.*, 2025). In safflower, *Carthamus tinctorius* L., breeding efforts focus on optimizing tocopherol profiles, with metabolic flux analysis identifying key enzymatic control points in tocopherol biosynthesis to enhance oxidative resistance (Golkar, 2014). Sunflower (*Helianthus annuus*) seeds predominantly contain α-tocopherol, constituting more than 90% of total tocopherols (Velasco *et al.*, 2014). Genetic modifications targeting HPPD, a key enzyme in the tocopherol biosynthesis pathway, have been shown to enhance α-tocopherol production, further emphasizing the importance of tocopherol biosynthesis in determining oil quality (Srinivasan *et al.*, 2022).

While strategies to enhance tocopherol content show promise for improving oil quality and stability across different crops, it is essential to consider potential trade-offs in crop yield and other agronomic traits that may result from genetic modifications and breeding approaches. Genetic diversity in linseed (Kaur *et al.*, 2024) provides a strong foundation for trait optimization. The variety 'Railinus' has been identified as a high-performing genotype, exhibiting significant levels of tocopherols and phytosterols, which are crucial for oil quality and stability (Matthäus and Özcan, 2017). In this study, Variety 06 was identified as a high-performing genotype (tocopherol: 431.94 mg/kg oil; phytosterol: 501.34 mg/100 g oil), demonstrating the potential of integrating multi-omics data with advanced breeding strategies. Transcriptomic analyses targeting key enzymes, such as tocopherol cyclase (VTE1) in tocopherol cyclization and Cytochrome P450 family 710 subfamily A (CYP710A) in sterol modification, could reveal regulatory hubs amenable to marker-assisted selection. Overexpression of VTE1 has been shown to significantly increase total tocopherol content in *Arabidopsis* leaves, underscoring its critical role in tocopherol biosynthesis (Kanwischer *et al.*, 2005). Similarly, the CYP710A family encodes sterol C-22 desaturases involved in the biosynthesis of stigmasterol from β-sitosterol, as shown in studies on *Arabidopsis* and tomato (Morikawa *et al.*, 2006). Moreover, predictive models have proven effective in assessing oilseed crop performance. The CAMEL model, developed for false flax, *Camelina sativa* L., successfully predicted seed yield, oil production, and key fatty acid accumulation under varying climatic conditions in Northern Italy (Cappelli *et al.*, 2019). Adapting similar simulation frameworks for linseed could enhance breeding strategies by optimizing oil quality across diverse environmental conditions.

Despite the advances presented here, the three-year dataset in this study may not fully capture long-term climatic trends and extended multi-year trials are necessary to validate stress-response patterns. Additionally, the influence of the soil microbiome on lipid metabolism, a crucial factor in linseed rhizosphere interactions, remains unexplored. Future research should also incorporate economic analyses of breeding high-tocopherol linseed for both nutritional and industrial applications.

5 Conclusions

This study shows that tocopherol and phytosterol contents in linseed are governed by a complex interplay of genetic and environmental factors. Notably, high temperatures reduce the content of these bioactive compounds. This could be due to the protective role of tocopherols and phytosterols as antioxidants against hot stress, leading to a decrease of their content in the plant. The variability encountered within varieties allows the possibility to increase the content of these two families of components by breeding. By selecting varieties with superior tocopherol and phytosterol profiles, it may be possible to develop linseed cultivars that not only offer improved nutritional and cosmetic benefits but also maintain oil quality over longer storage periods. Future research should focus on integrating advanced breeding techniques to further exploit this variability for improved crop performance under changing climatic conditions.

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Conflicts of interest

The authors declare no conflict of interest

Author contribution statement

Conceptualization, Alicia Ayerdi Gotor, Brigitte Thomaset and Larbi Rhazi; Data curation, Alicia Ayerdi Gotor, Florentin Donot and Rachid Sabbahi; Methodology, Florentin Donot and Larbi Rhazi; Supervision, Thierry Aussenac and Larbi Rhazi; Validation, Alicia Ayerdi Gotor, Florentin Donot, Brigitte Thomaset and Thierry Aussenac; Writing – original draft, Alicia Ayerdi Gotor, Florentin Donot, Rachid Sabbahi and Larbi Rhazi; Writing – review & editing, Alicia Ayerdi Gotor, Brigitte Thomaset, Thierry Aussenac, Rachid Sabbahi and Larbi Rhazi.

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