# Variation of Alkaloid Components of Lupin Seeds in 49 Genotypes of *Lupinus albus* L. from Different Countries and Locations

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Forty-nine genotypes of *Lupinus albus* seeds from different countries have been analyzed for their alkaloid content by thin-layer chromatography and gas chromatography–mass spectrometry. Twenty samples were sweet, while 29 were bitter, and the taste was positively correlated with the alkaloid content. The composition of the alkaloids showed that lupanine was the main alkaloid present, and variable amounts of albine, α-isolupanine, multiflorine, and 13-hydroxylupanine were also detected in the seeds.

**Keywords:** *Lupinus albus, alkaloid, albine, α-isolupanine, lupanine, multiflorine, 13-hydroxylupanine*

## INTRODUCTION

Quinolizidine alkaloids constitute a characteristic class of natural products in legumes, especially of the genera *Lupinus, Baptisia, Thermopsis, Genista, Cytisus, Chamaecytisus, Laburnum,* and *Sophora.* In general, all parts of a lupin plant contain alkaloids, although their biosynthesis is restricted to the green tissues, especially the leaves. The alkaloids occur in abundant amounts in the seeds, which are also a good source for protein and lipid.

In terms of chemical ecology, alkaloids confer important resistance to pathogens and herbivores and, therefore, are desirable (Wink, 1988). On the other hand, lupin alkaloids are bitter and toxic, which is undesirable if animal or human consumption is of primary concern (Culvenor and Petterson, 1986). The traditional approach is to breed for sweet lupins devoid of alkaloids, which has been successful. At present, low-alkaloid *Lupin* varieties are available from *L. albus, L. angustifolius, L. luteus, L. mutabilis,* and *L. polyphyllus.* The sweet varieties are useful for animal consumption (and will therefore be exploited in the future) but have the ecological disadvantage that they can only be cultivated successfully if predators are kept away by fences and pesticides. However, pesticide pollution of soil and water has caused severe environmental problems (Wink, 1990).

Since the quinolizidine alkaloids occur in complex mixtures, only chromatographic methods capable of high resolution are adequate for complete analysis (Wink, 1991; Harris and Wilson, 1988; Muzquiz et al., 1993). Capillary gas–liquid chromatography (GLC) provided separation of nearly all of the alkaloids present. GLC was used in combination with mass spectrometry for identification, while a nitrogen-specific detector was used to enhance sensitivity. A total of 49 genotypes of *L. albus* from different countries and locations were analyzed to investigate differences in total and individual alkaloids.

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## EXPERIMENTAL PROCEDURES

**Materials.** The seed materials were part of the European Communities ECLAIR Programme (European Collaborative Linkage of Agriculture and Industry through Research) (No. AGRE-0048) for genetic studies.

Forty-nine genotypes of *L. albus* seed samples were selected for examination on the basis of their range in agronomic character and origin.

*Bitter Ecotypes*: Turkish ecotypes, TR19, LA430; Greek ecotypes, GR40, GR28, GR45, GR42, GR56, GR55; Italian ecotypes, ITA2, LA240, ITA37, LA239, LA109, ITA56, ITA29, ITA18; Sudanese ecotype, LA338; Azorean ecotypes, AC127, AC21, AC24; Spanish ecotype, E31; Portuguese ecotype, LA549, Moroccan ecotype, LA147; Ethiopian ecotype, LA402; Egyptian ecotypes, LA397, LA378, LA390.

*Bitter Lines*: C199, LA300.

*Sweet Lines and Cultivars*: Lublanc (early spring cultivar); LA123 (spring cultivar, Multolupa); Lucky (late cultivar); Ares (semi-early spring cultivar); Lutop (early spring cultivar); Lucrop (semi-early spring cultivar); Adam (early winter cultivar); Lunoble (late winter cultivar); Kalina (very early spring cultivar); XA100; CH304.70 (determinate winter line) (dwarf winter line); C168 (semi-late winter line); CH46 (winter line); CH304.73 (determinate winter line); Lublanc P1 (spring cultivar); Lublanc P2 (spring cultivar); Lublanc P3 (spring cultivar); Lunoble H1 (winter cultivar); Lunoble H2 (winter cultivar); Lunoble H3 (winter cultivar).

Every ecotype or variety was ground to pass through a 100-mesh sieve (Tecator, Cyclotec 1093), and two samples were taken for each analysis. The chromatographic and spectrometric determinations were done three times on every sample. The chemicals, all of analytical or reagent grade, were supplied by Prolabo, Merck, or Fluka.

**Methods.** The extraction of the milled seed was as described in Muzquiz et al. (1993). Finely ground lupin seed (0.5 g) was homogenized in 5% trichloroacetic acid (3 × 5 mL) with an Ultra-Turrax and centrifugated at 700*g* for 5 min. After centrifugation, 1 mL of 10 M NaOH was added to the supernatant. The alkaloids were then extracted with dichloromethane (3 × 5 mL). The dichloromethane extract was evaporated to dryness, and the alkaloids were dissolved in 1 mL of methanol. A 0.5-mL aliquot of the extract was added to 0.5 mL of a solution of codeine in methanol (2 mg $mL^{-1}$), which was the internal standard. Each of the bitter samples was diluted 10-fold.

*Thin-Layer Chromatography.* The qualitative study of alkaloids was carried out by TLC. The concentrated alkaloids

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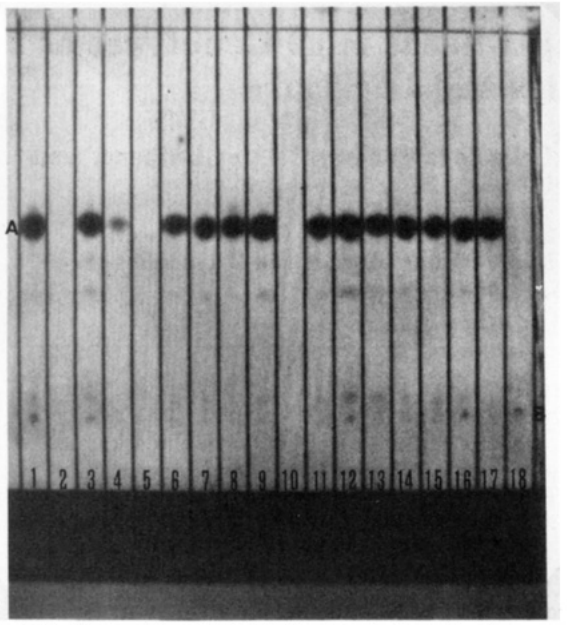


**Figure 1.** Separation of alkaloid extracts from *L. albus* sweet and bitter seeds by thin-layer chromatography using Whatman silica gel (LK6DF, 20 × 20 cm, 250 μm) plates, developed with chloroform/cyclohexane/diethylamine (6:4:1). (Lane 1) (A) lupanine standard; (lane 18) (B) 13-hydroxylupanine standard; (lanes 2, 5, 10) sweet varieties; (lane 4) semisweet variety; (others lanes) bitter ecotypes.

were dissolved in 1 mL of methanol. Samples were applied to Whatman silica gel (LK6DF, 20 × 20 cm, 250 μm) plates and developed with chloroform/cyclohexane/diethylamine (6:4:1). For visualization, ultraviolet light and Dragendorff's reagent were used. When the plates had dried, they were sprayed with Bouchardat's reagent.

*Capillary Gas–Liquid Chromatography.* The chromatography instrument used was a Perkin-Elmer Autosystem equipped with a phosphorus–nitrogen detector (PND) and Turbochrom for instrument control and data analysis. The column used was SPB-1 (30 m × 0.25 mm i.d., 0.25-μm film thickness), and helium was the carrier gas (1.38 bar). The temperatures of the injector and detector were 240 and 300 °C, respectively. The initial oven temperature was 150 °C, with a temperature ramp of 5 °C $min^{-1}$ to 235 °C and final hold time of 15 min at 235 °C.

The alkaloid standards used were 13-hydroxylupanine, isolupanine, and lupanine perchlorate. A calibration curve was

**Table 1. Total Alkaloid Content in Sweet *Lupinus* sp.**

| lines/ cultivars | total alkaloids (mg/100 mg of dm ± SE) | lines/ cultivars | total alkaloids (mg/100 mg of dm ± SE) |
|---|---|---|---|
| Lublanc | 0.05 ± 0.003 | CH304.70 | 0.08 ± 0.023 |
| LA123 | 0.42 ± 0.022 | C168 | 0.05 ± 0.002 |
| Lucky | 0.04 ± 0.001 | CH46 | 0.08 ± 0.004 |
| Ares | 0.03 ± 0.003 | CH304.73 | 0.06 ± 0.005 |
| Lutop | 0.01 ± 0.001 | Lublanc P1 | 0.03 ± 0.002 |
| Lucrop | 0.03 ± 0.001 | Lublanc P2 | 0.03 ± 0.001 |
| Adam | 0.04 ± 0.001 | Lublanc P3 | 0.03 ± 0.001 |
| Lunoble | 0.11 ± 0.007 | Lunoble H1 | 0.07 ± 0.001 |
| Kalina | 0.02 ± 0.001 | Lunoble H2 | 0.08 ± 0.001 |
| XA100 | 0.03 ± 0.001 | Lunoble H3 | 0.08 ± 0.001 |

**Table 2. Total Alkaloid Content in Bitter *Lupinus* sp.**

| ecotype | total alkaloids (mg/100 mg of dm ± SE) | ecotype | total alkaloids (mg/100 mg of dm ± SE) |
|---|---|---|---|
| TR19 | 1.70 ± 0.035 | ITA18 | 1.91 ± 0.052 |
| LA430 | 2.07 ± 0.045 | LA338 | 2.09 ± 0.021 |
| GR40 | 1.91 ± 0.035 | AC127 | 2.49 ± 0.038 |
| GR28 | 1.77 ± 0.050 | AC21 | 2.61 ± 0.161 |
| GR45 | 2.69 ± 0.055 | AC24 | 2.68 ± 0.140 |
| GR42 | 2.06 ± 0.052 | E31 | 2.23 ± 0.014 |
| GR56 | 1.92 ± 0.144 | LA549 | 1.86 ± 0.056 |
| GR55 | 2.54 ± 0.114 | LA147 | 2.14 ± 0.036 |
| ITA2 | 1.90 ± 0.019 | LA402 | 2.16 ± 0.100 |
| LA240 | 2.12 ± 0.016 | LA397 | 2.27 ± 0.048 |
| ITA37 | 0.10 ± 0.023 | LA378 | 1.63 ± 0.035 |
| LA239 | 2.12 ± 0.090 | LA390 | 1.52 ± 0.114 |
| ITA56 | 2.07 ± 0.052 | C199 | 1.78 ± 0.023 |
| ITA29 | 2.06 ± 0.047 | LA300 | 2.05 ± 0.022 |

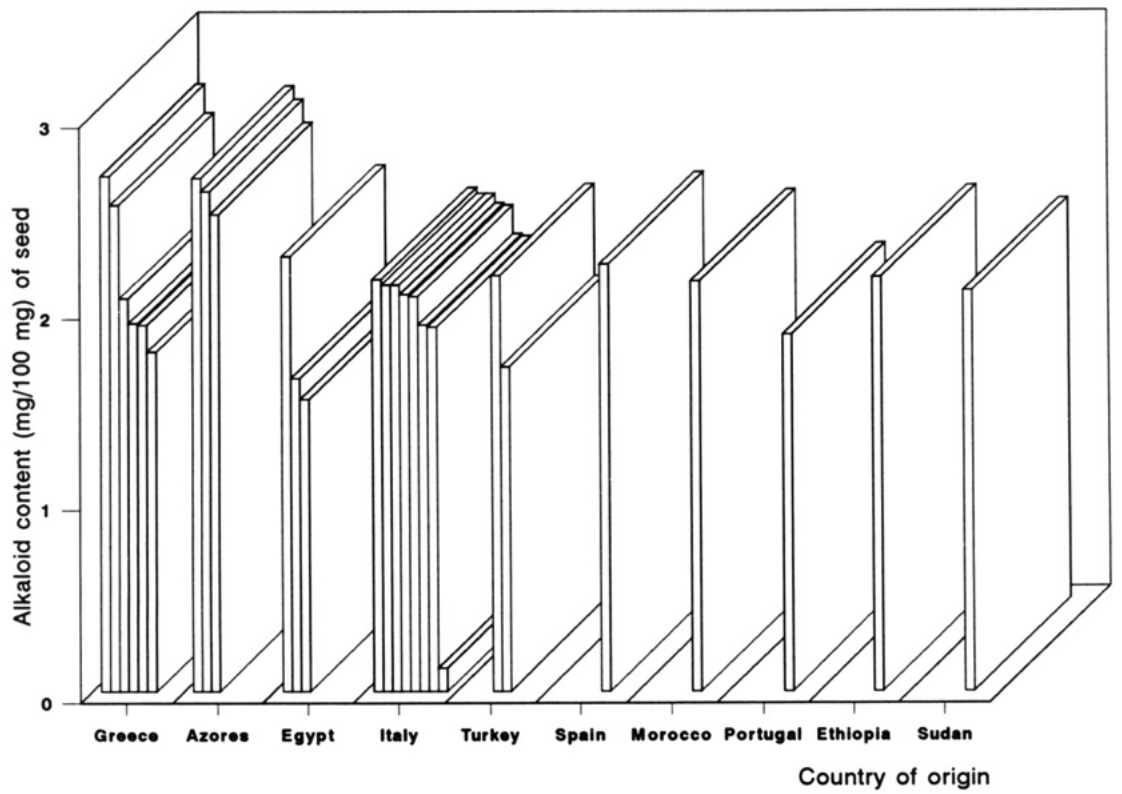


**Figure 2.** Representation of the alkaloid content of different *L. albus* ecotypes distributed by countries.

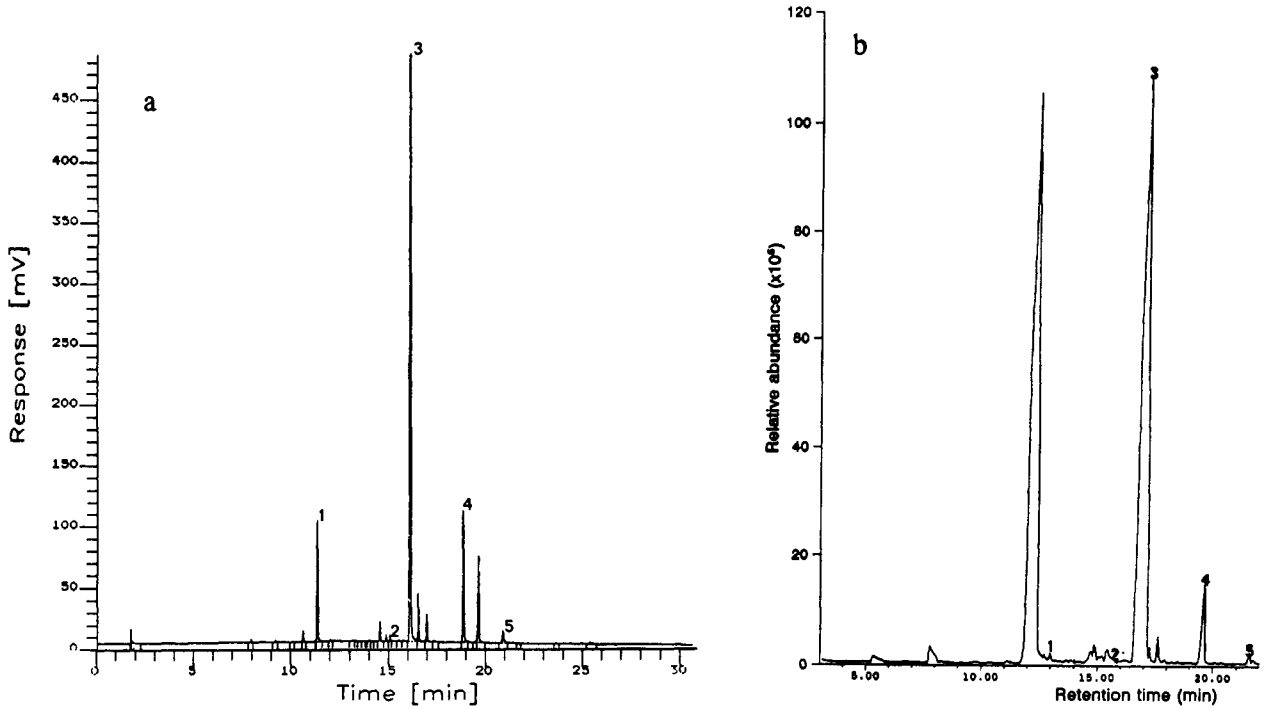


**Figure 3.** Separation of an alkaloid extract from *L. albus* bitter seeds by capillary gas–liquid chromatography: injector, 240 °C; detector, 300 °C; oven, 150–235 °C, 5 °C min$^{-1}$; carrier gas, helium; detection of alkaloids by (a) nitrogen-specific detector (PND) and (b) mass selective detector. Numbers of the GLC peaks correspond to those in Table 3.

**Table 3. Mass Spectral Data of *L. albus* Alkaloids Obtained from GLC Separations of Crude Alkaloid Extracts**

| peak no. | alkaloid | $M^+$ | characteristic ions (rel abundance) | reference |
|---|---|---|---|---|
| 1 | albine | 232 | 191 (100), 110 (70), 122 (49), 149 (45), 120 (22), 232 (21), 80 (20) | Meibner and Wink (1992) |
| 2 | α-isolupanine | 248 | 136 (100), 149 (52), 248 (33), 98 (32), 150 (31), 110 (19), 84 (16) | Meibner and Wink (1992) |
| 3 | lupanine | 248 | 136 (100), 149 (52), 98 (28), 150 (34), 248 (32), 110 (12), 84 (12) | Meibner and Wink (1992) |
| 4 | multiflorine | 246 | 134 (100), 246 (35), 136 (26), 110 (22), 149 (21), 97 (18), 83 (15) | Meibner and Wink (1992) |
| 5 | 13-hydroxylupanine | 264 | 152 (100), 246 (50), 165 (40), 264 (40), 134 (30) | Meibner and Wink (1992) |

**Table 4. Composition of Identified Alkaloids in 29 Genotypes of Bitter *L. albus***

| | mg/100 mg of dm ± SE | | | | |
|---|---|---|---|---|---|
| sample | albine | α-isolupanine | lupanine | multiflorine | 13-hydroxylupanine |
| TR19 | 0.20 ± 0.025 | 0.01 ± 0.001 | 1.12 ± 0.140 | 0.09 ± 0.014 | 0.06 ± 0.007 |
| LA430 | 0.21 ± 0.009 | 0.02 ± 0.001 | 1.57 ± 0.063 | 0.12 ± 0.008 | 0.07 ± 0.006 |
| GR40 | 0.17 ± 0.015 | 0.01 ± 0.001 | 1.32 ± 0.039 | 0.11 ± 0.009 | 0.06 ± 0.006 |
| GR28 | 0.23 ± 0.026 | 0.01 ± 0.001 | 1.12 ± 0.107 | 0.12 ± 0.011 | 0.04 ± 0.001 |
| GR45 | 0.23 ± 0.056 | 0.01 ± 0.002 | 1.71 ± 0.199 | 0.37 ± 0.073 | 0.07 ± 0.012 |
| GR42 | 0.13 ± 0.005 | 0.01 ± 0.000 | 1.48 ± 0.980 | 0.22 ± 0.005 | 0.03 ± 0.009 |
| GR56 | 0.16 ± 0.010 | 0.01 ± 0.001 | 1.29 ± 0.205 | 0.19 ± 0.011 | 0.01 ± 0.001 |
| GR55 | 0.18 ± 0.017 | 0.01 ± 0.001 | 1.88 ± 0.172 | 0.16 ± 0.014 | 0.04 ± 0.003 |
| ITA2 | 0.18 ± 0.010 | 0.01 ± 0.001 | 1.26 ± 0.062 | 0.16 ± 0.007 | 0.04 ± 0.002 |
| LA240 | 0.10 ± 0.003 | 0.01 ± 0.001 | 1.65 ± 0.031 | 0.10 ± 0.001 | 0.05 ± 0.001 |
| ITA37 | 0.02 ± 0.002 | 0.01 ± 0.001 | 0.05 ± 0.002 | 0.00 ± 0.000 | 0.00 ± 0.002 |
| LA239 | 0.16 ± 0.001 | 0.01 ± 0.001 | 1.43 ± 0.182 | 0.14 ± 0.002 | 0.08 ± 0.002 |
| LA109 | 0.20 ± 0.015 | 0.01 ± 0.001 | 1.29 ± 0.119 | 0.31 ± 0.032 | 0.04 ± 0.004 |
| ITA56 | 0.17 ± 0.010 | 0.01 ± 0.001 | 1.25 ± 0.061 | 0.29 ± 0.019 | 0.04 ± 0.004 |
| ITA29 | 0.20 ± 0.011 | 0.01 ± 0.001 | 1.41 ± 0.120 | 0.13 ± 0.005 | 0.04 ± 0.005 |
| ITA18 | 0.11 ± 0.005 | 0.01 ± 0.001 | 1.45 ± 0.088 | 0.09 ± 0.004 | 0.05 ± 0.002 |
| LA338 | 0.15 ± 0.020 | 0.01 ± 0.001 | 1.38 ± 0.125 | 0.25 ± 0.029 | 0.05 ± 0.005 |
| AC127 | 0.21 ± 0.017 | 0.01 ± 0.001 | 1.62 ± 0.061 | 0.25 ± 0.016 | 0.06 ± 0.008 |
| AC21 | 0.15 ± 0.022 | 0.01 ± 0.001 | 1.69 ± 0.216 | 0.37 ± 0.052 | 0.06 ± 0.008 |
| AC24 | 0.16 ± 0.009 | 0.01 ± 0.001 | 1.74 ± 0.155 | 0.41 ± 0.026 | 0.04 ± 0.002 |
| E31 | 0.33 ± 0.004 | 0.01 ± 0.001 | 1.26 ± 0.041 | 0.21 ± 0.005 | 0.05 ± 0.002 |
| LA549 | 0.20 ± 0.014 | 0.01 ± 0.001 | 1.16 ± 0.110 | 0.21 ± 0.019 | 0.04 ± 0.002 |
| LA147 | 0.12 ± 0.003 | 0.01 ± 0.001 | 1.62 ± 0.029 | 0.17 ± 0.005 | 0.03 ± 0.003 |
| LA402 | 0.21 ± 0.030 | 0.01 ± 0.001 | 1.39 ± 0.126 | 0.16 ± 0.017 | 0.07 ± 0.006 |
| LA397 | 0.29 ± 0.013 | 0.01 ± 0.001 | 1.45 ± 0.062 | 0.08 ± 0.003 | 0.05 ± 0.008 |
| LA378 | 0.13 ± 0.009 | 0.01 ± 0.001 | 1.04 ± 0.040 | 0.10 ± 0.006 | 0.09 ± 0.005 |
| LA390 | 0.14 ± 0.010 | 0.01 ± 0.001 | 0.94 ± 0.140 | 0.10 ± 0.013 | 0.07 ± 0.002 |
| C199 | 0.15 ± 0.004 | 0.01 ± 0.001 | 1.14 ± 0.050 | 0.15 ± 0.007 | 0.05 ± 0.001 |
| LA300 | 0.20 ± 0.005 | 0.01 ± 0.001 | 1.36 ± 0.070 | 0.11 ± 0.001 | 0.09 ± 0.040 |

prepared for lupanine. Response was linear over the range 0–1.250 mg mL$^{-1}$. The coefficient of determination of the alkaloid content was >0.99.

*Capillary Gas–Liquid Chromatography–Mass Spectrometry.* A Hewlett-Packard gas chromatograph (5890) coupled with a mass selective detector (5971) was used with G1034B software

and an MS ChemStation data system. For capillary GLC–MS the same capillary column and conditions as above were used.

## RESULTS AND DISCUSSION

In Figure 1, one can see the differences between sweet and bitter samples by TLC. The absence of alkaloids in the sweet varieties is evident.

Analysis of the results by GLC shows that large differences exist between the total content of alkaloids in the sweet and bitter samples (Tables 1 and 2). Among the bitter samples (Figure 2), the greatest concentrations of alkaloids are found in the two Greek ecotypes GR45 (2.69%) and GR55 (2.54%) and in the two Azorean ecotypes AC21 (2.61%) and AC24 (2.68%). The Italian ecotypes have very similar concentrations (1.90–2.1%), except ecotype ITA37 was very low (0.10%). This ecotype could be considered for new crossbreeding. The Spanish ecotype was similar to the other Spanish ecotypes reported by Muzquiz et al. (1993).

The sweet samples have alkaloid contents that are within the safe limit of toxicity (0.04–0.05%) for human and animal consumption (Keeler, 1989), except LA123 cv. Multolupa, which has 0.4% total alkaloids (Muzquiz et al., 1982, 1989) and the Lunoble samples which have approximately 0.07% total alkaloids (Table 1). These values are slightly greater than the accepted limit, but these genotypes should be further considered for improvement.

Among all of the bitter samples examined, five alkaloids were found including albine, α-isolupanine, lupanine, multiflorine, and 13-hydroxylupanine (Table 3; Figure 3). These alkaloids were tentatively identified from their mass spectra. In all samples lupanine was the main alkaloid. Albine and multiflorine were the other major alkaloids (Table 4). These alkaloids may provide pest protection for the seeds. These ecotypes may be exploited to obtain individual quinolizidine alkaloids in amounts sufficient for pharmacological and other biomedical purposes. However, their presence may make the seeds unsuitable for human and animal consumption.

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