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FLAX AND HEMP
LIN ET CHANVRE

Environmental and varietal impact on linseed composition and on oil unidirectional expression process

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Abstract – Effect of environmental factors and cultivar on linseed composition and subsequent oil expression has been studied. Ten linseed cultivars were grown in two different location (north and south of France) during years 2011, 2012 and 2013. Every year have been characterized in terms of pluviometry, sunshine hours and degree days, each climatic factor has been cumulated from flowering to harvest. A strong impact of cultivar on oil, C18:3 and starch content has been noticed whereas the growing location has only affected the starch and lipid contents. Year effect have been recorded on proteins, oil and C18:3 content. A principal component analysis has highlighted the correlation between the pluviometry and the C18:3 content. Oil content have not presented any correlation with environmental or composition factors. Oil expression using the seeds previously characterized has exhibited a strong correlation between C18:3 content and oil yield. No correlation between oil yield and other composition parameters has been observed. The analysis of variance conducted on yield has emphasized that yield variation was majority attributable to a year effect.

Keywords: Linseed / composition / environmental factors / oil expression / cultivar

Résumé – Impact de la variété et de l'environnement sur la composition des graines de lin et sur le pressage unidirectionnel. L'effet des paramètres environnementaux et de la variété sur la composition de la graine de lin et sur le procédé ultérieur de pressage a été étudié. Dix variétés de lin oléagineux ont été cultivées sur deux sites différents (nord et centre de la France) au cours des années 2011, 2012 et 2013. Chaque année de culture a été caractérisée, de la floraison des plants à la récolte des graines, en termes de cumul de précipitation, de cumul d'heures d'ensoleillement et de degrés jour de croissance (DJCF). Un fort effet variétal sur les teneurs en huile, C18:3 et amidon a été relevé tandis que le lieu de culture n'a affecté que la teneur en lipides et amidon. L'effet année a quant à lui été marqué pour les teneurs en protéines, huile et C18:3. Une analyse en composantes principales a mis en évidence le lien entre teneur en C18:3 et cumul de précipitations. La teneur en lipides n'a pu être corrélée à aucun des paramètres environnementaux ou de composition étudiés. Le pressage des graines précédemment caractérisées a montré une forte corrélation entre teneur en C18:3 et rendement. Aucune autre corrélation entre paramètres de composition et rendement de pressage n'a été notée. L'analyse de variance a mis en évidence que les variations de rendement observées étaient principalement imputables à un effet année.

Mots clés : Graines de lin / composition / facteurs environnementaux / pressage / cultivar

1 Introduction

Oil crops have enormous economic importance worldwide since vegetable oil consumption has increased by > 50% over the past decade and is expected to double by 2040 (FAOSTAT, 2014). The production of vegetable oils has represented around 176 million metric tons in 2014 (USDA – Oilseeds World Market and Trade, 2014) and the majority of oils we consumed are accumulated in seeds. These oil resources are

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used to 97% in the food industry leaving only 5 million tons for the energy market, chemistry and oleochemistry (Carlsson, 2009; Floros *et al.*, 2013; Hong *et al.*, 2012; Meier *et al.*, 2007).

Among these oil crops, flaxseed (*Linum usitatissimum* L.) is emerging as an important functional food ingredients and one of the richest sources of α-linolenic acid (18:3$^{cis\Delta9,12,15}$; ALA) praised for their health benefits (Akhtar, *et al.*, 2013; Goyal *et al.*, 2014; Hall *et al.*, 2006; Jhala and Hall, 2010; Singh *et al.*, 2011). Flaxseed typically accumulates 35–50% of the dry weight of their seed tissue as storage oil normally in the form of triacylglycerides (TAG) (Adugna *et al.*, 2004; Batta *et al.*, 1985; Vereshchagin and Novitskaya, 1965). This specific oil contains ~45 to 65% omega-3 polyunsaturated fatty acids depending of the genotypes (Adugna *et al.*, 2004; Batta *et al.*, 1985; Chandrawati *et al.*, 2014; Fofana *et al.*, 2010; McGregor and Carson, 1961; Thambugala *et al.*, 2013; Tonnet and Green, 1987; Westcott and Muir, 2003).

However, plants are cultured in different environments where they are exposed to various climate conditions and soils conditions with a direct repercussion on the seed filling and its final composition (Dilman and Hopper, 1943; Dybing *et al.*, 1966; Kirkhus *et al.*, 2013; Lafond *et al.*, 2008; Painter *et al.*, 1944; Westcott and Muir, 2003). In fact, several factors comprising temperature, rainfall, light, drought, ozone exposure, nitrogen deprivation, fertilizers may influence the nutritional quality of oilseeds (Baldini *et al.*, 2002; Flagella *et al.*, 2002; Green, 1986; Harris *et al.*, 1978; Rahimi *et al.*, 2011; Tripathi and Agrawal, 2013). Numerous works have reported the environmental effects on yield, oil content and fatty acids composition of oil, tocopherols, phytosterols, and phospholipids in oilseed plants (Froment *et al.*, 1999; Green, 1986; Trémolière *et al.*, 1982; Tripathi and Agrawal, 2013; Zubr and Matthaus, 2002). Cold is one of parameters directly involved in the increasing of omega-3 content in plant tissues maintaining membrane integrity and their fluidity (Guan *et al.*, 2014; Steindal *et al.*, 2015; Tonnet and Green, 1987).

The objective of this study was to evaluate the impact of environmental conditions on the chemical composition of seeds of different genotypes of flax. In this study, ten flax cultivars were systematically used and cultured during three consecutive years at two different locations. The effects of seasonal factors such as temperature, rainfall and sunshine on oil, starch and protein contents of flax seeds were determined. The impact of seed composition was also tested on the oil expression.

2 Materials and methods

2.1 Raw material

Ten linseed cultivars named L01, L02, L03, L04, L05, L06 and N01, N02, N03, N04 were cultivated in fields over three years (2011, 2012 and 2013) in France. Two locations were studied, one in the center of France (named south in the following) and one in Picardie (North). Due to meteorological issues, all cultivars have not been harvested in all locations for all years. Table 1 summarized the growing location and cultivars considered for each cultivation year.

2.2 Meteorological conditions

For each cultivation year and growing location, meteorological conditions were collected. This characterization was realized using meteorological data from the meteorological station nearest the growing location (*i.e.* Abbeville meteorological station for north location and Chartres for south; www.infoclimat.fr). Raw data (*i.e.* pluviometry, sunshine hours, maximal and minimal temperatures) were collected for each day between plant flowering (estimated at 50% of the field in flower) and seed harvesting. Degree day after flowering (DDAF) were calculated from temperature data using equation (1).

$$DDAF = \frac{T_{max} + T_{min}}{2} - T_{base} \qquad (1)$$

with T_{max} the daily maximal temperature, T_{min} the daily minimal temperature and T_{base} a reference temperature set at 5 °C for linseed.

For evaluating the impact of meteorological conditions on seed composition, these data were summed for all days between flowering and harvesting.

2.3 Seed characterization

Each biochemical characterization was performed in triplicate on three independent seed samples randomly chosen.

2.3.1 Oil content

Seed oil content was determined using a method adapted to low seed masses. 200 mg of seeds were oven dried at 104 °C for 24 h. Dried seeds were grinded one minute in isooctane using a ball mill (Precellys 24 lysis homogenization). Seed-solvent mix was then centrifuged (6000 rpm, 1 min). The upper phase containing oil and solvent was recovered and a second extraction on the bottom solid phase was performed. Supernatants were pooled and then evaporated under nitrogen. Recovered oil was then weighted and seed oil content was calculated on a dry basis. Reliability of the extraction method was assessed through comparison with standard soxhlet method (norm NF EN ISO 65) and quantitation obtained by a Cofrac certified laboratory (Cetiom) using NMR method (data not shown).

2.3.2 Protein content

Protein content was determined according to the Dumas method. The nitrogen content of the samples was quantified by combustion method using a LECO FP528 nitrogen analyzer (LECO France, Garges les Gonesse, France). One hundred milligrams of dried seeds was weighed in tin foil and analyzed in triplicate using the AOAC method 990.03. The protein–nitrogen conversion factor of 6.25 was used for the calculation of the protein content.

Table 1. Cultivar repartition according to year and location.

Year	Location	Cultivar									
2011	North	N01,	N02,	N03,	N04	L01,	L02,	L03,	L04,	L05,	L06,
2012	North	N01,	N02,	N03,	N04	L01,	L02,	L03,	L04,	L05,	L06,
	South	N01,	N02,	N03,	N04						
2013	North	N01,	N02,	N03,	N04		L02,	L03,	L04,		
	South	N01,	N02,	N03,	N04		L02,		L04		

Table 2. Meteorological conditions.

Growing location	Year	Sunshine hours (h)		Pluviometry (mm)		DDAF (°C)	
		Value	Mean	Value	Mean	Value	Mean
North	2011	630 ± 80	530 (490*)	250 ± 20	190 (160*)	1240 ± 50	1010 (890*)
	2012	450 ± 10		220 ± 20		930 ± 40	
	2013	520 ± 10		90 ± 20		860 ± 30	
South	2012	505 ± 10	570	260 ± 20	180	1050 ± 30	1110
	2013	638 ± 0		106 ± 0		1174 ± 0	
All	2012	480	530	240	170	990	1000
	2013	580		100		1010	

* Values in brackets are the means for years 2012 and 2013 for North growing location. ± values are standard deviations for the different cultivars.

2.3.3 Starch content

Starch content was determined using an enzymatic kit (Megazyme K-TSTA) on 100 mg of seeds.

2.3.4 Acid α-linolenic content

Fatty acid profile was established by transmethylation of triglycerides and further analysis by GC-FID. On 5 mg of oil, 100 μl of diethyl ether and 5 μl of tetramethyl ammonium hydroxide (in 25% methanol) were added. The mix was incubated at 50 °C for 5 min then the reaction was stopped by addition of decane. After centrifugation, the aqueous phase containing fatty acid methyl ester was recovered and diluted in heptane prior to GC analysis. Gas chromatography with Flame Ionization Detector (FID) was used for fatty acid quantitation. Fatty acids separation was performed using a capillary BPX-70 (30 m × 0.25 mm × 0.25 μm) column. 10 μl of sample was injected at 250 °C using an automatic injector (AOC-20i, Shimadzu). Oven temperature was set at 120 °C and was increased to 250 °C using a 15 °C/min slope. Then temperature was maintained for 2 min. 1.2 ml/min hydrogen flow at 67 kPa was used. Detection was realized at 280 °C. Data was collected and integrated by a GC solution v2.4 integration system (Shimadzu). α-linolenic acid content was calculated by the ratio of C18:3 peak surface to the total surface of the chromatogram.

2.4 Oil expression

Pressing experiments were conducted on a device especially designed for expression conducted on small seed quantities. 3.17 g of milled seeds were expressed at 100 bar for 1 h at 50 °C. The experimental setup was described in Savoire (2008). Expression was conducted on seeds dried at 5% residual moisture content.

2.5 Statistical analysis

All data were analyzed using R software through the R Studio interface. For principal component analysis, FactoMineR package was used, for ANOVA car package with type III sum of squares was chosen.

3 Results and discussion

Seeds of every year and location were analyzed for lipid, protein, starch content and α-linolenic content of oil was also determined. Data were correlated with meteorological growing conditions and cultivar considered to assess the influence of growing location, year and cultivar.

3.1 Meteorological conditions

Table 2 computes the meteorological data for each growing location and year. Each year has presented a particular climate. Year 2011 was characterized by high pluviometry, DDAF and sunshine hours. For this year, cultivation has only take place in the North. Comparing years 2012 and 2013, climate has evolved following similar tendencies in south and in North. Year 2012 was more rainy but also less sunny. DDAF evolution according to year was dependent of the growing location. DDAF has decreased between 2012 and 2013 in the North whereas it has increased in the South. South growing location has presented higher temperatures (highlighted by higher DDAF) and higher sunshine hours than North ones. Pluviometry was relatively closed for both locations.
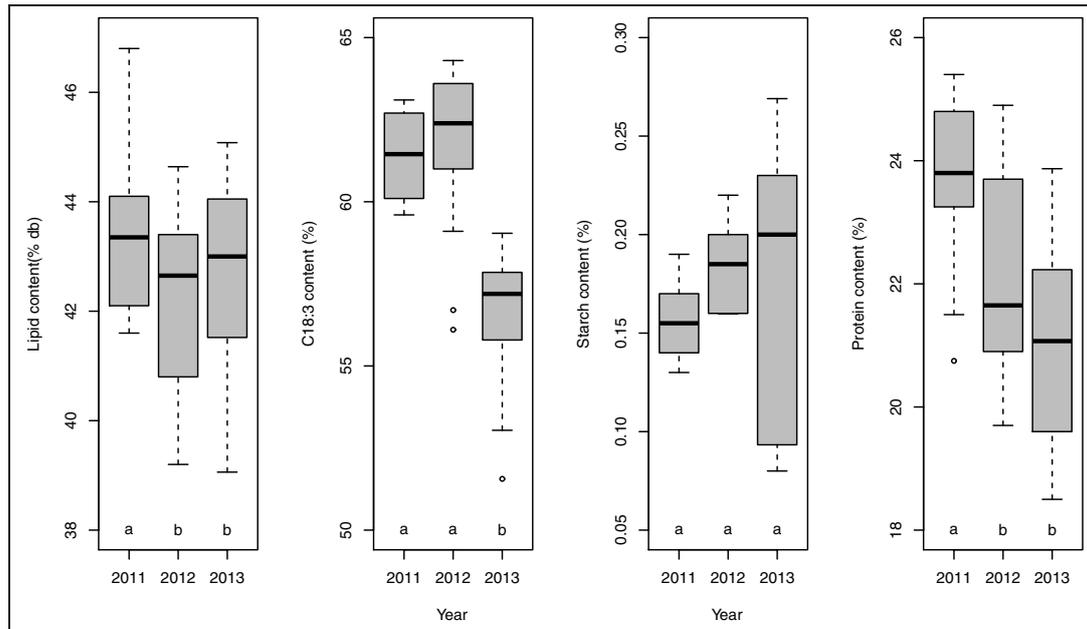
Fig. 1. Boxplots of year significant effect on lipid content, C18:3 content, starch content and protein content (means with the same letter are not significantly different according to Tukey HSD test at 95% confidence interval).

Table 3. *F* values and levels of significance of ANOVA of seed components.

Factor	Oil	Proteins	Starch	C18:3
Cultivar	12.24***	1.73[NS]	3.19*	4.45**
Year	7.16**	6.08**	4.69*	39.53***
Location	8.06**	1.33[NS]	5.25*	0.63[NS]

NS: non-significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3.2 Analysis of variance

First of all, data of composition were analyzed through ANOVA using year, growing location and cultivar as explicative factors. Table 3 summarized the ANOVA results.

Cultivar remains the principal factor affecting oil, starch and C18:3 content of flax seeds (Tab. 3, Fig. 2). Oil content in flax seeds were reported between 30 to 50% of the dry weight containing 40 to 66% of C18:3 with a level highly dependent on the genetic variation between linseed genotypes (Adugna *et al.*, 2004; Batta *et al.*, 1985; Mc Gregor and Carson, 1961; Tonnet *et al.*, 1987; Vereshchagin and Novitskaya, 1965; Westcott and Muir, 2003). As for other plant species, the flax cultivars tend to maintain their rank in regard to oil content and fatty acid composition whatever the culture conditions (Ghamkhar *et al.*, 2010; McGregor and Carson, 1961; Rahimi *et al.*, 2011). Thus, the flax variability is specifically exploited to identify common molecular markers linked to oil content and fatty acids in order to establish efficient strategies for marker-assisted breeding of flax (Chandrawati *et al.*, 2014; Soto-Cerda *et al.*, 2014).

Year had a significant impact on all considered responses. This impact could be attributed to different meteorological conditions during growing. Mean composition of the seeds according to year is presented in Figure 1. For flax as

for various other plant species, the seed storage compounds (proteins, starch, fatty acids) are subjected to modifications that can be easily explained by seasonal variations (Adugna and Labuschagne, 2002, 2003; Dilman and Hooper, 1943; Fieldsend and Morison, 2000; Kirkhus *et al.*, 2013; McGregor and Carson, 1961; Westcott and Muir, 1996). The year interaction effects are often considered as the most important environmental factors affecting yield and seed components.

For lipid content, Tukey HSD test has highlighted a pairwise difference between year 2011 and years 2012–2013. This could be due to the higher DDAF and sunshine hours for year 2011 compare to other years. However, the large variation depending in particular of the genotype was observed for each year (Figs. 1 and 2). This result is in accordance with literature in which seasonal conditions are generally reported to significantly affect seed oil content and oil composition (McGregor and Carson, 1961; Vollmann *et al.*, 2007; Westcott and Muir, 1996). However, it is on contradiction with the results obtained by Kirkhus and collaborators (2013) on camelina (a plant rich in C18:3) where no difference has been observed on seed oil content during 3 years of camelina cultures in Norway.

In our study and for C18:3 content, year 2013 is significantly different from year 2011 and 2012. This difference could be correlated with the lower pluviometry observed in 2013 compare to the other years (100 mm instead of 240/250 mm). No data has been reported on the modification of flax seed composition under an excess or a limitation of rainfall during plant development. Generally, a low temperature during seed filling promotes the accumulation of C18:3 in triglycerides (TAG) and phospholipids of flax seeds (Green, 1986; Tonnet and Green, 1987; Velasco and Fernandez-Martinez, 2002). However, in our study, only a slightly decrease of temperature was observed between 2012 and 2013 (990 °C compared with 1010 °C; Tab. 2 – DDAF)
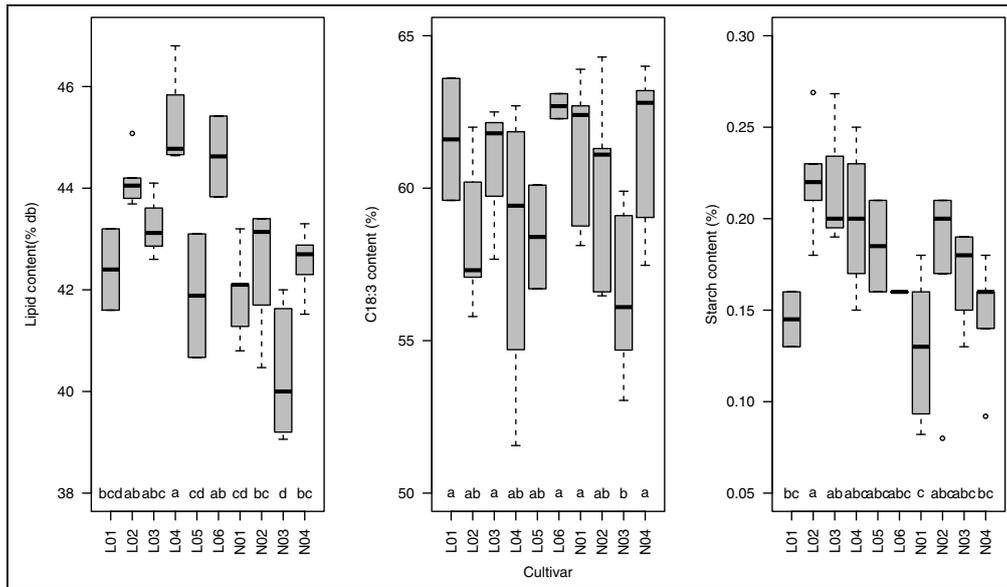
Fig. 2. Boxplots of cultivar significant effect on lipid content, C18:3 content and starch content (means with the same letter are not significantly different according to Tukey HSD test at 95% confidence interval).
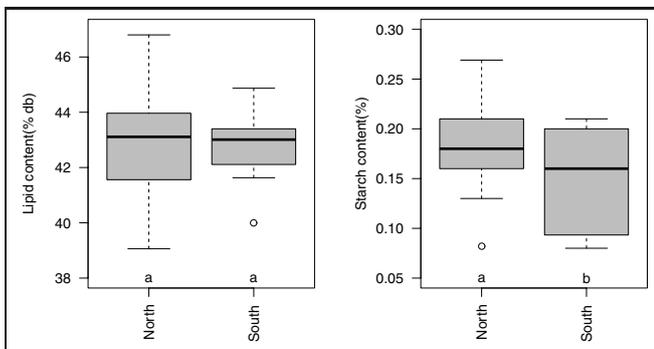

Fig. 3. Boxplots of location significant effect on lipid content, and starch content (means with the same letter are not significantly different according to Tukey HSD test at 95% confidence interval).

not enough to explain the observed differences (Fig. 1). In the studies on camelina, an oilseed plant rich in linolenic fatty acids, Vollmann and collaborators (2007) have clearly shown that low temperatures and high precipitation during the seed filling increase the production of linolenic fatty acids in seeds of different camelina genotypes. This result seems to confirm our observations on linseed (Fig. 1). In contrast, Kirkhus and collaborators (2013) mentioned that the highest levels of C18:3 were obtained in camelina seeds when high temperatures and excessive rainfall were recorded before and during flowering and low rainfall and high temperature observed during seed filling and maturation period. In fact, lots of works indicated a regulation of omega-3 desaturases enzymes dependent on the growth temperature. At low temperature, the transcription of fatty acid desaturase genes (FAD) in various organs are shown to be induced (Soto-Campos *et al.*, 2014; Yurchenko *et al.*, 2014).

For protein content, year 2013 is significantly different from years 2011 and 2012. Unfortunately, no data has been reported in the literature excepted that the ratio lipid/protein increased to low temperatures concomitantly with an increased C18:3 level in phospholipid content (Behzadipour *et al.*, 1998). However, Roche in 2005 has clearly reported a positive effect of high temperatures on the accumulation of proteins in sunflower seeds when plants were cultured in greenhouse under controlled conditions. In our study, the temperatures observed in 2011 (Tab. 1: DDAF 1240 °C) during the seed filling were higher than those recorded for 2012–2013 (Fig. 1). This could explain the high level of proteins in our flax seeds in 2011.

Starch content was also affected by year according to ANOVA. Unfortunately, the Tukey had not evidenced any difference between years. This could be attributed to the very large variation in starch content observed in year 2013 (Fig. 1). Beyond these considerations about starch content, the very low absolute value of starch contents (below 0.3%) should be noticed. In oilseed embryos, starch is generally transiently synthetized in the early stages of seed development and then transformed in lipids and proteins (Andriotis *et al.*, 2010; Eastmond and Rawsthorne, 2000; Troufflard, 2004; Streb and Zeeman, 2012). In flax, the maxima of starch content occurs around 20/25 days after flowering and can be up to 0.015 mg/embryo. At maturity stage, starch content of 0.002 mg/embryo are reported (Troufflard, 2004). So the variations of starch content observed in this study could be attributed to different maturity stage of the harvested seeds and/or to a varietal effect. However, starch content has varied in a very low range of values.

Considering cultivar effect, it was only noticed for lipid, C18:3 and starch content. Tukey test has permitted to highlight some special cultivars that are more likely to be different from other ones for lipid content (Fig. 2). Thus cultivars N03 and L04 have presented lipid contents significantly different from several varieties (N02, N04, L03, L02, L06 and L04 for N03 cultivar and N01, N02, N03, N04, L01 and L05 for cultivars L04, respectively). For C18:3 content only N03

Table 4. Correlation coefficients between variables and PCA dimensions.

Variable	PCA dimensions		
	1	2	3
Lipids	0.449*	0.033	0.887*a
Proteins	0.731*a	−0.015	−0.038
C18:3	0.678*	−0.612*	0.008
Pluviometry	0.770*a	−0.477*	−0.296
Sunshine	0.392*	0.875*a	−0.102
DDAF	0.778*a	0.560*	−0.139

* Statistically significant correlation coefficient. a Variable with $\cos^2$ higher than 0.5.
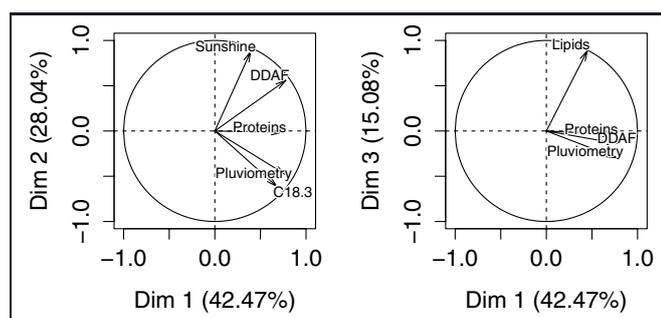
Fig. 4. Variables projection on PCA dimensions 1–2 and 1–3 (only variables with $\cos^2 > 0.5$ are presented).

cultivar seemed different from a large part of other cultivars and for starch content, only difference between cultivars L02 and N01 has been noticed. As previously mentioned, the cultivar effect is the major parameter affecting storage compounds of flax seeds (McGregor and Carson, 1961; Westcott and Muir, 1996; Vollmann *et al.*, 2007). In our study, the flax cultivar L04 seems to be highly interesting for its high lipid level in seeds.

According to ANOVA, culture location has only affected the lipid and the starch contents of the seeds. However, the Tukey test realized on the lipid content results have not evidenced a significant difference between mean lipid contents observed in North and in South. For starch content, South has appeared more favorable for lower starch content indicating a higher maturity of the seeds.

3.3 Principal component analysis

In order to establish correlations between climatic growing conditions and seeds composition, a principal component analysis (PCA) was performed using DDAF, pluviometry and sunshine hours as explicative variables in addition to composition variables affected by growing year (oil, proteins and C18:3). Starch was not taken into account in this study due to the very low starch content in the seeds. The correlation coefficients defining the three PCA factors (explaining 85% of observed variance) were presented in Table 4 and the projection of explicative variables on the PCA axis were on Figure 4. For projection, only variables with $\cos^2$ higher than 0.5 were

represented for more readability. The projection axis were described each by different variables. Indeed, dimension 3 was only described by lipid content, dimension 2 mainly by sunshine hours and dimension 1 by pluviometry, DDAF, protein and C18:3 contents. From Figure 4, the positive correlation between pluviometry and C18:3 could be evidenced as well as the independence of lipid content from all studied variables. Positive correlation between DDAF and sunshine hour was also noticed. This last observation is quite normal as DDAF is a measure of the total heat available for vegetables during seed growing and, in temperate countries, sunshine hour and ambient temperature evolves in a same way. Proteins content was described by dimensions 1 and was correlated (but in a lesser extent than C18:3) with pluviometry and also DDAF. Roche in 2005 has clearly reported a positive effect of temperatures on the accumulation of proteins in sunflower seeds when plants were cultured in greenhouse under controlled conditions.

During PCA treatment, the quantitative variables year, growing location and cultivars were added as supplementary variables. It has appeared that cultivar was significantly correlated with PCA dimension 3. The cultivars L04, L02 and N03 have shown projection on dimension 3 being significantly different from 0, this projection was positive for L04 and L02 and negative for N03. So cultivars L04 and L02 have presented higher lipid contents than the overall cultivars whereas cultivar N03 had lower lipid content. The supplementary variable growing location has been correlated with PCA dimension 2. South was positively correlated with dimension 2 and North negatively. So North was more rainy less sunny than south and C18:3 content was higher in North. In oilseed crops, the higher levels of polyunsaturated fatty acids are often observed when low temperatures occur during seed filling period (Dybing and Zimmerman, 1965; Green, 1986; Velasco and Fernandez-Martinez, 2002; Vollmann *et al.*, 2007). Kirkhus *et al.* (2013) have also shown that C18:3 content in camelina seeds could be highly affected by the regime of precipitations. A period with high rainfall during flowering and then low rainfall during seed filling contribute to favour the accumulation of C18:3 in seeds.

Concerning PCA dimension 1, a correlation with growing year has been established. Year 2011 was statistically positively correlated with axis 1 and year 2013 was negatively. So year 2011 has contained more proteins and C18:3 than year 2013 harvest. In 2011, the temperatures were high and contributed to accumulation of proteins and oil in flax seeds (Tab. 1, Fig. 1). For this year, it has also been reported high pluviometry during flax seed filling (Tab. 1) that could explain the high level of C18:3 in seeds and is in accordance with the results of Kirkhus on camelina (Kirkhus *et al.*, 2013).

A plot of active individuals according to PCA dimensions was performed (Fig. 5). This type of plot permits to distinguish the individuals using supplementary variables as explaining factors. Clear distinction could be performed according to year and growing location in the projection plan using dimensions 1 and 2. For cultivars, distinction was more difficult and projection plan with dimensions 2 and 3 would be preferred. In addition to individuals, confidence ellipses were also plotted. For cultivars, confidence ellipses were only plotted for cultivars whose correlation with dimension 3 was statistically significant (L02, L04 and N03).
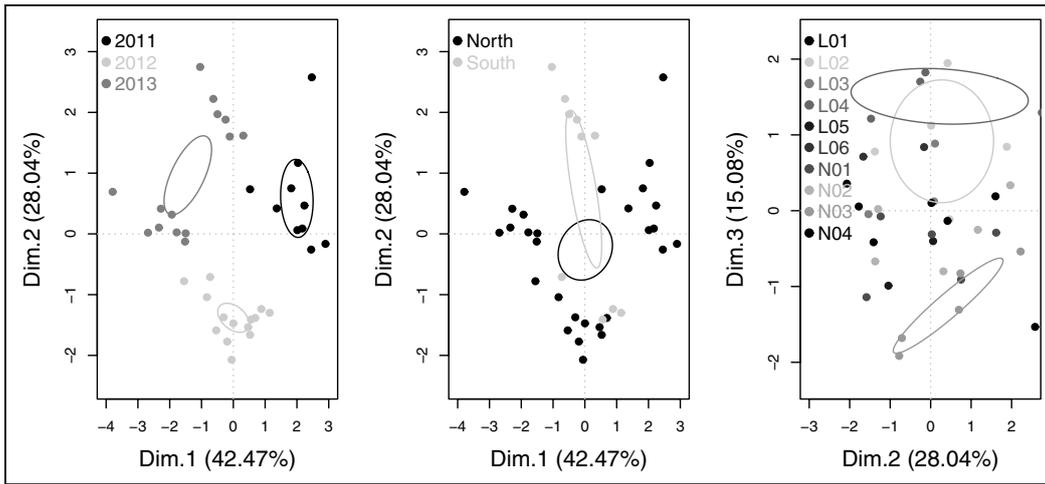
Fig. 5. Individuals representation on PCA dimensions with confidence ellipses. Confidence ellipses are only represented for cultivars L02, L04 and N03.

Table 5. *F* values and levels of significance of ANOVA of oil yield.

Factor	Oil yield
Cultivar	2.05[NS]
Year	30.02[***]
Location	2.49[NS]

NS: non-significant; *$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.*

As previously mentioned, these results indicate a clear impact of year on the quality of seeds (C18:3). The location seems also to be a dominant source of variation (Fig. 4). The flax L02 and L04 lines are the best for their lipid and C18:3 contents whereas the N03 line has poor performance whatever the culture conditions (Fig. 5).

3.4 Oil expression

Oil expression was carried out on previously described seeds. The oil yield obtained was analyzed using the same statistical methods as in the first part of this article. ANOVA conducted on oil yield has highlighted the significant impact of growing year whereas growing location and cultivar were not significant (Tab. 5). So extraction process was only dependent (among studied variables) of growing year. This year effect on oil expression was previously observed on linseed for pressing seeds at different maturity stages (Savoire, 2008). The absence of cultivar impact on linseed oil expression is more surprising as this effect has been largely described in the literature on various oilseeds species. This effect has notably been observed by Dedio and Dorrell (1977) on unidirectional press and also by Zheng *et al.* (2003) and Rombaut (2013) on continuous screw presses.

The boxplot of mean oil yield according to year was plotted in Figure 6. Graphical interpretation of Figure 6 and Tukey HSD test have permitted to evidence oil yield differences between year 2013 and years 2011–2012. In addition, oil yield for year 2013 has presented higher dispersions.
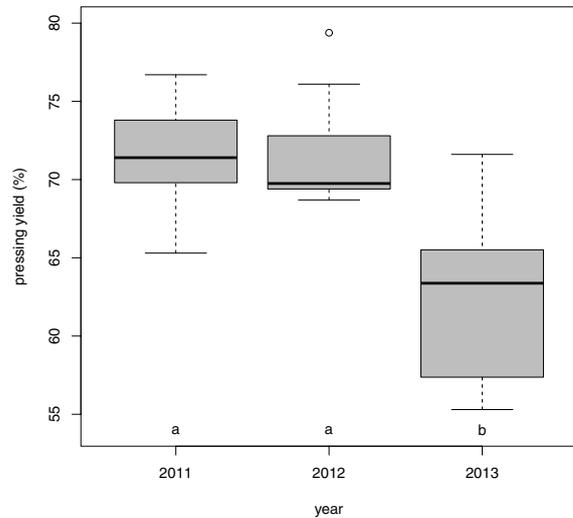

Fig. 6. Boxplots of year significant effect on oil yield.

In our study, year has a significant impact on storage compounds (lipids, proteins) in seeds (Fig. 1). It could be supposed that a correlation between lipids and the extraction yield could exist.

From ANOVA results, cultivar and growing location had no effect on oil yield. Despite the few study available on cultivar effect on oil expression, all existing studies conclude to an impact of cultivar on oil yield. Contrary to our results, Savoire (2008) found an effect of cultivar on yield of linseed oil. Thus among 14 varieties, only two stood out with a maximum and a minimum yield. According to (Rombaut, 2013; Savoire, 2008; Zheng *et al.*, 2003), the reason for this variation can be attributed to a negative correlation between oil yield and oil content of the seed, or to an effect of seed geometry. The absence of cultivar effect in our study could be explained by the large variation in oil yield observed for each cultivar. Indeed, for almost each studied cultivar, oil yield varied from 60 to 75% depending on year and growing location. So eventual cultivar impact was hidden by year effect. Similar conclusion could be
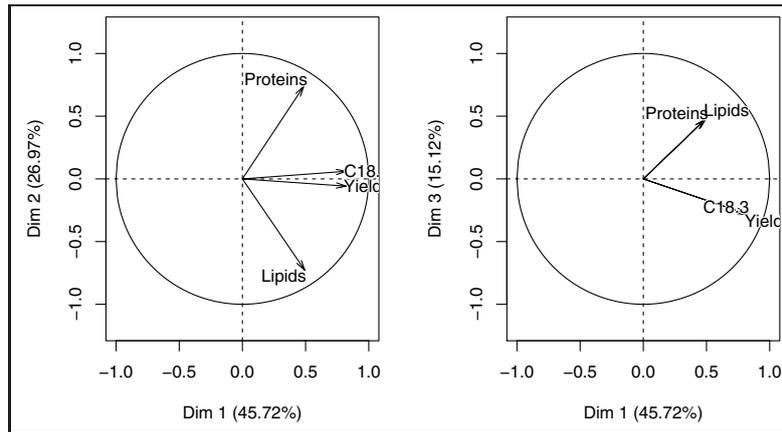
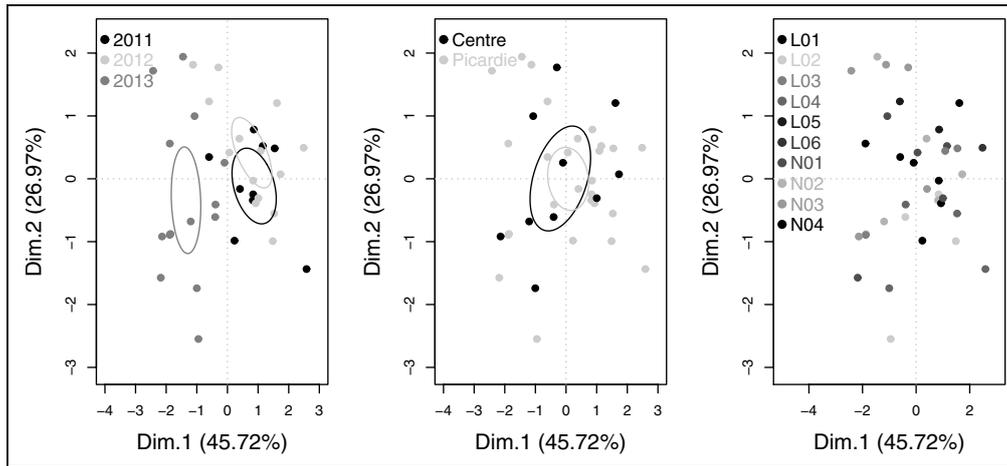**Fig. 7.** Variables projection on PCA dimensions 1–2 and 1–3 (PCA yield).

Fig. 8. Individuals representation on PCA dimensions (PCA yield).

formulated to explain the lack of significance of growing location on oil yield. The effect of growing location on the oil yield has not been widely studied. However in Dedio and Dorrell's study (1977) on linseed oil unidirectional expression, a location impact was noticed. In our study, the two growing location are only distant from 170 km, this two location could be too close to evidence a strong growing location impact on seed post treatment.

As the growing year has impacted lipid, protein and C18:3 content (*cf*. Tab. 3), the relation between these composition variables and the oil yield was studied through PCA (In the following, notably for table and figure, this PCA will be referred as PCA yield). The correlation coefficients defining the three PCA yield factors (explaining 87% of observed variance) are presented in Table 6 and the projection of explicative variables on the PCA axis are on Figure 7. A strong correlation between C18:3 content and oil yield has appeared through this PCA yield analysis. In axis 1 definition, the variables accounting for the majority of the contribution were oil yield and C18:3 content. So oil yield was highly dependent of C18:3 content and to a lesser extent from protein and lipid contents. Analysis of other dimensions have mainly highlighted the correlation between lipid and protein contents. So far there have been no studies linking the overall composition of the seed to the ef-

Table 6. Correlation coefficients between variables and PCA dimensions (PCA yield).

Variable	PCA dimensions		
	1	2	3
Oil yield	0.822[*a]	–0.058	–0.281
Lipids	0.493[*]	–0.728[a*]	0.475[*]
C18:3	0.822[*a]	0.061	–0.282[*]
Proteins	0.485[*]	0.736[a*]	0.470

[*] Statistically significant correlation coefficient. [a] Variable with $\cos^2$ higher than 0.5.

ficiency of pressing. One study was interested by the impact of linseed mucilage content on oil expression but no correlation between these particular molecule and process has been established (Savoire, 2008).

Individuals were plotted in the projection plan defined by the dimensions 1 and 2 of PCA; year, cultivar and growing location were used as illustrative variables (Fig. 8). Using year as illustration, distinction between years 2013 and years 2011 and 2012 could be observed. For growing location, no distinction between South and North has appeared. Supplementary analysis of PCA dimension has highlighted that growing year was

significantly linked to dimension 1. Years 2011 and 2012 were positively linked to dimension 1 and year 2013 negatively. In addition, cultivar N03 was also negatively linked to dimension 1. The second dimension was linked to cultivar (negatively with cultivars L04 and L02). For the third dimension, positive link with cultivar L04 and year 2013 was noticed and negative link with year 2012 and cultivar N01.

Correlation between oil yield and C18:3 content has been evidenced from PCA analysis. Physical interpretation of this correlation could not be found and it would be possible that a non-studied physical or biochemical factor is responsible for oil yield variations.

4 Conclusion

Growing year, location and cultivar effect on linseed composition has been studied. Year impact was mainly noticed on oil, C18:3 and protein content. Analysis of year impact through meteorological factors (pluviometry, sunshine and DDAF) has only permitted to evidence the pluviometry influence on C18:3. For other biochemical factor, no link between their content in the seed and the meteorological growing conditions has been observed. Raw material impact on seed processing (expression) has been evaluated and only year effect was detected. Correlation between oil yield and C18:3 content has been noticed but no satisfactory explanation have been found.

So the impact of cultivar and culture conditions on seed composition and transformation was evaluated. In this study, two flax lines (L02, L04) presented a robust behaviour with the best lipid and C18:3 content and one line (N03) a poor performance whatever the culture conditions (year, location). These two most stable lines could be exploited for industrial use.

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