

Lupin Seed for the Crop-Livestock Food Chain

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Abstract

The ban on animal protein supplements in animal nutrition and, particularly, the increase in the demand for alternative protein sources to replace soybean meal in organic farming has stimulated research on GM-free feeds and renewed interest in grain legumes. Of these lupin appears to be one of the more interesting and promising crops for ruminant diets, due to its key role in low-input cropping systems and its high protein content. The aim of this study was to evaluate the productive and nutritional characteristics of several sweet varieties of lupin seeds cultivated in the Mediterranean region. The trial was carried out during the years 2007/08 on a volcanic soil in East Sicily, employing 8 sweet cultivars belonging to *Lupinus albus*, *L. angustifolius* and *L. luteus* species. Yield and its components (pod plant⁻¹, seed pod⁻¹, 1000 seed weight) were evaluated. Crude protein, fibre fractions and alkaloid content were also determined. *L. albus* exhibited the highest yields (2.2 t ha⁻¹), with an intermediate value of pod x plant (15.4). *L. luteus* yielded 1.5 t ha⁻¹ and *L. angustifolius* 0.5 t ha⁻¹. *L. luteus* showed higher levels of crude protein (34.2%) although the greatest protein yield per hectare was recorded in *L. albus* (0.7 t ha⁻¹). *L. angustifolius* showed the highest mean values of NDF (28.1%) and ADF (25%), whereas *L. luteus* the lowest mean values of ADL (1.75%). Six alkaloids were detected and quantified, among which, the Iso-lupanine always was the most abundant (1.5 mg 100g⁻¹, on average). Lower concentrations of quinolizidine were observed in *L. luteus* (0.9 – 1.4 mg 100g⁻¹) as compared with *L. angustifolius* (1.5 – 5.5 mg 100g⁻¹) and *L. albus* (3.8 – 7.5 mg 100g⁻¹). Alkaloid content was always lower than the safe limit of toxicity (< 0.20‰) indicated for human and animal consumption.

Key-words: Lupins, yield, protein, fibre fractions, alkaloids.

Introduction

The ban on animal protein supplements (European Commission Decisions No. 98/272/CE and 2000/374/CE) has increased the need to research different feeding systems able to satisfy the dietary requirements of livestock whilst safeguarding the quality of animal production (Vicenti et al., 2009). This has induced farmers to use soybeans which have a particularly high protein content and are therefore one of the most used and valuable components in ruminant and non ruminant feed formulation (Woodworth et al., 2001; Graham et al., 2002;

Kerley and Allee, 2003). However, the ban on use the soy which comes from genetically modified crops in organic farming (Reg. CE 1804/99), has greatly stimulated research on GM-free feeds, which are able to satisfy protein requirements in animal nutrition, and renewed the interest in low input grain legumes, especially for eco-sustainable crop-livestock systems. Moreover grain legumes are strategically important not only in decreasing the marked deficit of high-protein feedstuff (Annicchiarico and Carroni, 2009), but also in increasing the sustainability of crop-livestock systems through the safeguarding of soil fertility, the reduction

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of greenhouse gas emission, and the reduction of nitrogen fertilizers use, etc. (Carrouée et al., 2003; Jensen and Hauggaard-Nielsen, 2003). Last but not least, the increase of domestic production of GMO-free plant proteins represents a way to reduce Italian dependence on feedstuff imports. Among legumes, lupin appears to be an interesting and promising crop. Since it has a winter cycle, it may play a key role in crop rotation (Postiglione et al., 1995) especially as it has a high protein content compared to the other winter legumes (Hill, 1977; Sujak et al., 2006). These characteristics increased its use in ruminant diet, since lupin can replace soybean meal even for high-producing lactating cows (van Bernelveld, 1999; Froidmont and Bartiaux-Thill, 2004). In addition, lupin has been studied as a human foodstuff (Reinhard et al., 2006) because of its potential to be used as an ingredient in functional and healthy food products due to its hypocholesterolaemic potential (El-Adawy et al., 2001; Duranti et al., 2008). However, spontaneous lupins contain many antinutritional components such as saponins, tannins and flavonoids (Sirtori et al., 2004). It also has bitter principles. Among these, more than 150 alkaloids of the quinolizidine, piperidine and indole group are known to appear at concentrations up to 6%, conferring resistance to pathogens and grazing (Wink, 1988). For this reason, plant breeders have developed so called “sweet lupins” with low alkaloid content (below 0.05%), for human and animal diet (Reinhard et al., 2006).

Lupin is, widely cultivated in Central and Eastern Europe and in Australia, but almost absent in the Mediterranean basin (Faostat, 2009) due, above all, to its restricted soil adaptability (acid or sub-acid soil). Anyway, in many Mediterranean environments this species could

represent an alternative crop, especially in agricultural systems oriented toward organic productions with high traceability (Abbate, 2001; Arnoldi, 2004; Scarafoni et al., 2007).

Nowadays, the economic sustainability of this legume depends on its ability to maintain or reduce its alkaloid content and to increase its grain yield (Dronne, 2003). With this in mind, the aim of this study was to evaluate the productive and nutritional characteristics of eight sweet varieties of lupins (*L. albus*, *L. angustifolius* and *L. luteus*) cultivated in the Mediterranean environment. Particular attention was paid to the detection and quantification of the quinolizidine alkaloids, in order to promote the use of this crop within the Mediterranean livestock food chain.

Materials and methods

The trial was carried out in 2007/08 on a volcanic soil in East Sicily, Italy (Acireale, CT, 16 m a.s.l.) whose characteristics are reported in Table 1, using 8 sweet cultivars belonging to *Lupinus albus*, *L. angustifolius* and *L. luteus* (table 2). Plots of 6 m² (3 x 2 m) 3 times replicated in a randomized block design were adopted. Sowing was executed on 27 December 2007

Table 1. Main characteristics of soil.

Sand (%)	84.80
Silt (%)	9.90
Clay (%)	5.30
pH	8.5
N total (‰)	1.8
P ₂ O ₅ total (ppm)	167.72
K ₂ O exchange. (ppm)	788.31
Conductivity μS/cm 25 °C	770
Organic carbon (%)	3.11
Total limestone (g/kg)	30.0

Table 2. Provenance of the different varieties of lupin.

Latin name	Common name	Cultivars	Provenience
<i>L. albus</i>	White lupin	Luxor	Australia
<i>L. albus</i>	White lupin	Rosetta	Australia
<i>L. angustifolius</i>	Narrow-leafed lupin	Wonga	Australia
<i>L. angustifolius</i>	Narrow-leafed lupin	Jindalee	Australia
<i>L. angustifolius</i>	Narrow-leafed lupin	Sonet	Poland
<i>L. luteus</i>	Yellow lupin	Dukat	Poland
<i>L. luteus</i>	Yellow lupin	Mister	Poland
<i>L. luteus</i>	Yellow lupin	Taper	Poland

with a plant density of 80 plant m^{-2} on a ploughed and fertilized soil with 120 kg ha^{-1} of P_2O_5 . Seeds were harvested on 28 May 2008 for all varieties. Plant height and yield components (pod $plant^{-1}$, seed \times pod $^{-1}$ and 1000 seed weight) were evaluated using ten plants for each plot, while total yield was determined on the two central rows of the plot. The date of the main growth stages was also recorded according to the scale proposed by Dracup and Kirby (1996).

Crude protein content and fibre fraction (Neutral Detergent Fibre, Acid Detergent Fibre, Acid Detergent Lignin) percentages were determined using AOAC official methods of analysis (2005).

Alkaloids were extracted as described by Muzquiz et al. (1994). As control for the alkaloid content, we used *Lupinus albus* L. var. Multitalia, an Italian old sweet variety. Two grams of defatted bean flour were homogenized with an Ultraturrax homogeniser for 1 min in 20 mL of trichloroacetic acid (5%) and centrifuged at 700 $g\ min^{-1}$ for 5 min. The extraction was repeated twice and the supernatants decanted while the precipitate was discarded (Oboh et al., 1998). The alkaloids in the sample extract were extracted with dichloromethane and analysed by HRGC-MS (Nossack et al., 2000) performed using a 5973 inert mass selective detector (Agilent technologies USA), coupled to a 6890N GC (Agilent technologies USA). The capillary column used was a 95% methyl, 5% phenylpolysiloxane, HP-5 (Agilent J&W GC Columns) of 30 m length, 0.25 mm i.d., 0.25 μm film thickness. Samples were injected using the split mode (1:10) and the injection volume was 1 μl , the injection temperature was 240 $^{\circ}C$, with HRGC-MS interface temperature both at 250 $^{\circ}C$. The acquisition was from m/z 50 to 300 and the source operated in EI mode at 70 eV. The column temperature was programmed to rise from 150 $^{\circ}C$, at 5 $^{\circ}C/min$, to 235 $^{\circ}C$ (held during 15 min). Helium was used as carrier gas, at the average linear velocity of 35cm/sec and at a flow of 1mL/min.

The alkaloids (Sparteine, Angustifoline, Lupanine, a-Iso-lupanine, 13 a-Hydroxylupanine and 11,12-Deidrelupanine) quantification was performed in full-scan mode by internal standard method using caffeine as an analytical standard. *Lupinus* extracts were diluted to 1.0 mL in methanol and 0.3 mL of a caffeine stan-

dard solution (1 mg mL^{-1}) was added to the sample, which was analysed by GC-MS (Nossack et al., 2000). In the standard solutions the Limit Of Quantification (L.O.Q.; $S/N > 7$) was of 0.02 mg 100 g^{-1} for Sparteine and 0.04 mg 100 g^{-1} for all other alkaloids.

All data were submitted to the analysis of variance. When ANOVA indicated a significant treatment effect, Tukey's HSD test was performed to determine which treatment means differed significantly. Moreover, Principal Component Analysis (PCA) was performed on productive and nutritional parameters (Statistix Software). Principal components with eigenvalues > 1.0 were selected and correlation values > 0.6 were considered as relevant for the PCA.

Results and discussion

Biological cycle lasted 153 days for all the varieties studied (Fig. 1). Differences arose when the single stages were taken into account. Early flowering was recorded in Luxor of *L. albus*, Sonet of *L. angustifolius* and Taper of *L. luteus* (63-66 days after emergence), while late flowering (74 days after emergence) was recorded on Jindalee of *L. angustifolius* and Dukat of *L. luteus*.

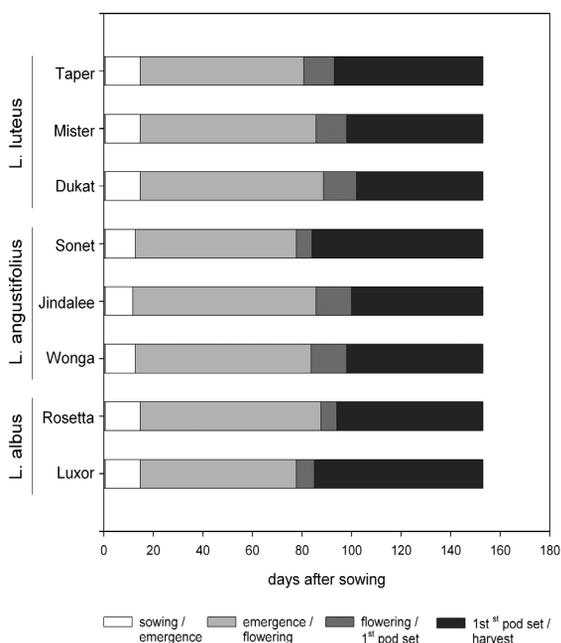


Figure 1. Duration of the main phenological stages of the studied varieties.

Table 3. Main agronomic traits of the studied varieties of lupin.

Species	Varieties	Height	Pod plant ⁻¹	Seed pod ⁻¹	1000 seed weight (g)	Yield (t ha ⁻¹)
<i>L. albus</i>	Luxor	93.9 a	16.0 b	3.7	272.7 a	2.4 a
<i>L. albus</i>	Rosetta	104.5 a	14.8 b	3.7	266.7 a	2.0 a
<i>L. angustifolius</i>	Wonga	73.5 bc	23.4 ab	3.8	101.0 b	0.5 c
<i>L. angustifolius</i>	Jindalee	80.1 b	23.4 ab	3.5	108.0 b	0.5 c
<i>L. angustifolius</i>	Sonet	58.9 d	33.5 a	3.7	125.3 b	1.5 b
<i>L. luteus</i>	Dukat	71.8 bcd	22.7 ab	3.7	111.0 b	1.4 b
<i>L. luteus</i>	Mister	79.5 b	19.9 ab	3.6	117.7 b	1.6 b
<i>L. luteus</i>	Taper	63.9 cd	13.5 b	3.5	104.3 b	0.8 c

Mean values with different letters are significantly different for $P < 0.05$.

Significant differences emerged among the species and varieties in relation to morphological and productive parameters. *L. albus* showed the highest values in terms of plant height (99.2 cm on average) and plant yield (2.2 t ha⁻¹ on average) and also the lowest value of pod plant⁻¹ (Tab. 3). Dukat and Mister of *L. luteus* and Sonet of *L. angustifolius* yielded on average 1.5 t ha⁻¹, whereas the remaining cultivars never exceeded 0.8 t ha⁻¹. The number of seeds pod⁻¹ was quite similar in all the studied varieties (3.7 on average). No correlation was found between flowering date and yield.

Yield values were in agreement with that obtained on *L. albus* and *L. angustifolius* by several authors (Postiglione et al., 1993; Pritoni, 1993; Annichiarico et al., 2003) in North and central Italy, even though a pick of production up to 4.0 t ha⁻¹ were recorded. Lopez-Bellido et al., (2000), in a pluriannual trial carried out in

southern Spain with 60 plant m⁻², found on *L. albus* comparable yield (2.5 t ha⁻¹) and number of seeds x pod (3.4), but with a greater 1000 seed weight (480 g) and lower number of pods x plant (5.2). Higher yield (2.9 t ha⁻¹) was obtained in *L. angustifolius* by Bohm et al. (2008) in North Germany.

Among the different species of lupins (Fig. 2), significantly higher levels of crude protein were observed in *L. luteus* (34.2%, on average). According to Gdala et al., (1996), significantly lower values of crude protein were obtained in the *L. angustifolius* (29.0%, on average). Generally, the crude protein content of the studied cultivars showed lower values compared to those reported in other studies (Bohm et al., 2008; Sauvant et al., 2002; Sujak et al. 2006). Taking into account the total protein yield, *L. albus*, even if characterised by a lower protein percentage compared to *L. luteus*, showed the highest protein production reaching values of 0.7 t ha⁻¹, due to its superior productive performance. This value is slightly higher compared to what obtained by Postiglione et al. (1993) in Italy, but lower as compared to Bohm et al. (2008) in Germany. The lower productive and qualitative traits recorded in our trial compared to what was obtained in central Europe may be due to the terminal drought imposed by the Mediterranean climate; including water shortages in spring and during the grain formation period which can play a prominent role in determining yield and quality (Dracup et al., 1998; Lopez-Bellido et al., 2000).

As regards the fibre fractions (Fig. 3), *L. angustifolius* showed higher mean values of NDF (28.1%, on average) and ADF (25%, on average), specifically in Wonga (NDF = 31.5%; ADF = 27.7%), than those observed in *L. luteus*

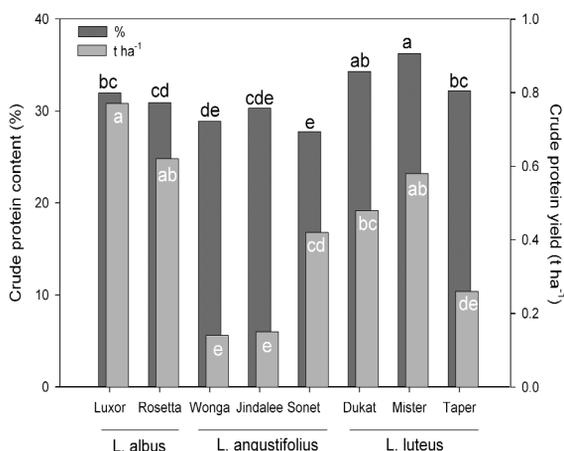


Figure 2. Crude protein content (%) and crude protein yield (t ha⁻¹) of the studied varieties. Different letters indicate significant value for $P < 0.05$.

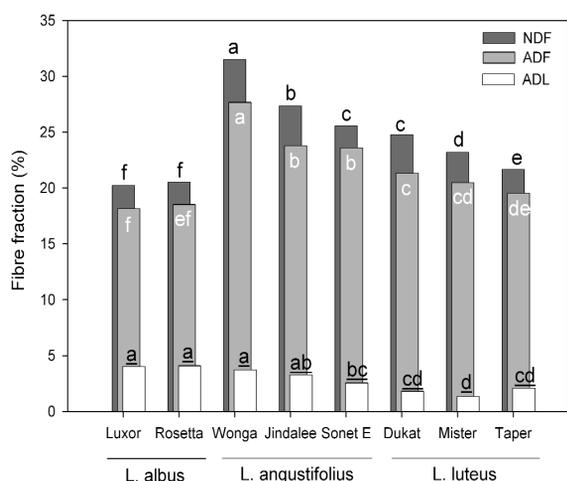


Figure 3. NDF, ADF and ADL fibre fractions (%) of the studied varieties. Different letters indicate significant value for $P < 0.05$.

(NDF = 23.2%; ADF = 20.4%, on average) and *L. albus* (NDF = 20.37%; ADF = 18.33%, on average). Both cultivars of *L. albus*, Wonga and Jindalee of *L. angustifolius* showed significantly higher mean values of ADL (3.9%, on average), when compared to Sonet of *L. angustifolius* (2.9%, on average) and *L. luteus* (1.8%, on average). The results concerning the fibre fractions of *L. albus* were similar to those reported by NRC (1998) for sweet white lupin, but higher than data reported by Singh et al. (1995) and by Sauvant et al. (2002). Moreover, data regarding these fractions in *L. angustifolius* were higher than those reported by Sauvant et al. (2002), and those regarding *L. luteus* were similar to those reported by Gdala et al. (1996).

As reported in Table 4, six alkaloids were detected and quantified: Sparteine, Angustifoline, Lupanine, α -Iso-lupanine, 13 α - Hydroxylupanine and 11,12-Deidrelupanine. The analysis of the results showed that the studied samples had much lower alkaloid content than that reported by Gdala et al. (1996) and Reinhard et al. (2006); these values are much lower than the safe limit of toxicity ($< 0.20\%$), indicated for human and animal consumption by the health authorities of Great Britain, France and Australia (Boschin et al., 2008). Very low concentrations of quinolizidine alkaloids were observed in all the varieties ($3.0 \text{ mg } 100\text{g}^{-1}$) compared to the control Multitalia ($166.4 \text{ mg } 100\text{g}^{-1}$). The Lupanine was the most represented alkaloid ($1.5 \text{ mg } 100\text{g}^{-1}$, on average of the tested varieties), whereas, Iso-lupanine was the least represented alkaloid ($0.22 \text{ mg } 100\text{g}^{-1}$, on average of the tested varieties) (table 4); these results are in agreement with the observations of Muzquiz et al. (1994) in the alkaloid profile of *L. albus* and with those of Boschin et al. (2008) in nine alkaloid-poor varieties of *L. albus* and *L. angustifolius* grown in two climatically contrasting Italian sites. The chromatographic analysis allowed to identify the presence of Iso-lupanine only in *L. albus*; in *L. angustifolius* and *L. luteus* the Iso-lupanine content was below the instrumental limit of quantification ($0.04 \text{ mg } 100 \text{ g}^{-1}$) (Tab. 4). Regardless the reported lupanine content was too low to play any important biological activity within human and animal metabolisms (Keeler, 1989). The Angustifoline, rather uncommon in *L. albus*, was identified in both cultivars Luxor and Rosetta as reported by

Table 4. Composition of quinolizidine alkaloids ($\text{mg} \cdot 100\text{g}^{-1}$) in the studied varieties of lupin.

Species	Varieties	1	2	3	4	5	6	7
<i>L. albus</i>	Multitalia	0.21 c	3.35 a	0.91 a	149.9 a	9.91 a	2.12 a	166.4 a
<i>L. albus</i>	Luxor	ND	0.63 bc	0.25 b	3.3 b	1.34 bc	2.00 a	7.5 b
<i>L. albus</i>	Rosetta	ND	0.49 bc	0.18 b	1.8 b	0.38 c	0.92 b	3.8 b
<i>L. angustifolius</i>	Wonga	ND	0.23 c	ND	0.74 b	0.56 c	ND	1.5 b
<i>L. angustifolius</i>	Jindalee	ND	1.12 b	ND	1.79 b	2.6 b	ND	5.5 b
<i>L. angustifolius</i>	Sonet	0.059 d	ND	ND	1.19 b	0.87 bc	ND	2.1 b
<i>L. luteus</i>	Dukat	0.97 ab	ND	ND	ND	ND	ND	1.0 b
<i>L. luteus</i>	Mister	0.71 bc	ND	ND	0.05 b	0.11 c	ND	0.9 b
<i>L. luteus</i>	Taper	1.36 a	ND	ND	ND	ND	ND	1.4 b

(1) Sparteine, (2) Angustifoline, (3) α -Isolupanine, (4) Lupanine, (5) 13 α - Hydroxylupanine, (6) 11,12-Deidrelupanine, (7) Sum of alkaloids.

Mean values with different letters are significantly different for $P < 0.05$.

ND = value below the L.O.Q.

Table 5. Correlation matrix.

Variable	PC 1	PC 2	PC 3
Pods plant ⁻¹	-0.375	0.626	0.353
seeds pod ⁻¹	0.185	0.207	0.899
1000 seed weight	0.934	-0.252	0.215
seed yield	0.667	-0.510	0.407
protein	-0.163	-0.778	-0.203
NDF	-0.512	0.796	0.110
ADF	-0.508	0.824	0.158
ADL	0.779	0.471	0.091
alk. 1	-0.594	-0.700	-0.279
alk. 2	0.613	0.506	-0.523
alk. 3	0.955	-0.233	0.147
alk. 4	0.940	0.298	-0.044
alk. 5	0.423	0.701	-0.501
alk. 6	0.935	-0.208	0.090
alk. 7	0.896	0.243	-0.323

Wink et al. (1995) and in *L. angustifolius* (Tab. 4). The 11,12-Deidrelupanine was quantified only in the *L. albus*, whereas, in the other species was below the instrumental limit of quantification (0.04 mg 100 g⁻¹).

Even if a pair comparison among tested species and *Multitalia*, used as control, was not possible since this last crop was not cultivated in the same environment, on the whole, the quinolizidine alkaloids of the 8 sweet cultivars belonging to *Lupinus albus*, *L. angustifolius* and *L. luteus* showed considerably lower values. A

large part of this difference may be ascribed to the remarkable breeding improvement of the new varieties.

To identify patterns in our data set and to express the data in such a way as to highlight its similarities and differences, we applied the multivariate statistical technique of the Principal Components Analysis. PCA showed three principal components accounting, as a whole, for 89.2% of the total data variability. The first one, which explained 46.9% of the variation, was associated mainly with productive traits (1000 seed weight and yield), ADL fraction and with the majority part of the alkaloids detected; component two, responsible for 29.2% of the variation, was mainly represented by protein, NDF and ADF; component three contributed for 13.1% of the variation and it is exclusively associated with seed pod⁻¹ character (Tab. 5). The graphic representation of the relationships among varieties and parameters shows an evident distinction among the species (Fig. 4). *L. luteus* clearly stand out for high negative PC1 and PC2 values, corresponding mainly to high protein content and low alkaloid content, except for alkaloid 1. *L. angustifolius* showed high PC2 values, mainly associated with low protein content and high NDF and ADF fractions. *L. albus* was characterized by a high value of PC1 linked

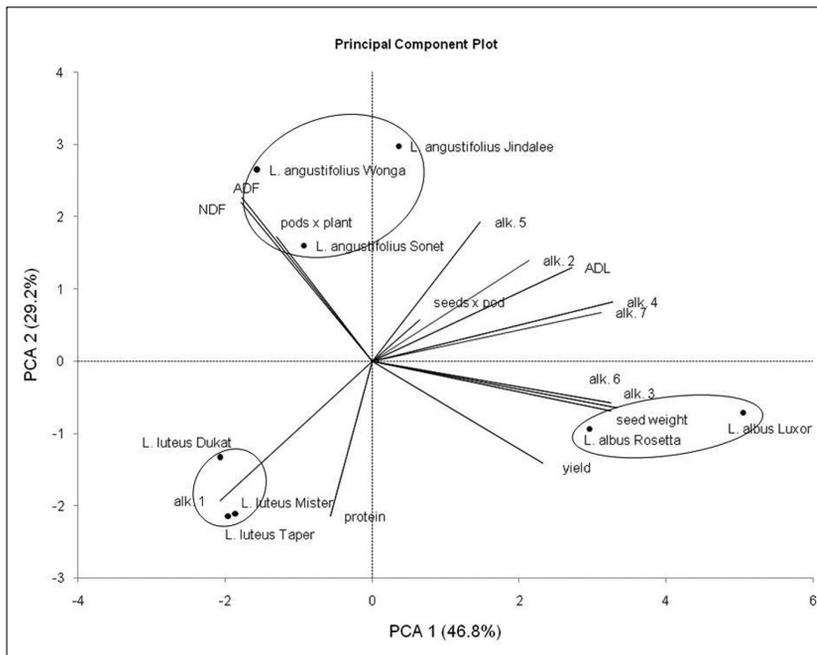


Figure 4. Scatter plot of the studied varieties based on PC1 and PC2. The rays connecting the traits to the biplot origin are referred to as trait vectors. The vector length of a trait measures the magnitude of its effect (positive or negative).

with high seed production and 1000 seed weight as well as the presence of Isolupanine and Deidrelupanine alkaloid.

Conclusions

Both *L. albus* varieties (Luxor and Rosetta) emerged with the highest grain and protein yields. Mister and Dukat of *L. luteus* were interesting not only for their high protein content, but also for their low lignin and total alkaloid content.

On the whole, our data confirms the possibility of using lupin as an alternative protein source in the Mediterranean crop-livestock food chain, in particular in organic husbandry where the feed represents a restraining factor due to the ban of using GM feeds.

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