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Effect of genotype and environment on fatty acid composition of *Lupinus albus* L. seed

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Abstract

Six cultivars of *Lupinus albus* L. (white lupin) were grown in two subcontinental-climate environments and one Mediterranean-climate environment in Italy, to assess the influence of genotypic (G) and genotype × environment (GE) interaction effects on grain yield and grain content of oil, total saturated fatty acids (FAs), polyunsaturated FAs, monounsaturated FAs, and ω−3/ω−6 FA ratio. The variance of genotypic effects was much larger than the GE interaction variance for all variables, except for grain yield, indicating that oil content and FA composition of different varieties can be assessed reliably in just a few test environments. Gas-chromatographic analyses highlighted that linoleic acid and α-linolenic acid were in the range 1.76–4.76 mg/g flour (7.79–15.81% of total FAs) and 1.17–3.14 mg/g flour (5.40–10.36% of total FAs), respectively. As a consequence, the analysed lupin seeds exhibited a very favourable ω−3/ω−6 FA ratio, ranging from 0.49 to 0.79.

Keywords: *Lupinus albus*; Oil composition; Linoleic acid; α-Linolenic acid; ω−3/ω−6 fatty acid ratio; Growing environment

1. Introduction

The health authorities of many countries promote the intake of foods containing high amounts of ω−3 fatty acids (FAs) and a favourable ω−3/ω−6 fatty acid (FA) ratio (http://www.efsa.eu.int/science/nda/nda_opinions/catindex_en.html; Simopoulos, 2003; West Suitor & Meyers, 2006). In fact, ω−3 FAs play very important roles in physiology, especially during foetal and infant growth, in particular in the formation of the central nervous system and retina (Bourre, 2003; Bowen & Clandinin, 2005), and for the prevention of cardiovascular diseases, being antithrombotic, anti-inflammatory, antiarrhythmic and favouring plaque stabilisation (Galli & Marangoni, 2006; Hu et al., 1999; SAS, 1999; Simon, Pong, Bernert, & Browner, 1995).

The seed of *Lupinus albus* L. (white lupin) contains 9–14% oil, whose composition includes about 50–60% oleic acid, 16–23% linoleic acid, and 8–9% α-linolenic acid (Bhardwaj, Hamama, & Merrick, 1998; Bhardwaj, Hamama, & van Santen, 2004; Jimenez, Cubero, & de Haro, 1991). As a consequence of these values, the ω−3/ω−6 FA ratio falls in the range 0.4–0.6. The increasing selection of sweet varieties (containing very low amounts of quinolizidine alkaloids) has recently widened the possibility of using white lupin seeds in human or livestock nutrition (Huyghe, 1997; Petterson, 1998). Considering the favourable content of both proteins and fibres, several companies in Europe have started to produce and commercialise food products, such as pasta, bread, and imitation meat products from lupin. These foods, in which lupin ingredients are used as a replacement for either animal/plant proteins or cereal flours (Bez, Schott, & Seger, 2005; Doxastakis et al., 2006; Seger & Bez, 2005), may, in principle, offer

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opportunities to improve both the daily intake of α-linolenic acid and of FAs with a favourable ω−3/ω−6 ratio.

In a previous paper (Boschin, D'Agostina, Annicchiarico & Arnoldi, 2007) we reported on the influence of the environment on the FA composition of *O*-acyl lipids of *L. albus* seeds. End users and plant breeders need to know whether the quality of lupin grain lots may also be affected by the genotype and, if sizeable genetic differences exist, whether they are consistent across environments or are subject to genotype × environment (GE) interaction. The main objective of this work was, therefore, to assess the extent of genotypic and GE interaction effects on the FA composition of *L. albus* cultivars grown in subcontinental or Mediterranean-climate conditions. Since it is not possible to differentiate between effects of soil and climatic conditions, environment is comprised of both soil and climatic conditions.

2. Materials and methods

2.1. Materials

Hexane and methanol were HPLC grade, diethyl ether was 95% purity and certified as peroxide-free; they were purchased from Baker (Deventer, Netherlands). Water was produced with a Milli-Q Water Purification System (Millipore, Billerica, MA). Sodium methoxide in methanol (1%) was freshly prepared, dissolving 0.34 g of metallic sodium in 100 ml of HPLC grade methanol. The following fatty acid methyl esters (FAMEs) were purchased from Fluka (Sigma–Aldrich, St. Louis, MO): methyl pentadecanoate (99.5% purity), methyl palmitate (99.5%), methyl palmitoleate (99.0%), methyl heptadecanoate (99.7%), methyl stearate (99.5%), methyl oleate (99.0%), methyl linoleate (98.5%), methyl linolenate (99.0%), methyl eicosanoate (98.0%), methyl *cis*-11-eicosenoate (98.5%), methyl docosanoate (99.0%), and methyl *cis*-13-docosenoate (99.0%).

2.2. Sampling

Six cultivars were studied: AB47 (Spain); Lucille, Ludet and Luxe (France); Multitalia and one ecotype collected in the Molise region (Italy). All of them, except the ecotype, have a low content of quinolizidine alkaloids. They were grown in Lodi (Lombardy) in 2002–2003 and Sanluri (Sardinia) in 2003–2004. Lodi has a subcontinental-climate with cold winters, whereas Sanluri has a Mediterranean-climate with mild winters and terminal drought stress. Optimal soil characteristics for lupin growth, i.e., sub-acid pH and low content of $CaCO_3$ (Dracup, Turner, Tang, Reader, & Palta, 1998), existed at both sites. Lodi included two environments represented by an early (23th October 2002; LE environment) and a late (7th November 2002; LL environment) autumn sowing. Sowing dates were arranged on main plots, and cultivars on subplots, of a split-plot experimental design with three replications. Sanluri's experiment was autumn-sown (10th November 2003, S environment), adopting a randomized complete block design with three replications. Information on the crop management in each environment is provided elsewhere (Annicchiarico, Carroni, & Iannucci, 2004; Annicchiarico, Iannucci, & Filippi, 2003). Chemical analyses were carried out on two field replicates per cultivar in each growing environment on random samples of grain collected at crop maturity. Data from three replications were used for grain yield assessment.

2.3. Extraction of the crude oil

Lupin seeds were dehulled and ground in a household mill; 12 g of flour were extracted with hexane (300 ml) for 6 h in a Soxhlet apparatus using cellulose extraction thimbles (123 mm × 45 mm o.d.; 43 mm i.d.; Whatman International, Brentford, UK). The solvent was then evaporated under reduced pressure. The oil content was gravimetrically determined and expressed as weight percentage (%) of lupin flour.

2.4. Preparation of fatty acid methyl esters (FAMEs)

FAMEs were prepared by transmethylation of *O*-acyl lipids using CH_3ONa in CH_3OH (1%) according to the official method published in the Official Journal of the European Union (Annex XB, 05/09/1991 No. L248/44). This is the official method used for the preparation of FAMEs from *O*-acyl lipids when the unsaponifiable matter is less than 3%. One gram of oil was suspended in 20 ml of CH_3OH; 1 ml of a 30 mg/ml hexane solution of methyl heptadecanoate (C17:0) was added as internal standard (IS), in order to quantify the FAs; then 1.75 ml of 1% CH_3ONa in CH_3OH was added under reflux; the mixture was then heated under reflux for 3 h. The FAMEs were extracted with diethyl ether and 1 ml of a 30 mg/ml methyl pentadecanoate (C15:0) solution in hexane was added as a second IS to evaluate the FAMEs recovery. The solvent was evaporated under reduced pressure and the residue was diluted in hexane to give a 10 mg/ml solution, which was analysed by GC–FID.

2.5. GC–FID analysis

The FAMEs were analysed with a DANI 86.10HT gas chromatograph (DANI Instruments, S.p.A., Cologno Monzese, Italy) equipped with a flame ionization detector (FID) (gas pressure: H_2 at 1 bar; air at 1 bar). An SP-2340 column (60 m × 0.25 mm i.d. × 0.2 μm film thickness) (Supelco, Bellefonte, PA) was used. Analyses were performed in splitless mode, using a PTV injector (operating conditions: 45 °C for 30 s, then heating to 250 °C in 12 s); carrier gas He (1.4 bar; flow rate 1.1 ml/min), auxiliary gas N_2 (0.8 bar; flow rate 0.9 ml/min). The detector temperature was set at 250 °C; the temperature program was: 16 min at 160 °C, from 160 °C to 210 °C at 1.5 °C/min,

then 20 min at 210 °C. The analyses were processed with Star GC Workstation software (Version 5.52; Varian, Inc., Palo Alto, CA). Each analysis was performed at least in triplicate. Peaks were identified by comparison of retention times with those of standard compounds. The quantification was performed both with (a) a normalisation method and (b) by using two internal standards.

(a) Normalisation method: The percent of a single FA was calculated from the ratio of individual peak area multiplied by the proper correction factor to the sum of all FA areas, using the following formula:

$$\%FA_i = \left[(\text{Area } FA_i \times Cf_i) \Big/ \sum_i \text{Area } FA_i \right] \times 100$$

where Cf is the proper correction factor: 1.06 for palmitic acid, 2.00 for palmitoleic acid, 1.79 for stearic acid, 2.50 for oleic acid, 3.54 for linoleic acid, 2.41 for α-linolenic acid, 1.65 for arachidic acid, 1.89 for 11-eicosenoic acid, 1.58 for behenic acid, and 1.92 for erucic acid, previously calculated.

The percentage values of the considered groups of FAs were obtained from the summation of the percentages of the appropriate FAs: TSFA, sum of the percentage values of total saturated FAs, i.e., palmitic acid + stearic acid + arachidic acid + behenic acid; MUFA, sum of the percentage values of monounsaturated FAs, i.e., palmitoleic acid + oleic acid + 11-eicosenoic acid + erucic acid; PUFA, sum of the percentage values of polyunsaturated FAs, i.e., linoleic acid + α-linolenic acid.

(b) Internal standard method: Single FA absolute quantities were expressed as mg/g lupin flour, obtained by using the two internal standards; in particular methyl pentadecanoate (C15:0) was used for evaluating the recovery of the extraction procedure and methyl heptadecanoate (C17:0) was used to quantify each FA, using the following formula:

$$Q_i = (Q_{st} \times \text{Area}_i)/(Cf_i \times \text{Corrected Area}_{st})$$

where Q_i is the content of the FA, Q_{st} is the content of methyl heptadecanoate, Area_i is the peak area of the FA, Cf_i is the correction factor of the FA, and $\text{Corrected Area}_{st}$ is the area of methyl heptadecanoate adjusted for the recovery value.

2.6. Statistical analysis

An analysis of variance was performed for grain yield (expressed at 13% seed moisture), oil content (expressed as weight percentage of lupin flour), percent content of TSFA, MUFA and PUFA, and the ω−3/ω−6 FA ratio (expressed as the ratio α-linolenic acid/linoleic acid), assessing the variation among cultivars and environments and the occurrence of GE interaction. The relative extent of genotypic and GE interaction effects was assessed by estimating the relevant components of variance through a restricted maximum likelihood method, assuming that the cultivars were a random sample of the white lupin material available for cultivation (while environment was kept as fixed factor). The relationship between grain yield, oil content and the variables related to FA composition of the cultivars was assessed by simple correlation analysis, using values of cultivars averaged across environments. Statistical Analysis System (SAS, 1999) software was used for all analyses.

3. Results and discussion

3.1. Oil and fatty acids content

The oil content for individual cultivar-environment combinations ranged from 7.12% to 11.50% (Table 1). These values appear to be in good agreement with literature data: 4.86% and 7.2–8.2% for *L. albus* grown in US (Bhardwaj, 2002; Bhardwaj et al., 2004), 5.95% and 10.74% for two Turkish cultivars (Erbas, Certel, & Uslu, 2005; Uzun, Arslan, Karhan, & Toker, 2007), although the different cultivars and growing conditions prevent a direct quantitative comparison.

The percent composition of the FAs in individual cultivars/environment combinations is presented in Table 1. On average, the FAs ranked in the following order of abundance: oleic acid (C18:1) > palmitic acid (C16:0) > linoleic acid (C18:2) > α-linolenic acid (C18:3) ≈ 11-eicosenoic acid (C20:1) ≈ behenic acid (C22:0) > stearic acid (C18:0) ≈ erucic acid (C22:1) > arachidic acid (C20:0) > palmitoleic acid (C16:1). Concerning TSFA, the percentage of palmitic acid was in the range 15.17–19.85%, which is about twice the value of 7.6% reported for an Egyptian cultivar (Uzun et al., 2007) and higher than the mean value of 11.6% observed for Turkish samples (Erbas et al., 2005); stearic acid was in the range 1.34–3.56% (Table 1), in good agreement with literature values (Bhardwaj et al., 2004; Erbas et al., 2005; Uzun et al., 2007). In respect to MUFA, oleic acid was the most abundant FA, with a content falling in the range 40.8–50.5%, whereas erucic acid was in the range 0.78–4.84%. Regarding PUFA, the content of linoleic acid was in the range 7.79–15.81% and that of α-linolenic acid in the range 5.31–10.36%, with a consequent ω−3/ω−6 FA ratio in the range 0.49–0.79. Literature data for linoleic acid content are generally much higher than ours since they exceed 20% [22.4% in Erbas et al. (2005); 20.3% in Uzun et al. (2007), and 23.48% in Bhardwaj (2002)], whereas those of α-linolenic acid are comparable [8.7% in Erbas et al. (2005), 9.2% in Uzun et al. (2007), and 9.68% in Bhardwaj (2002)]. Once again the different experimental conditions prevent a deeper quantitative discussion.

3.2. Effects of genotype and environment

Test locations differed sharply for frequency and extent of winter frosts, which implied severe cold stress in the subcontinental-climate site and negligible stress in the

Table 1
Oil content and fatty acid composition (expressed as percentage value ± S.D.) of six cultivars of *Lupinus albus* in three environments

Cultivar	Env.	Oil content	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
AB47	LE	9.24 ± 1.89	16.25 ± 0.42	0.29 ± 0.12	1.74 ± 0.43	50.46 ± 0.27	9.49 ± 0.45	5.40 ± 0.18	1.28 ± 0.02	6.93 ± 0.05	5.20 ± 0.12	2.96 ± 0.01
	LL	9.11 ± 0.70	16.37 ± 0.35	0.43 ± 0.01	1.94 ± 0.24	49.86 ± 0.46	9.78 ± 0.06	5.50 ± 0.01	1.26 ± 0.10	6.87 ± 0.40	5.20 ± 0.12	2.78 ± 0.02
	S	9.86 ± 0.07	16.83 ± 0.02	0.50 ± 0.05	2.63 ± 0.03	47.98 ± 0.45	9.98 ± 0.03	6.58 ± 0.13	1.65 ± 0.11	6.46 ± 0.64	5.44 ± 0.21	1.95 ± 0.02
Ecotype	LE	9.94 ± 0.61	16.47 ± 0.26	0.54 ± 0.02	2.27 ± 0.02	48.73 ± 0.87	7.79 ± 0.40	5.56 ± 0.11	1.55 ± 0.02	6.78 ± 0.38	6.25 ± 0.63	4.06 ± 0.39
	LL	10.21 ± 0.71	17.73 ± 1.26	0.60 ± 0.18	1.34 ± 0.40	47.50 ± 0.07	8.29 ± 0.22	5.91 ± 0.50	1.31 ± 0.24	5.92 ± 0.34	6.56 ± 0.51	4.84 ± 0.16
	S	11.50 ± 1.37	15.75 ± 0.18	0.50 ± 0.01	2.95 ± 0.03	45.35 ± 0.03	10.46 ± 0.07	6.60 ± 0.23	1.89 ± 0.13	6.60 ± 0.13	7.20 ± 0.29	2.70 ± 0.08
Lucille	LE	9.11 ± 0.20	19.25 ± 0.45	0.62 ± 0.08	2.02 ± 0.16	44.17 ± 0.17	13.40 ± 0.16	7.38 ± 0.30	1.27 ± 0.05	5.16 ± 0.29	4.83 ± 0.01	1.90 ± 0.63
	LL	8.78 ± 0.06	19.53 ± 0.10	0.79 ± 0.29	2.07 ± 0.03	44.87 ± 0.93	12.38 ± 0.05	7.33 ± 0.15	1.46 ± 0.37	4.97 ± 0.20	4.81 ± 0.06	1.78 ± 0.15
	S	9.35 ± 0.14	19.85 ± 0.76	0.54 ± 0.21	2.52 ± 0.04	45.09 ± 0.30	11.83 ± 0.01	7.52 ± 0.21	1.43 ± 0.01	4.99 ± 0.17	4.87 ± 0.25	1.35 ± 0.01
Ludet	LE	9.55 ± 0.11	17.23 ± 0.66	0.48 ± 0.14	1.34 ± 0.74	43.98 ± 0.16	13.80 ± 0.20	9.04 ± 0.48	1.33 ± 0.16	5.62 ± 0.15	5.20 ± 0.32	1.98 ± 0.39
	LL	9.92 ± 0.38	18.02 ± 0.06	0.80 ± 0.08	1.80 ± 0.03	41.82 ± 0.60	13.64 ± 0.17	8.95 ± 0.60	1.20 ± 0.05	6.28 ± 0.40	5.25 ± 0.26	2.25 ± 0.19
	S	8.31 ± 0.01	17.74 ± 0.30	0.59 ± 0.02	1.80 ± 0.01	40.76 ± 0.29	15.81 ± 0.11	10.36 ± 0.36	1.15 ± 0.04	5.01 ± 0.23	5.36 ± 0.20	1.43 ± 0.05
Luxe	LE	8.45 ± 0.21	18.57 ± 0.10	0.77 ± 0.01	2.83 ± 0.05	48.08 ± 0.29	10.74 ± 0.15	5.31 ± 0.01	1.74 ± 0.11	4.92 ± 0.02	5.57 ± 0.28	1.47 ± 0.03
	LL	8.80 ± 0.11	18.47 ± 0.04	0.74 ± 0.01	2.72 ± 0.07	49.84 ± 0.42	10.07 ± 0.05	5.34 ± 0.09	1.61 ± 0.04	4.86 ± 0.25	4.99 ± 0.28	1.36 ± 0.21
	S	10.45 ± 1.07	16.88 ± 0.02	0.51 ± 0.02	3.56 ± 0.02	47.21 ± 0.17	12.80 ± 0.01	6.55 ± 0.32	1.99 ± 0.07	4.57 ± 0.19	5.15 ± 0.01	0.78 ± 0.01
Multitalia	LE	7.12 ± 2.31	16.69 ± 0.27	0.64 ± 0.13	1.41 ± 0.75	48.49 ± 1.78	8.81 ± 0.10	6.94 ± 0.29	1.25 ± 0.01	6.69 ± 0.36	5.56 ± 0.11	3.52 ± 0.34
	LL	8.36 ± 0.69	16.75 ± 0.93	0.36 ± 0.09	1.70 ± 0.05	47.46 ± 0.21	9.43 ± 0.01	7.40 ± 0.13	1.20 ± 0.10	6.31 ± 0.07	5.94 ± 0.25	3.44 ± 0.02
	S	10.29 ± 0.16	15.17 ± 0.02	0.44 ± 0.01	2.42 ± 0.01	46.55 ± 0.31	11.27 ± 0.03	8.78 ± 0.05	1.42 ± 0.01	6.35 ± 0.07	5.48 ± 0.15	2.14 ± 0.04

Env. = Environment: LE = Lodi – Early autumn; LL = Lodi – Late autumn; S = Sanluri.

Table 2
Climatic variables for two test locations of six cultivars of *Lupinus albus*

Location	Cropping year	Abs. min. temperature (°C)	No. frost days	Spring rainfall (Mar. 1–Jun. 15; mm)
Lodi	2002–2003	−7.8	54	83
Sanluri	2003–2004	−2.2	10	268

Mediterranean site (Table 2). Sanluri displayed higher spring rainfall than Lodi (Table 2), but the sites did not differ for grain yield (Table 3), likely because of the higher evapotranspiration demand in spring of the Mediterranean environment.

The comparison among mean values of environments indicated that the seed content of oil, TSFA, MUFA, and PUFA was influenced by the environment (i.e., the location), but not by the sowing date within the Northern Italy site, whereas the ω−3/ω−6 FA ratio was not influenced by the environment (Table 3).

Significant ($p < 0.05$) variation among cultivars across environments was detected for all traits (Table 4). GE interaction effects occurred for all traits except for oil content, but their size for FA composition or ω−3/ω−6 FA ratio was always smaller than that of purely genetic effects (Table 4). For instance, the genotypic variance was about eight-fold greater than the GE interaction variance for PUFA content and the ω−3/ω−6 FA ratio (Table 4). The relatively small size of GE effects suggests that breeders' knowledge on oil or FA content of genotypes may reliably be based on a few testing environments. The high broad sense heritability of these traits, which may be inferred by the much larger extent of purely genotypic compared with GE effects, facilitates the genetic progress for specific quality features, such as oil content or PUFA amount. A more extensive study performed in Australia on cultivars of *L. angustifolius* produced similar indications, as the genotypic variance component exceeded the sum of the GE interaction variance components, relative to genotype × location, genotype × year and genotype × location × year interaction effects (Cowling & Tarr, 2004).

Grain yield was subjected to GE interaction effects of similar size to genotypic effects (Table 4). This result is largely due to the different and partly incompatible agronomic traits associated with adaptation to subcontinental- and Mediterranean-climate environments. In particular, late-flowering materials (such as the cultivars Lucille, Ludet and Luxe) tend to escape late frosts and, thereby, be more adapted to cold-prone environments (Annicchiarico & Iannucci, 2007). However, the later crop maturity relative to early-flowering types (such as the Spanish or Italian genotypes), makes them more susceptible to the terminal drought stress that is a feature of Mediterranean environments.

The genetic variation across environments for oil content was fairly modest, the only two cultivars significantly different were Ecotype and Multitalia (Table 5). On the

Table 3
Location, sowing time, cropping year, and mean values of grain yield (t/ha), seed oil content (weight % on lupin flour), TSFA, MUFA, PUFA (expressed as % of total FA), ω−3/ω−6 FA ratio (α-linolenic/linoleic acid) across three cropping environments for six cultivars of *Lupinus albus*

Location	Sowing time	Cropping year	Grain yield (t/ha)	Oil content (%)	TSFA (%)	MUFA (%)	PUFA (%)	ω−3/ω−6 FA ratio
Lodi	Early autumn	2002–2003	3.49a	8.90b	26.18b	56.54a	17.28b	0.629a
Lodi	Late autumn	2002–2003	3.61a	9.19b	26.54ab	56.12a	17.34b	0.640a
Sanluri	Mid autumn	2003–2004	3.54a	9.96a	26.86a	53.39b	19.75a	0.644a
			0.083	0.25	0.15	0.15	0.10	0.008

Means followed by different letters differ at $p < 0.05$, according to Newman and Keuls' test.

Table 4
Estimated genotypic and genotype-environmental components of variance for grain yield, seed oil content weight % on lupin flour), TSFA, MUFA, PUFA (expressed as % of total FA), ω−3/ω−6 FA ratio (α-linolenic/linoleic acid) across three cropping environments for six cultivars of *Lupinus albus*

Component of variance	Degrees of freedom	Grain yield (t/ha)	Oil content (%)	TSFA (%)	MUFA (%)	PUFA (%)	ω−3/ω−6 FA ratio	Degrees of freedom
Genotype	5	0.310**	0.294*	1.782**	12.390**	11.001**	0.00856**	5
Genotype × Environment (GE)	10	0.339**	0.319NS	0.366**	0.967**	1.246**	0.00101*	10
Pooled error	18	0.125	0.781	0.264	0.255	0.126	0.00082	30

*,**, NS = significant at $p < 0.01$, $p < 0.05$ and not significant, respectively. Environmental effects, considered as fixed, accounted for two degrees of freedom.

Table 5
Name, and mean values of grain yield (t/ha), seed oil content (weight % on lupin flour), TSFA, MUFA, PUFA (expressed as % of total FA), ω−3/ω−6 FA ratio (α-linolenic/linoleic acid) across three cropping environments for six cultivars of *Lupinus albus*

Cultivar	Grain yield (t/ha)	Oil content (%)	TSFA (%)	MUFA (%)	PUFA (%)	ω−3/ω−6 FA ratio
AB47	3.07b	9.40ab	25.26cd	59.16a	15.58e	0.598c
Ecotype	4.11a	10.55a	27.09b	58.04b	14.87f	0.687b
Lucille	3.09b	9.08ab	27.97a	52.08d	19.95b	0.592c
Ludet	3.18b	9.26ab	25.80c	50.33e	23.87a	0.655b
Luxe	3.45b	9.23ab	28.03a	55.04c	16.94d	0.512d
Multitalia	4.40a	8.59b	24.99d	57.46b	17.54c	0.783a
Standard error	0.12	0.36	0.21	0.21	0.14	0.012

Means followed by different letters differ at $p < 0.05$, according to Newman and Keuls' test.

other hand, all the ω−3/ω−6 FA ratios were significantly different and therefore were strongly related to genotype. Genetic characteristics also affected the FA content, whereas the oil content and grain yield seemed only marginally influenced (Table 5).

Higher-yielding material tended to higher ω−3/ω−6 FA ratio, whereas no correlation was observed between grain yield and oil content or other variables related to FA composition (Table 6). The latter variables were unrelated also to oil content (Table 6). These indications, which are encouraging for the simultaneous selection for grain yield, oil content and good FA composition of oil, are just preliminary, owing to the limited sample of tested genotypes. The fairly wide genetic variation and the modest GE interaction, and their effects on FA composition, reinforce the interest in selecting for grain nutritional quality and exploiting the genetic differences already available.

Table 6
Coefficients of correlation among seed oil content, TSFA, MUFA, PUFA, ω−3/ω−6 FA ratio (α-Linolenic/linoleic acid) and grain yield of six *Lupinus albus* cultivars

Variable	Grain yield	Oil content
Grain yield	–	0.09 NS
TSFA	−0.24	0.29 NS
MUFA	0.48	0.28 NS
PUFA	−0.42	−0.41 NS
ω−3/ω−6 FA ratio	0.74 +	−0.09 NS

+, NS = significant at $p < 0.10$ and not significant, respectively. Cultivar values averaged across three cropping environments.

3.3. Nutritional remarks

This work indicates that a drawback of white lupin is the presence of small amounts of erucic acid (Codex Alimentarius, 2001). Using the same definition applied to rapeseed oil, a few genotypes, i.e., Lucille and Luxe, may be classified as "low-erucic acid", since erucic acid is under the threshold of 2% in all environments. This negative feature, however, appears to be marginal since lupin oil is not a commercial product; on the other hand, the selection of erucic-free or low-erucic acid genotypes would be certainly desirable.

A major objective of this study was to investigate how the genotype and the environment influence some positive nutritional features of lupin seed, in particular

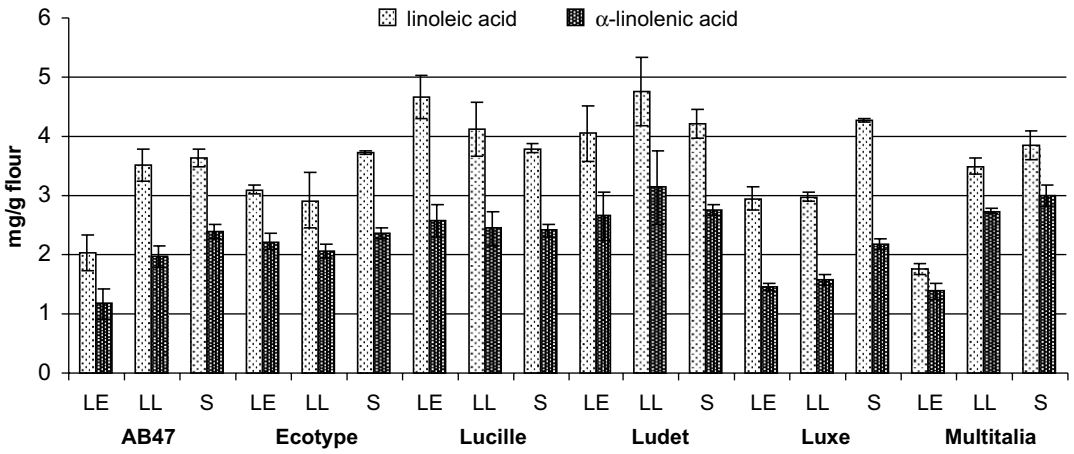

Fig. 1. Content of linoleic acid and α-linolenic acid expressed in mg/g lupin flour of six cultivars of *L. albus* in three cropping environments (LE = Lodi – Early autumn; LL = Lodi – Late autumn; S = Sanluri).

the α-linolenic acid and linoleic acid content, and the ω−3/ω−6 FA ratio.

Since up to now lupin oil is not a commercial product, lupin lipids can only be taken through foods containing lupin ingredients, mostly lupin flour. It becomes therefore relevant to know the absolute α-linolenic acid and linoleic acid content expressed as mg in g of lupin flour (Fig. 1).

The linoleic acid content of lupin flour ranged from 1.76 (Multitalia LE) to 4.76 mg/g (Ludet LL), whereas the α-linolenic acid content ranged from 1.17 (AB47 LE) to 3.14 mg/g (Ludet LL). Although these values are not very high, they may contribute to the total daily intake of these essential FAs (Boschin, D'Agostina, Annicchiarico, & Arnoldi, 2007).

The ω−3/ω−6 FA ratio ranged between 0.49 (Luxe LE) and 0.79 (Multitalia LE and LL). It is interesting to observe that Multitalia, showing the highest ω−3/ω−6 FA ratio, is also top-ranking for grain yield response across environments (Table 5).

The average ω−3/ω−6 FA ratio of lupin genotypes considered in this investigation was in all cases distinctly higher than that of most vegetable oils, e.g., canola oil (0.45), olive oil (0.13), soybean oil (0.15), and walnut oil (0.20) (Belitz & Grosch, 1999).

This high ω−3/ω−6 FA ratio is typical of *L. albus*, whereas other lupin crops, such as *L. angustifolius* and *L. luteus*, have lower ω−3/ω−6 FA ratios, due to a much higher linoleic acid content (34–48% and 45–48%, respectively) (Favini, Domenichini, & Fedeli, 1980).

A right balance between the daily intake of α-linolenic and linoleic acid is important, since linoleic acid is the precursor of arachidonic acid and its 2-series prostaglandins, which are considered as pro-inflammatory compounds (Simopoulos, 2003), whereas α-linolenic acid is the precursor of ω−3 long-chain polyunsaturated fatty acids (LC PUFA), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These latter PUFA are the intermediates of the biosynthesis of anti-inflammatory mediators, such as prostaglandins of 3-series and neuroprotectin D1 (Das, 2007). In these processes, α-linolenic acid and linoleic acid are competing for the same enzymes, desaturases and elongases, and an adequate α-linolenic acid intake is important, both to increase the production of ω−3 LC-PUFA and to reduce the adverse effects of arachidonic acid and its eicosanoids (Zhao et al., 2004).

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