GENOTYPIC AND GENOTYPE-ENVIRONMENTAL EFFECTS ON FATTY ACID COMPOSITION OF *LUPINUS ALBUS* L. SEED

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ABSTRACT

The influence of genetic variables (G) and genotype × environment (GE) interactions on grain yield and grain content of oil, total saturated fatty acid (TSFA), polyunsaturated FA (PUFA), monounsaturated FA (MUFA) and ω3/ω6 fatty acid (FA) ratio of the seeds of different cultivars of *Lupinus albus* L. sown in subcontinental and Mediterranean climate conditions in Italy was investigated. The fatty acid composition was determined by GC-FID analysis of the methyl esters obtained by trans-methylation of the extracted oil. The quantification was performed either with the normalisation or the internal standard method to obtain the absolute quantities of single FAs. The results were submitted to statistical evaluation, using the Newman and Keuls' test, to identify significant differences among samples, and by variance analysis, to highlight the possible variation between cultivars and environments and the occurrence of GE interaction. Genotypic features (G) were shown to be relatively constant across environments, since G × GE interactions are lower than G interactions for all variables. A relevant nutritional feature of lupin seed is the content of PUFAs, i.e. linoleic acid and α-linolenic acid, and, as a consequence, the favourable ω3/ω6 FA ratio, ranging from 0.49 to 0.79, much higher than that of most vegetable oils.

KEYWORDS

Lupinus albus, oil composition, α-linolenic, ω3/ω6 ratio, growing environment

INTRODUCTION

Several experimental, clinical, and epidemiological studies indicate that ω3 fatty acids (FA) are protective against cardiovascular diseases (Hu *et al.* 1999) and that they play a fundamental role during the foetal and infant development, in particular in the formation of the central nervous system and retina (Bowen *et al.* 2005). These considerations have prompted the Health Authorities of several countries to suggest specific recommended daily intakes (RDI) for ω3 FA (West Suitor *et al.* 2006). Beside the absolute ω3 FA intake, another important dietary parameter is the ω3/ω6 FA ratio, whose favourable values range from 0.5 to 1 (Simopoulos, 2003). *Lupinus albus* L. (white lupin) belongs to the small set of vegetables whose lipid composition appears to be very favourable in this respect (Bhardwaj *et al.* 2004; Boschin *et al.* 2008): its seed contains about 9-14% oil, being 50-60% oleic acid, 16-23% linoleic acid, and 8-9% α-linolenic acid. Thus, lupin-based foods offer opportunities for improving the ω3/ω6 FA ratio and to increase the daily intake of α-linolenic acid. The objective of this study was to evaluate the extent of genotypic and genotype x environment interaction on the FA composition of seeds of *L. albus* grown in subcontinental and Mediterranean-climate conditions.

MATERIALS AND METHODS

Six cultivars were investigated: AB47 (Spain); Lucille, Ludet, and Luxe (France), Multitalia and one ecotype collected in the Molise region (Italy). They were grown in Lodi (Lombardy) in 2002-2003 at two sowing times, early autumn (LO e.a.) and late autumn (LO e.a.) and in Sanluri (Sardinia) in 2003-2004 sown in mid autumn (SA m.a.). Lupin seeds were dehulled and ground to a fine flour which was extracted with hexane (300 mL) in a Soxhlet apparatus for 6 hours. Fatty acid methyl esters (FAMEs) were prepared by trans-methylation 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 num L248/44). The FAMEs were analysed with a DANI 86.10HT gas-chromatograph equipped with a Flame Ionization Detector (FID). The detector temperature was set at 250°C; a SP-2340 column (60 m x 0.25 mm i.d. x 0.2 μm film thickness) was used. Analyses were performed in split-less mode using a PTV injector (operative conditions 45°C for 30 sec, then heating to 250°C in 12 sec). Operating conditions were: carrier gas He (1.4 bar), auxiliary gas N_2 (0.8 bar). 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 the Star GC Workstation software (Varian, version 5.52). Peaks were identified

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by comparison of retention times with those of standard compounds. The percent fatty acid composition was calculated from the ratio of individual peak area to summation of all fatty acid areas after determination of the correction factors. The absolute quantities (expressed as mg/g flour) were obtained by using two internal standards (Boschin *et al.* 2007). ANOVA analysis and Newman and Keuls' test were performed for oil content, grain yield, percentage FA composition and ω3/ω6 ratio of all six cultivars.

RESULTS AND DISCUSSION

The per cent composition in each cultivar is presented in Fig. 1. On average, the FAs ranked in the following order of the abundance oleic acid (C18:1) > palmitic acid (C16:0) > linoleic acid (C18:2) > α-linolenic (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).

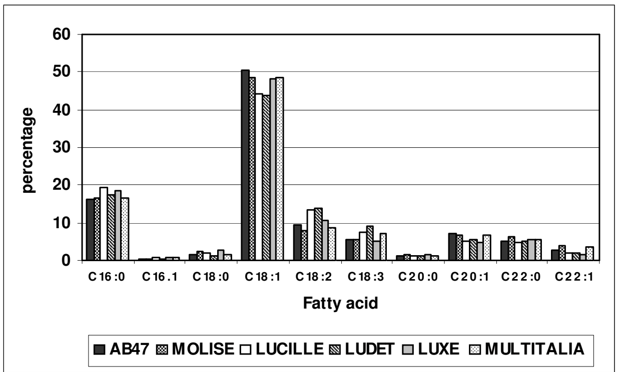

Fig. 1. Fatty acid percentage composition of six cultivars of *L. albus* sowed in Lodi early autumn in the cropping season 2002-2003.

NUTRITIONAL REMARKS

A relevant nutritional aspect regards the absolute content of linoleic acid and α-linolenic acid: the former is in the range 1.76-4.76 mg/g flour (7.8-15.8% total FA) and the latter in the range 1.17-3.14 mg/g flour (5.4-10.4% total FA). As a consequence, lupin oil has a very favourable ω3/ω6 ratio ranging between 0.49 (Luxe) and 0.79 (Multitalia). This latter, besides showing the highest ω3/ω6 ratio, gave also the highest grain yield (4.40 t/ha). The average ω3/ω6 ratio of the lupin varieties considered here is always distinctly higher than that of most vegetable oils, such as canola oil (0.45), olive oil (0.13), soybean oil (0.15) and walnut oil (0.20) (Belitz *et al.* 1999). This high value is typical of *L. albus*, whereas other lupin crops, i.e. *L. angustifolius* and *L. luteus*, have lower ω3/ω6 ratio, due to a much higher linoleic acid content (Boschin *et al.* 2007). A major drawback of *L. albus* oil is the presence of a small amount of erucic acid (Codex Alimentarius, 2001), although all our samples satisfy the maximum residue limit fixed by the Italian Health Authority of 5% in oils and oil derivatives for human nutrition and animal feeding (Law 659/1980). The selection of erucic-free or low-erucic acid genotypes would certainly be desirable.

EFFECTS OF GENOTYPE AND ENVIRONMENT

The comparison among mean values of environments indicates that the oil content and composition (TSFA, MUFA, PUFA) are influenced by location but not by sowing time. The ω3/ω6 ratio and the grain yield were not affected by location (Table 1).

Table 1. Mean values of grain yield, seed oil content, FA composition, and ω3/ω6 FA ratio of six *L. albus* cultivars grown in three environments.

	Early Lodi	Late Lodi	Mid San.	Pooled error
Grain yield (t/ha)	3.49a	3.61a	3.54a	0.083
Oil content (%)	8.90b	9.19b	9.96a	0.25
TSFA (%)	26.18b	26.54ab	26.86a	0.15
MUFA (%)	56.54a	56.12a	53.39b	0.15
PUFA (%)	17.28b	17.34b	19.75a	0.10
ω3/ω6 ratio	0.629a	0.640a	0.644a	0.008

Early Lodi: early autumn; Late Lodi: late autumn; Mid San : Sanluri mid autumn; means with different letters differ at $p < 0.05$ (Newman and Keuls' test).

Table 2. Estimated genotypic (G) and genotype-environmental (GE) components of variance for grain yield, seed oil content, FA composition, and ω3/ω6 FA ratio of six *L. albus* cultivars grown in three environments.

Comp. of var	G	GE	Error
D of Freed.	5	10	18
Grain yield (t/ha)	0.310**	0.339**	0.125
Oil content (%)	0.294*	0.319 NS	0.781
TSFA (%)	1.782**	0.366**	0.264
MUFA (%)	12.390**	0.967**	0.255
PUFA (%)	11.001**	1.246**	0.126
ω3/ω6 ratio	0.00856**	0.00101*	0.0008
df.	5	10	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

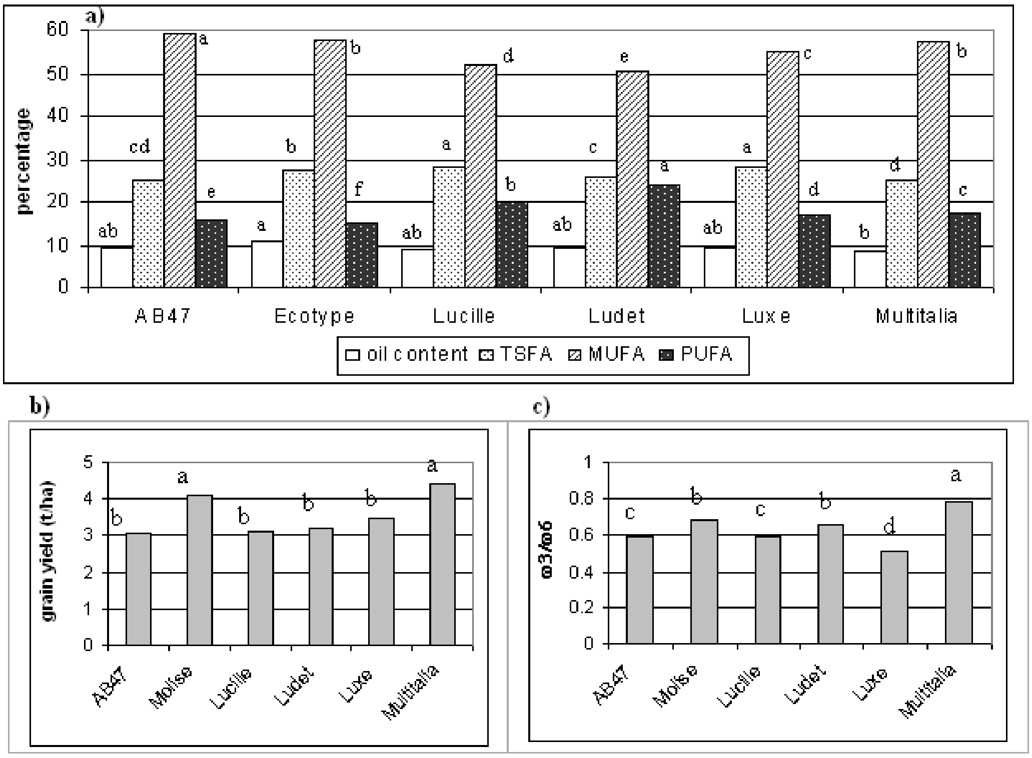

Fig. 2. a) mean values of oil content and FA composition; b) mean values of grain yield and c) mean values of ω3/ω6 FA ratio of six *L. albus* cultivars grown in three environments. Different letters indicate significant differences between means (Newman and Keuls' test).

Significant ($p < 0.05$) variation among cultivars was detected for all traits (Table 2). GE interaction effects were significant for all traits except oil content, but GE variance components were smaller than G effects for oil content and oil composition traits. The GE variance component for grain yield had similar magnitude as the G component. The genetic variation across environments for oil content is fairly modest (only Ecotype and Multitalia differ significantly from one another; Fig. 2a). The ω3/ω6 ratios are significantly different and strongly related to genotype; moreover higher-yielding material tends to higher ω3/ω6 ratio (Fig. 2b).

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LITERATURE CITED

Belitz, H.D. and W. Grosch. 1999. Legumes. In: Belitz, H.D. and W. Grosch. (Eds.) Food Chemistry. Berlin. Springer. pp. 694-715.

Bhardwaj, H.L., A.A. Hamama and E. van Santen. 2004. Fatty acids and oil content in white lupin seed as affected by production practices. J Am Oil Chem Soc 81: 1035.

Boschin, G., A. D'Agostina, P. Annicchiarico and A. Arnoldi. 2007. The fatty acid composition of the oil from *Lupinus albus* cv. Luxe as affected by environmental and agricultural factors. Eur Food Res Technol. 225: 769.

Boschin, G., A. D'Agostina, P. Annicchiarico and A. Arnoldi. 2008. Effect of genotype and environment on fatty acid composition of *Lupinus albus* L. seed. Food Chem 108: 600.

Bowen, R.A.R. and M.T. Clandinin. 2005. Maternal dietary 22:6n-3 is more effective than 18:3n-3 in increasing content in phospholipids of glial cells from neonatal rat brain. British Journal of Nutrition 93: 601.

Codex Alimentarius. 2001. Codex standard for named vegetable oils CX-STAN 210-1999, vol. 8.

Hu, F.B., M.J. Stampfer, J.E. Manson, E.B. Rimm, A. Wolk, G.A. Colditz, C.H. Hennekens and W.C. Willett. 1999. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. Am J Clin Nutr 69: 890.

Simopoulos, A.P. 2003. Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. World Rev Nutr Diet 92: 1.

West Suitor, C. and L.D. Meyers. 2006. Dietary Reference intakes research synthesis workshop summary. Institute of Medicine of the National Academies. Washington D.C. The National Academies Press.