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# Searching for Low Alkaloid Forms in the Andean Lupin (*Lupinus mutabilis*) Collection

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**Abstract**

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The Andean lupin (*Lupinus mutabilis* Sweet) is the only cultivated species of the genus *Lupinus* originating from South America. Attempts were made to introduce this species to European soil and climatic conditions. The main disadvantages of the Andean lupin include too long and non-uniform maturation of pods in a plant, dropping flowers and pod buds, and a high alkaloid content (up to 5%). The aim of this study was to identify in post-mutagen treated material genotypes with low alkaloid content in seeds, which would be useful for improving *L. mutabilis* by breeding. The iodine test was annually performed to test the presence of alkaloids in seeds, using Lugol's solution (I in KI). Based on the turbidity of the test samples, the investigated plants were divided into four groups, labelled as follows: G – individuals with a high content of alkaloids (very bitter), P – individuals with an intermediate content of alkaloids (bitter), PS – individuals with a low alkaloid content (fairly sweet) and S – individuals with a very low alkaloid level (sweet). For further propagation, mainly individuals from the S and PS groups were selected, and by part from the group P, while all bitter forms (G group) were rejected. Thirty chosen lines were examined for the content and composition of alkaloids by gas chromatography. The alkaloid content did not exceed 0.1% in 13 favourable lines, and even two lines had less than 0.05%. The seeds of the studied material contained 7 to 9 different alkaloids. The prevailing alkaloids in the seeds of the Andean lupin were 4-hydroxylupanine and lupanine. Significant progress has been achieved in reducing the content of alkaloids in subsequent generations of the investigated representatives of *Lupinus mutabilis* from South America.

**Keywords**: Andean lupin; qualitative composition of alkaloids; selection; total content of alkaloids

The Andean lupin (*Lupinus mutabilis* Sweet) is the only cultivated species of the genus *Lupinus* originating from South America. In the Andean region there are two ecotypes of this species. The first, called "Lupinos" or "Chocho", grows in Colombia, Ecuador and northern Peru. The Andean lupin was domesticated more than 3000 years ago. Its disadvantage is a high alkaloid content in cultivated populations. Debittering of seeds in order that they served as a component of the diet has been carried out from the ancient times by dipping the seeds in jute bags in the current of a stream, through which water-soluble alkaloids were tapped, a practice continued so far in many parts of the Andes (Calligari *et al*. 2000; Von Baer 2008). *L. mutabilis* has up to 50% protein content and up to 20% oil content in the seeds. The protein is rich in cysteine and relatively rich in lysine, but has a deficiency of methionine. It can be

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a valuable addition to wheat flour. The obtained oil is rich in unsaturated fatty acids, especially in linoleic acid. In comparison with other species of lupin crops, the Andean lupin better tolerates drought, cold weather and soil acidification (Cowling *et al.* 1998). It also has advantages from the breeding and agricultural perspective, which is a soft and thin seed coating as compared with other species of the genus, non-shedding and indehiscent pods, and the last trait being particularly important in the case of mechanical harvesting. In addition, the plants are thermoneutral. Andean lupin belongs to the group of nematode enemies, helping to reduce the incidence of soil nematodes in crop fields. Therefore, for centuries it has been used as part of the crop rotation system (lupin – potatoes – barley – lupin) in South America (Mujica *et al.* 2002). Moreover, this lupin species can produce 40–50 t/ha and 1750 kg protein biomass. Within the Andean region it is grown in monoculture at an altitude of 3000 m above sea level. Usually, the size of the plot does not exceed 2000 $m^2$, although *L. mutabilis* is occasionally cultivated on a larger area of about 1–2 ha. Seed yield from larger plots is low, estimated to be in the range of 0.6–1.0 t/ha, whereas on experimental plots the yields reach 3–5 t/ha (Caligari *et al.* 2000; Falconi 2012). From the aspect of cultivation in the temperate climate zone, the main disadvantages of the Andean lupin include too long and non-uniform maturation of pods within a plant, drooping flowers and pod buds, and high alkaloid content up to 5% (Święcicki & Nawrot 2004). Genotypes with the determined type of vegetative growth and without susceptibility to diseases including anthracnose and pests are desirable (Adomas *et al.* 2015).

Information about low alkaloid forms of the Andean lupin appeared in the literature as early as in 1992 (Wink 1992). However, these forms are characterized by a poor yield. A significant achievement in this regard was made in Chile by E. von Baer, who bred the Inti variety in which the alkaloid content in seeds amounted to 0.0075% (Gross *et al.* 1988). Under the Polish climatic conditions this variety enters the period of generative growth very late, which results in incomplete maturation, reflected in very weak setting of pods and seeds, and sometimes in their total lack. In the 90s of the last century, at the Przebedowo Plant Breeding Station mutants with reduced alkaloid content (personal communication) were obtained through mutagenesis using N-nitroso-N-methylurea and sodium azide (Stawiński & Rybiński 2001). These mutants were also characterized by weaker vitality in terms of development of both the vegetative and the generative part. In order to obtain more viable offspring open pollination was done but it led to a loss some of the characteristics of low alkaloid content. Other investigations aimed at acquisition through mutagenesis of *L. mutabilis* forms characterized by lower alkaloid content were initiated in the 80s of the last century by Sawicka-Sienkiewicz (Sawicka 1993).

The aim of this study was to identify in *L. mutabilis* material, after inducing mutations, total content and qualitative composition of alkaloids in seed. Selection of low alkaloid mutants would be useful for improving *L. mutabilis* for further breeding purposes.

## MATERIAL AND METHODS

In the last years of the 20th century, the Przebędowo Plant Breeding Station obtained – through chemical mutagenesis – low alkaloid mutants of the Andean lupin (Stawiński & Rybiński 2001). Iodine test identification was performed for the presence of alkaloids in seeds, using 1.2% Lugol's solution (I in KI). Single seeds representing the analysed plants were placed in distilled water (4 ml) in test tubes and then the samples were boiled for 90 min in a water bath. Three drops of I in KI were added to the samples after cooling. Based on the intensity of the test sample turbidity, the investigated individual plants were divided into four groups, labelled as follows:

G – individuals with a high content of alkaloids above 1.0% (very bitter) – very turbid solution,
P – individuals with an intermediate content of alkaloids 0.3–1.0% (bitter) – turbid solution,
PS – individuals with a low alkaloid content 0.15–0.3% (fairly sweet) – slightly turbid,
S – individuals with a very low alkaloid content below 0.15% (sweet) – clear solution.

The division into groups S, PS, P and G was adopted from alkaloid content (%) in the seeds of *Lupinus angustifolius* L. based on the intensity of turbidity (Forbes & Beck 1954).

In the years 2011–2013 the Plant Breeding Station started to select those forms on a broader scale using isolators during the flowering time (against insect pollination). For further propagation, individuals from the S and PS groups were mainly selected, and part of the P group, while all bitter forms (G group) were rejected. The following procedure presented below shows the way of mutant propagation and selection:

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– 1995 mutants were induced,
– 1995 to 2009 individual mutants were reproduced and those with low alkaloid content (S and PS) were selected based on the iodine test – open pollination was provided to increase viability,
– 2010 – 103 chosen plants (that produced 1784 seeds) were analysed for the alkaloid level based on the iodine test (11 plants belonged to S group, 21 to PS, 46 to P, 25 to G),
– 2011 – 720 seeds were sown representing plants belonging to S, PS and P groups and only 119 plants were obtained that were assigned to S and PS groups (which produced 1784 seeds) – open pollination; lack of seeds from plants of P group,
– 2012 – 1392 chosen seeds (under isolation) were sown and plants were evaluated according to the iodine test – 58 plants (that produced 4176 seeds) were assigned to S group, 75 to PS, 277 to P and 982 to G; the first evaluation of ten chosen plants for total alkaloid content and profile of alkaloids was done,
– 2013 – 920 seeds (from plants belonging only to S group) were sown, under isolation; the obtained plants were evaluated according to the iodine test (87 plants classified to S group, 96 to PS, 737 to P); the following test of 30 chosen plants representing S, PS, P and G groups was done for total alkaloid content and qualitative composition of alkaloids.

In 2012 and 2013, 10 and 30 lines respectively, the traditional morphotypes were examined for the total content and qualitative composition of alkaloids in seeds (Figure 1 and 2, Table 1, 2). In this material three lines (No. 5, 6 and 7) originating from Australia were tested for comparison with different genetic background. Those lines were characterized by lower alkaloid content and early maturation. Seed samples for analyses were collected after harvest in full maturity. Alkaloid extraction was performed by the procedure described by Kamel *et al.* (2015).

Total quinolizidine alkaloid (QA) values were calculated as the sum of the individual QA (sparteine, ammodendrine, isolupanine, tetrahydrorhombifoline,

Table 1. The total content and qualitative composition of alkaloids in the seeds of 10 chosen lines of Andean lupin and statistical parameters in 2012 (%)

| Group | Total alkaloid content | Sparteine | Ammodendrine | Isolupanine | Tetrahydro-rhombifoline | Lupanine | 4-hydroxylupanine | Multiflorine | 13-hydroxy-lupanine | 4,13-dihydroxylupanine |
|---|---|---|---|---|---|---|---|---|---|---|
| S | 0.0573 | 1.17 | 1.13 | 3.39 | 19.12 | 19.09 | 34.19 | 10.93 | 8.69 | 2.29 |
| PS | 0.2033 | 0.21 | 0.25 | 1.63 | 14.95 | 9.22 | 64.73 | 2.80 | 1.88 | 4.36 |
| PS | 0.2942 | nd | 0.18 | 1.31 | 13.66 | 17.11 | 51.56 | 1.32 | 6.53 | 8.32 |
| PS | 0.1539 | nd | 0.22 | 1.78 | 13.89 | 14.44 | 59.65 | 1.15 | 4.04 | 4.83 |
| PS | 0.1980 | 2.31 | 0.31 | 1.43 | 8.94 | 27.09 | 36.21 | 9.05 | 6.87 | 7.80 |
| S | 0.0985 | 0.35 | 1.03 | 2.32 | 23.26 | 7.36 | 33.88 | 16.03 | 6.64 | 9.14 |
| S | 0.1499 | 0.71 | 0.61 | 2.27 | 13.40 | 15.65 | 41.23 | 10.96 | 6.56 | 8.61 |
| PS | 0.1425 | nd | 0.37 | 2.05 | 23.66 | 6.57 | 40.07 | 4.56 | 11.73 | 11.00 |
| P | 0.9190 | 1.40 | 0.06 | 0.67 | 4.90 | 34.43 | 38.49 | 10.79 | 5.04 | 4.22 |
| P | 0.8022 | 3.54 | 0.05 | 0.84 | 4.69 | 57.33 | 15.22 | 0.72 | 15.33 | 2.28 |
| SE | 0.10 | 0.38 | 0.12 | 0.25 | 2.11 | 4.90 | 4.49 | 1.70 | 1.22 | 0.97 |
| Mean | 0.30 | 1.38 | 0.42 | 1.77 | 14.05 | 20.83 | 41.52 | 6.83 | 7.33 | 6.29 |
| Min | 0.06 | 0.21 | 0.05 | 0.67 | 4.69 | 6.57 | 15.22 | 0.72 | 1.88 | 2.28 |
| Max | 0.92 | 3.54 | 1.13 | 3.39 | 23.66 | 57.33 | 64.73 | 16.03 | 15.33 | 11.00 |
| SD | 0.30 | 1.19 | 0.38 | 0.79 | 6.68 | 15.49 | 14.19 | 5.38 | 3.84 | 3.06 |
| CV | 100.20 | 85.70 | 91.00 | 44.90 | 47.60 | 74.40 | 34.20 | 78.80 | 52.40 | 48.70 |

SE – standard error; SD – standard deviation; CV – coefficient of variability; nd – not detected; S – individuals with a very low alkaloid content; PS – individuals with a low alkaloid content; P – individuals with an intermediate content of alkaloids

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lupanine, 4-hydroxylupanine, multiflorine, 13-hydroxylupanine, 4,13-dihydroxylupanine) expressed on seed dry weight (DW) basis.

For evaluation of total variability, the standard deviation (Statistica 10.0), variability coefficient and range of variability (Min, Max) were calculated. In order to check relations between total content and qualitative composition of alkaloids in seeds correlation coefficients were calculated (Table 3).

## RESULTS AND DISCUSSION

The tested material was characterized by the lack of stability in terms of alkaloid content in seeds. In the second year of selection (2012) through isolation during the flowering time we found that only 4.16% of individual plants were assigned to S group based on the iodine test. Results of the mean and statistical parameters calculated for total alkaloid content and qualitative composition of alkaloids in seeds for 10 randomly selected lines are presented in Table 1. Data showed that screening with the iodine test was almost compatible with total alkaloid content determined by gas chromatography (Table 1), except one case. Nine of QA were detected in different proportions. Sparteine was not detected in free lines. The 4-hydroxylupanine and lupanine were the main alkaloids in seeds.

In the third year of intense selection, seeds were collected from 920 individuals derived from 58 plants from S group. We found that in 19.9% of them (87 out of 920 individuals) a very low alkaloid level was identified. The remaining plants were classified to the groups PS and P – 96 and 737 individual plants, respectively. The most important task would be further possible derivation of homozygous lines, which would help determine the inheritance of this trait in the Andean lupin and allow the stabilization of alkaloid content in seeds. Difficulties in the acquisition of homozygous lines which would be alkaloid-free ("sweet") stem also from the complex pattern of inheritance of this trait (controlled by recessive genes). Difficulties in obtaining homozygous low alkaloid lines probably consist in a complex way of inheritance of this trait (may be recessive genes). Erik von Baer crossed "bitter" forms with "sweet" ones, and he received only 12% of individual plants with low alkaloid content in the $F_2$ generation (von Baer 2011).

Table 2 shows the values of total alkaloid content in 30 lines of the Andean lupin. The alkaloid content did not exceed 0.1% in 13 favourable lines, and two lines had less than 0.05% (Figure 1). The iodine test coincides in 77% of cases with the determination of the total alkaloid content carried out by gas chromatography. Differences in assays identified for several lines qualified on the basis of iodine test for the S group instead of PS or P and PS group instead of P. This test was found useful in the selection of Andean lupin in terms of reduced alkaloid content in seeds. It should be emphasized that it is possible to analyse a large number of plants in a relatively short

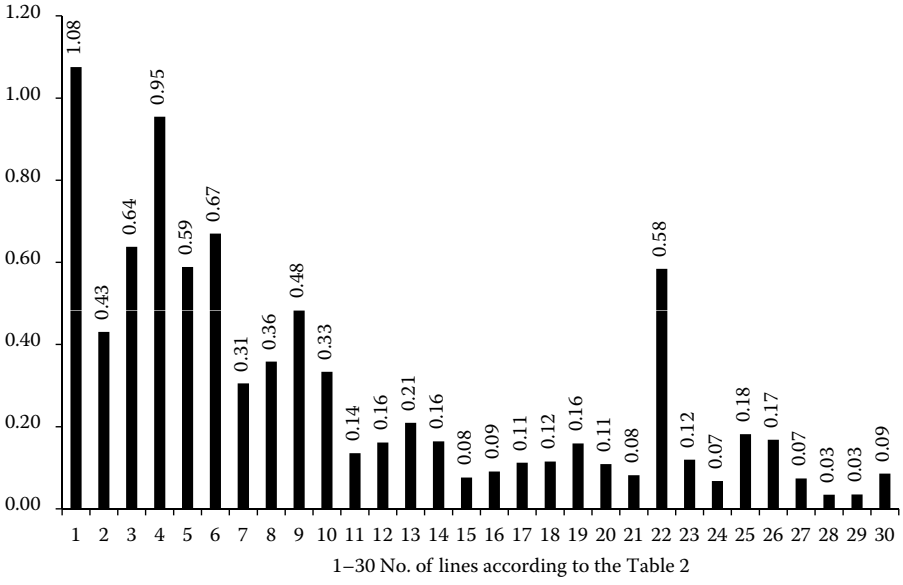


Figure 1. Total alkaloid content (in %) in 30 lines of *Lupinus mutabilis*

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Table 2. Characteristics of the qualitative composition of alkaloids in the investigated material in 2013 (%)

| No. | Group | Sparteine | Ammodendrine | Isolupanine | Tetrahydrorhombifoline | Lupanine | 4-hydroxylupanine | Multiflorine | 13-hydroxylupanine | 4,13-dihydroxylupanine |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | G | 1.21 | 0.22 | 0.83 | 7.30 | 26.99 | 47.84 | 6.82 | 4.19 | 4.6 |
| 2 | P | 6.65 | 0.59 | 0.98 | 5.85 | 30.30 | 37.73 | 1.37 | 12.06 | 4.47 |
| 3 | P | 12.83 | 0.13 | 1.44 | 6.21 | 42.65 | 19.78 | 4.40 | 9.36 | 3.2 |
| 4 | P | nd | 1.18 | 1.01 | 7.39 | 28.76 | 44.92 | 1.39 | 7.15 | 8.21 |
| 5 | P | nd | 0.26 | 1.10 | 9.71 | 20.67 | 54.91 | 2.90 | 3.03 | 7.43 |
| 6 | P | 2.93 | 0.11 | 0.86 | 6.60 | 21.70 | 49.50 | 7.88 | 3.87 | 6.56 |
| 7 | P | 0.91 | 0.21 | 1.82 | 11.39 | 18.61 | 41.10 | 9.78 | 6.11 | 10.08 |
| 8 | PS | 5.49 | 0.66 | 0.93 | 6.30 | 28.96 | 42.90 | 1.16 | 9.73 | 3.88 |
| 9 | PS | 1.79 | 0.37 | 1.91 | 17.02 | 17.95 | 42.15 | 8.57 | 5.49 | 4.76 |
| 10 | PS | 3.57 | 0.17 | 1.10 | 10.91 | 16.95 | 39.80 | 15.26 | 3.49 | 8.75 |
| 11 | S | nd | 0.42 | 1.45 | 16.52 | 11.66 | 54.24 | 2.81 | 4.93 | 7.97 |
| 12 | S | 2.16 | 0.26 | 1.13 | 13.12 | 14.15 | 44.73 | 11.49 | 4.23 | 8.74 |
| 13 | S | 0.38 | 0.21 | 2.23 | 11.31 | 19.93 | 41.67 | 9.36 | 5.98 | 8.93 |
| 14 | S | 2.02 | 0.23 | 1.23 | 14.43 | 9.71 | 37.38 | 17.56 | 4.22 | 13.22 |
| 15 | S | 0.49 | 0.24 | 2.10 | 15.19 | 11.19 | 41.36 | 10.43 | 8.09 | 10.9 |
| 16 | S | 0.82 | 0.22 | 1.84 | 11.39 | 16.65 | 39.29 | 14.44 | 6.44 | 8.91 |
| 17 | S | nd | 0.28 | 1.49 | 23.68 | 4.31 | 39.97 | 8.18 | 5.43 | 16.66 |
| 18 | S | nd | 0.29 | 1.78 | 25.84 | 3.64 | 37.54 | 7.38 | 5.84 | 17.68 |
| 19 | S | nd | 0.17 | 1.71 | 20.19 | 6.37 | 47.29 | 5.14 | 4.55 | 14.57 |
| 20 | S | nd | 0.3 | 1.41 | 20.12 | 4.62 | 44.75 | 8.38 | 5.44 | 14.98 |
| 21 | S | 1.20 | 0.23 | 1.64 | 10.75 | 15.60 | 38.35 | 17.57 | 6.88 | 7.77 |
| 22 | S | nd | 0.11 | 1.03 | 7.59 | 23.78 | 56.12 | 1.74 | 4.08 | 5.55 |
| 23 | S | 1.05 | 0.18 | 1.86 | 10.73 | 18.83 | 41.52 | 11.97 | 6.03 | 7.82 |
| 24 | S | nd | nd | 2,00 | 16.39 | 12.73 | 43.76 | 10.73 | 6.57 | 7.82 |
| 25 | S | 0.77 | nd | 2.03 | 9.77 | 21.95 | 43.48 | 10.14 | 4.95 | 6.91 |
| 26 | S | 2.26 | 0.15 | 1.79 | 8.69 | 24.32 | 37.48 | 11.50 | 6.07 | 7.74 |
| 27 | S | nd | nd | 1.21 | 9.86 | 21.43 | 54.89 | 2.29 | 5.51 | 4.81 |
| 28 | S | nd | nd | 2.63 | 19.96 | 7.25 | 37.06 | 12.26 | 6.98 | 13.86 |
| 29 | S | nd | nd | 2.38 | 21.60 | 7.93 | 41.31 | 12.12 | 4.95 | 9.70 |
| 30 | S | 0.52 | 0.27 | 1.78 | 14.22 | 15.24 | 36.97 | 11.57 | 8.22 | 11.22 |
| Mean | | 2.61 | 0.30 | 1.56 | 13.00 | 17.49 | 42.66 | 8.55 | 6.00 | 8.92 |
| SD | | 0.73 | 0.05 | 0.09 | 1.02 | 1.64 | 1.30 | 0.87 | 0.36 | 0.69 |
| CV | | 117.8 | 75.7 | 31.0 | 42.90 | 51.30 | 16.7 | 55.80 | 33.12 | 42.60 |

SD – standard deviation; CV – coefficient of variation; nd – not detected; G – individuals with a high content of alkaloids; P – individuals with an intermediate content of alkaloids; PS – individuals with a low alkaloid content; S – individuals with a very low alkaloid content

time and at a low cost. The successfully conducted selection has allowed choosing desired genotypes for further genetic and breeding works.

We found 7 to 9 alkaloids in the examined material (Table 2, Figure 2). Some lines did not contain any sparteine or ammodendrine. Much more alkaloids

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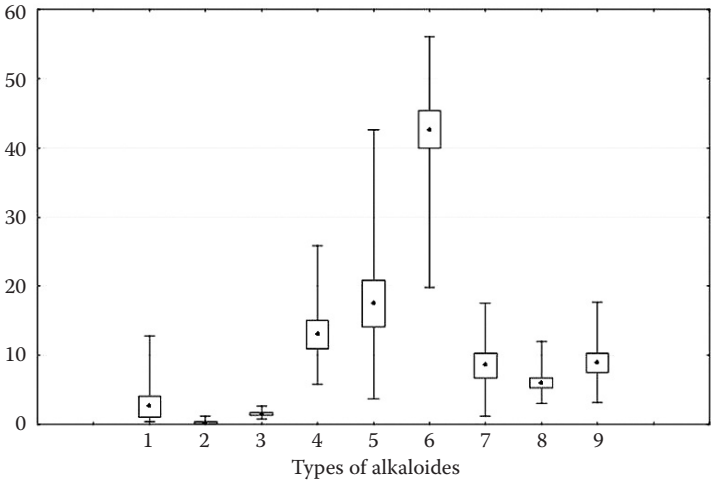


Figure 2. Percentages of individual alkaloids in the total sum of alkaloids in tested *L. mutabilis* lines

1 – sparteine, 2 – ammodendrine, 3 – isolupanine, 4 – tetrahydrorhombifoline, 5 – lupanine, 6 – 4-hydroxylupanine, 7 – multiflorine, 8 – 13-hydroxylupanine, 9 – 4,13-dihydroxylupanine

were detected than in the narrow-leafed and yellow lupins – in our own annual studies of 100–110 lines, in which the presence of 4–5 alkaloid compounds was ascertained. The predominant alkaloid in the Andean lupin was 4-hydroxylupanine, which did not occur in the yellow and narrow-leafed lupins. The 30 tested lines of *L. mutabilis* were characterized by ranges of alkaloids from 19.78% to 56.12%. Another identified alkaloid was lupanine (3.64–42.62%), usually the dominant alkaloid in *L. angustifolius*. Other alkaloids, which were not detected either in yellow or in narrow-leafed lupin, were tetrahydrorhombifoline (5.85–25.84%), 4,13-dihydroxylupanine (3.80–17.68%) and 13-hydroxylupanine (3.03–12.06%), and multiflorine (1.16–17.57%), the latter occurring also in the narrow-leafed lupin. Literature sources reported the identification of other alkaloids in the Andean lupin seeds: α-isosparteine, β-isosparteine, α-isolupanine, 5,6-dehydrolupanine, 13-(angeloyloxy) lupanine (Adomas *et al.* 2015). In respect of these alkaloids, the evaluated Andean lupin material is comparable with the yellow lupin, but less abundant in sparteine and ammodendrine, and isolupanine which also occurs in the narrow-leafed lupin. The above analyses from 2012 and 2013 of the total content and qualitative profiles of alkaloids in Andean lupin seeds (Table 1 and 2, Figure 1 and 2) showed a much broader variation than that hitherto described in the literature. In future it seems possible to decrease total alkaloid content via the elimination of some of them.

Table 3 shows correlation coefficients of those alkaloids with the positive influence on the total alkaloid content of ammodendrine, lupanine and negative with isolupanine, tetrahydrorhombifoline, multiflorine and 4,13-dihydroxylupanine. The relationships between the occurrences of the various alkaloids of Andean lupin are also interesting. The most significant positive dependence was found for 13-hydroxylupanine and sparteine, ammodendrine and lupanine. The 4,13-dihydroxylupanine was negatively correlated with sparteine and lupanine. Multiflorine was negatively correlated with ammodendrine, lupanine and 4-hydroxylupanine while positively with isolupanine.

In order to increase the genetic variability of the Andean lupin, mutations have been induced successfully. For example by inducing mutations, Römer (1994) received mutants with the determined type of growth (epigonal). Many years' selection has produced promising lines with larger seeds, higher yield and high contents of protein and oil. Line No. 246 (Chile) was first harvested mechanically, and it has been used to feed farm salmon (von Baer 2011). The final result of the work done in Chile was acquisition through crossing of a new variety Pinta (Inti × SCG9), which is characterized by a low content of alkaloids, high protein and oil contents, and also larger seeds (von Baer 2011). The problem of low alkaloid content in *L. mutabilis* is still an open question, as already pointed out by Williams *et al.* (1984), who identified the recessive allele *mutal* of the gene *Mutal*. This allele in homozygous constitution was responsible for development of plant seeds with the alkaloid content of 2–3 g/kg; the vegetative part of plants was organoleptically very sweet as well. Also in *L. angustifolius* this feature was conferred by recessive alleles. Moreover, it has turned out that it is dependent on environmental conditions, including pH (Jansen *et al.* 2012). According to Boersma *et al.* (2005), reduced alkaloid content in *L. angustifolius* is controlled by three alleles of different loci.

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Table 3. Correlation coefficients for the total content and qualitative composition of alkaloids in the seeds of Andean lupin

| | Total alkaloids content | Sparteine | Ammodendrine | Isolupanine | Tetrahydrorhombifoline | Lupanine | 4-hydroxylupanine | Multiflorine | 13-hydroxylupanine |
|---|---|---|---|---|---|---|---|---|---|
| Sparteine | 0.32 | | | | | | | | |
| Ammodendrine | 0.44* | 0.10 | | | | | | | |
| Isolupanine | –0.63* | –0.31 | –0.43* | | | | | | |
| Tetrahydrorhombifoline | –0.59* | –0.49* | –0.22 | 0.54* | | | | | |
| Lupanine | 0.66* | 0.69* | 0.27 | –0.45* | –0.89* | | | | |
| 4-hydroxylupanine | 0.15 | –0.61* | 0.02 | –0.33 | –0.06 | –0.16 | | | |
| Multiflorine | –0.49* | –0.18 | –0.44* | 0.47* | 0.27 | –0.44* | –0.40* | | |
| 13-hydroxylupanine | –0.06 | 0.53* | 0.38* | 0.09 | –0.22 | 0.39* | –0.55* | –0.22 | |
| 4,13-dihydroxylupanine | –0.51* | –0.49* | –0.09 | 0.37* | 0.83* | –0.85* | –0.12 | 0.35 | –0.22 |

*Significant at $P = 0.05$

The recessive allele *iuc* (*iucundis*) decreases alkaloid content in seeds to approximately 0.06% of their dry weight. The presence of *depr* (*depressus*) allele causes an extremely low alkaloid content (about 0.01% of the seed dry weight), while *es* (*esculentus*) allele confers an intermediate alkaloid content (Święcicki & Święcicki 1995). Therefore, it seems necessary to conduct wider research on the heritability of this trait in the Andean lupin. The acquisition of stable "sweet" forms should be feasible both through increasing the genetic variation by induced mutations and searching for appropriate recombinants via interspecific hybridization. It is possible that further development of biotechnological methods utilizing achievements in the field of molecular biology and in vitro cultures will contribute to work on the mapping of the Andean lupin genome. The application of biotechnological tools should be helpful in obtaining homozygous material, which will enable further progress in domestication of this species in European conditions.

Not only low alkaloid forms but also bitter ones are of practical use. Recent reports indicate the importance of lupin alkaloids in medicine. Attempts to use alkaloids extracted from the lupin proved to be helpful in the treatment of patients with high blood pressure or those suffering from diabetes (Wink 2011; Baldeón *et al.* 2012). This type of health problems is very serious in developing countries, for instance in Ecuador it has been found to be the main cause of death in the last decade (Baldeón *et al.* 2012). *L. mutabilis* introduced to the diet has been noted to bring about an increase in the blood glucose level. The studies demonstrate that cooked Andean lupin seeds or medicaments containing the alkaloids isolated from this species give a positive impact on improving the health of diabetics. This finding emphasizes the role of the macro- and micronutrients present in the Andean lupin seeds (Baldeón *et al.* 2012).

## CONCLUSIONS

A significant progress has been achieved in reducing the content of alkaloids in subsequent generations of the investigated representatives of *L. mutabilis* from South America.

It has been found that the predominant alkaloids in the seeds of the Andean lupin are 4-hydroxylupanine and lupanine.

The tested material was characterized by systematic segregation of this trait, which demonstrates the need to derive homozygous lines with low alkaloid content in the seeds for further progress in acclimatization and domestication of *L. mutabilis* under the European conditions.

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