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RESEARCH ARTICLE

Quantitative and qualitative content of alkaloids in seeds of a narrow-leafed lupin (*Lupinus angustifolius* L.) collection

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Abstract Alkaloids represent the main antinutritional factor in lupins. The total content and qualitative composition of four major alkaloids in *L. angustifolius* L. were analyzed. The material included 329 accessions from the Polish collection divided into three classes of origin: wild lines, cultivars, and other man-made accessions. A very broad differentiation was found in terms of total alkaloid content—from 0.0005 to 2.8752 % of seed dry weight. In most cases, cultivars were characterized by a sharply decreased content of alkaloids, even below 0.01 % of seed dry weight. The average proportions of individual alkaloids were also very differentiated: lupanine—0.98 to 73.0 % of total alkaloid content (mean 46.4 %), 13-hydroxylupanine—15.6–71.1 % (mean 35.6 %), angustifoline—0–49.8 % (mean 15.5 %) and isolupanine—0–34.0 % (mean 2.5 %). The above mean values are probably typical for this species. In some accessions, lupanine and 13-hydroxylupanine accounted for 90–100 % of all major alkaloids. The average content of isolupanie (2.5 % of all alkaloids) allows it to be consider a major alkaloid of *L. angustifolius*, but quite frequently a ratio below 1 % or even its absence was stated. The three classes of origin were divided into three significantly different groups based on total alkaloid content as well as individual alkaloid content. Among wild lines, high alkaloid accessions were most numerous, but among cultivars it was low alkaloid accessions. The last class also contained numerous accessions with the lowest content of individual alkaloids. The influence of the content of individual alkaloids on total alkaloid content was also investigated in the wild lines and cultivars.

Keywords Antinutrional factors · Quinolizidine alkaloids · Lupin seed quality · Genetic resources valorization · *Lupinus angustifolius* L.

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Introduction

Lupins are grown for their green mass as a manure and animal fodder and for their seeds as human food and animal fodder. Some lupin species were used in ancient times, but it was only in the twentieth century that wild traits were eliminated or improved, e.g. hard seed coat, shattering pods and high alkaloid content in seeds (Brummund and Święcicki 2011). Decreasing the alkaloid content was a basic condition for the use of lupins as fodder. Wild accessions of narrow-leafed lupin contain 0.4–3.0 % of alkaloids in the dry weight of their seeds and 0.3–0.5 % in the dry weight of their green forage (Święcicki and Święcicki 1995; Brummund and Święcicki 2011). First, low alkaloid narrow-leafed lupins were selected by Sengbusch in 1927/1928 and then genes responsible for low alkaloid content (*iucundus, depressus* and *esculentus)* were presented by Hackbarth and Troll (1956). The gene *iuc* (*iucundus*) decreases alkaloid content in seeds to approximately 0.06 % of their dry weight, the gene *deper* (*depressus*) gives a very low alkaloid content (about 0.01 % of the seeds' dry weight), while *es* (*esculentus*) leads to an intermediate alkaloid content (Hackbarth and Troll 1956). It is suggested that, for feeding purposes, a safe alkaloid content is below 0.02 % of the seeds' dry weight (Cowling et al. 1998). International Union for the Protection of New Varieties of Plants (UPOV) regulations divide cultivars into two groups: sweet (low alkaloid) and bitter (high alkaloid)—based on the colorimetric method (UPOV Guidelines 2004). A cultivar description quite often presents a precise alkaloid content in seeds, for example in 2013 1.165 % of the dry weight for bitter cv. Karo and 0.013 % of the dry weight for sweet cv. Regent (Synthesis RCCT 2014).

Besides the total alkaloid content, their qualitative composition is also important. Individual alkaloids have different toxicities and the various *Lupinus* species differ in their qualitative composition (Wink et al. 1995; Wink 2011). Each species contains so-called major alkaloids (abundance >1 % of total alkaloids) and minor alkaloids (abundance <1 % of total alkaloids) (Wink et al. 1995). In the narrow-leafed lupin, the major alkaloids are lupanine, 13-hydroxylupanine and angustifoline (Petterson 1998; Kurlovich et al. 2002). According to Petterson (1998), the following quinolizidine alkaloid profile is characteristic for sweet cultivars of narrow-leafed lupin: lupanine (42–59 %), 13-hydroxylupanine (24–45 %), angustifoline (7–15 %) and isolupanine (1–1.5 %).

A main source of initial material for breeding are collections of genetic resources. Their usefulness depends on the range of gathered variation and its valorization. The Polish collection of the genus *Lupinus* is one of the largest—1169 accessions mostly belonging to lupin crops (Święcicki et al. 2015). The aim of this study was an analysis of the total content and qualitative composition of alkaloids in seeds of the Polish collection of narrow-leafed lupin.

Materials and methods

Plant material

The total of 329 narrow-leafed lupin accessions (maintained as pure lines) from the genus *Lupinus* collection of the Polish Gene Bank at Wiatrowo, Poznan Plant Breeders Ltd, were investigated. The collection covers the following origin classes:

- CO—including 143 wild and primitive populations originating from places of natural distribution and collection missions,
- XD—including 108 accessions created by man as an effect of selection after crossings or induced mutations for different goals (not cultivars),
- CV—including 78 accessions, most improved by man, i.e. past and present registered cultivars.

The above collection was divided into two parts (subsets). The first part covered 154 accessions (89 CO, 31 XD, 34 CV), the second 175 accessions (54 CO, 77 XD, 44 CV). Accessions from both parts were regenerated in field experiments in 2011 and 2012, respectively. Both experiments were carried out in a completely randomized design with two replications, plot size 1 m^2, 100 seeds per plot. Controls in both years were bitter cv. Karo, Bc (Wt 95,964) and sweet cv. Baron, Sc (Wt 96,210). Seed samples for analyzes from two replications were collected after harvest in full maturity of each plot. Whole plots were harvested, 100 g seeds was sampled and 10 g was milled.

Alkaloid extraction

Dried lupin meal (0.5 g) was homogenized in 5 ml of 5 % (w/v) trichloroacetic acid (TCA). The suspension was sonicated at room temperature for 15 min followed by centrifugation at 5000 rpm for 10 min at 5 °C. The supernatant was decanted and the pellet was dissolved in 5 mL of 5 % (w/v) TCA. All steps were repeated twice and the extracts were combined.

An aliquot of the supernatant (15 mL) was subsequently alkalized with 1 mL of 10 M sodium hydroxide and extracted three times with dichloromethane (15 mL). The organic extracts were dried over anhydrous sodium sulfate, collected in a flask containing 20 µg of the internal standard (caffeine) and the solvent was evaporated *in vacuo*. The residues were reconstituted in 200 µL of dichloromethane.

Alkaloid analysis

Quinolizidine alkaloid (QA) extracts were separated on a ZB-5 silica capillary column (30 m × 0.25 mm × 0.25 µm; Phenomenex) with He as the carrier gas (1 mL min^{-1}; split ratio 1:20; injection port 250 °C; detection 300 °C) using GC. QA were analyzed using the gas chromatography technique with the oven temperature program starting at 180 °C, 2 min isothermal, 5 °C min^{-1} to 300 °C, 10 min isothermal. QA identification was performed via a comparison of the retention times of alkaloid standards obtained from the Institute of Bioorganic Chemistry, Polish Academy of Sciences. Quantitative analysis was carried out using a calibration curve made for lupanine with caffeine as the internal standard. Total QA values were calculated as the sum of the individual QA (lupanine, 13-hydroxylupanine, angustifoline and isolupanine) expressed on seed dry weight (DW).

Statistical analyzes

For each experiment, a single-factor analysis of variance (Gomez and Gomez 1984) was applied to test hypotheses concerning the differences among the lines for four studied alkaloids. On the basis of the analysis of variance, the interesting contrasts between the control cultivars (Bc and Sc) and the three classes of accession origin (CO, XD and CV) were estimated and tested using the F–statistic. Moreover, for the major alkaloids and three classes of accession origin the Gabriel procedure (Gabriel 1964) was used for the division of the set of accessions in each class into three significantly different groups with high, medium and low total and individual alkaloid content. As a measure of the linear dependence between the total content of alkaloids and its four major components, the correlation coefficients were calculated.

Results and discussion

The results of analyzes of the total alkaloid content (in % of the seed DW) and quantitative composition (four major alkaloid contents in % of the seed DW and its % in total alkaloid content) of all narrow-leafed lupin accessions are presented in the table attached to the Database of the Polish *Lupinus* Collections (http://www.igr.poznan.pl/uploads/resources/Lupinus%20angustifolius.pdf).

As a result of the analyzes of variance, a highly significant ($P = 0.01$) differentiation between accessions was stated in terms of total alkaloid content as well as the content of individual alkaloids. This allowed comparisons between interesting classes of accessions for individual alkaloids to be conducted, and also the division of accessions from each class of origin and year into significantly different groups.

The estimations and results of comparison testing between the mean values for controls and the mean values for accessions from individual classes of origin for both years are presented in Table 1. Compared were also mean values between years (2011–2012) for individual classes of origin, Bc and Sc controls for total and individual alkaloids content. In almost all cases differences were statistically non-significant ($P = 0.005$). For example, for total alkaloid content: Bc—0.28, Sc—0.01, CV—0.02, XD—0.20 and CO—0.12. Exceptions were significant results when comparing XD for lupanine and isolupanine. The results of the division of accessions into significantly different groups for total alkaloid content and the content of individual major alkaloids are presented in Table 2. Selected examples for the total alkaloid content and qualitative composition of major alkaloids in seeds of the *L. angustifolius* L. collection are shown in Table 3. Table 4 presents: the content of four major alkaloids, total alkaloid content in % of the seed DW and the individual alkaloid content in % of total alkaloid content in each significantly different group for two classes of origin (CO and CV). The highest (highly significant) correlation coefficients between these alkaloids and the total alkaloid content are also included.

In Table 1, a comparison of the mean contents of two controls—Bc and Sc—points to a clearly higher content in Bc, for both total and individual alkaloids. A comparison of origin classes to Sc shows a higher content of each class, but statistically significant

Table 1 Estimates and results of contrast testing between mean alkaloid content (total alkaloid content and individual alkaloids–lupanine, 13-hydroxylupanine, angustifoline and isolupanine in % of the seed DW) in the *L. angustifolius* L. collection

Compared classes and controls	Total alkaloid content		Lupanine		13-hydroxylupanine		Angustifoline		Isolupanine	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Bc-Sc[1]	1.14	1.40*	0.52	0.86*	0.37	0.32	0.22	0.19	0.02	0.03
CV-Sc	0.46	0.46	0.23	0.24	0.14	0.13	0.08	0.07	0.01	0.01
XD-Sc	0.84	1.03*	0.40	0.54	0.27	0.29	0.16	0.18	0.01	0.02
CO-Sc	1.52*	1.63*	0.60*	0.68*	0.61*	0.64*	0.29*	0.30*	0.01	0.01
CV-Bc	−0.68	−0.94	−0.29	−0.61	−0.23	−0.19	−0.15	−0.12	−0.02	−0.02
XD-Bc	−0.29	−0.37	−0.12	−0.31	−0.10	−0.03	−0.07	−0.02	−0.01	−0.01
CO-Bc	0.38	0.23	0.09	−0.18	0.24	0.31	0.07	0.11	-0.01	−0.01
XD-CV	0.39*	0.57*	0.17*	0.30*	0.13*	0.16*	0.08*	0.10*	0.003	0.01*
CO-CV	1.07*	1.17*	0.37*	0.43*	0.47*	0.50*	0.21*	0.23*	0.006*	0.005*
CO-XD	0.68*	0.60*	0.20*	0.13*	0.34*	0.35*	0.13*	0.13*	0.003	−0.001

* Significant level $P = 0.01$

[1] *Sc* sweet control, *Bc* bitter control

differences exist only when comparing CO-Sc in both years (sub-sets) for total content and three major alkaloids. In the case of comparing classes to Bc, only the CO class slightly exceeds the content of Bc.

Among the 329 accessions of the Polish narrow-leafed lupin collection a very broad differentiation was stated in terms of the total alkaloid content in seeds from 0.0005 % (accession Wt 96,128) to 2.8752 % of the seed DW (Wt 95,708). Mean content in Sc (Wt 96,210 = cv. Baron) was 0.0320 % DW and in Bc (Wt 95,964 = cv. Karo) was 1.3011 % DW.

In the CO class, the differentiation of total alkaloid content was from 0.0163 % (Wt 95,931) to 2.8752 % (Wt 95,708) of the seed DW. Most accessions originated from Mediterranean countries (Spain, Portugal, Italy and Morocco)—regions of natural distribution (Gladstones 1974). Exceptions of unknown origin were collected from Australia and Germany. Statistical analyzes identified three significantly different groups in both years (Table 2). The most numerous groups are the second (including Bc) and the first group. The third group contains wild lines with decreased alkaloid content: about 1.1523 % (Wt 95,845)–1.0089 % (Wt 95,848) of the seeds DW in 2012 and 0.4592 % (Wt 95,916 E)–0.0163 % (Wt 95,931) of the seed DW in 2011 (less than in the Sc). In both years, the third group accounted for only 5 and 7 accessions, respectively. Five CO lines in 2011 with alkaloid content below 0.5 % of the seed DW shows that wild and primitive populations can also contain a decreased alkaloid content.

As a result of using Gabriel's procedure, a division of XD accessions into three significantly different groups was effected in both years. The range of total alkaloid content differed from 0.0005 % (Wt 96,128) to 2.4402 % (Wt 95,777) of the seed DW. This class covers accessions created for different breeding and research aims. Besides accessions with extremely low alkaloid content it also includes accessions with above 1.5 % of alkaloids in seed DW. Most numerous (49 accessions in both years) was the third group with decreased alkaloid content, including Sc, as well as having the accession with the lowest alkaloid content—0.0005 % of the seed DW (Wt 96,128). The number of accessions in this group shows that an important aim in the narrow-leafed lupin domestication is the decreasing of alkaloid content, although selected accessions with increased alkaloid content are also maintained in gene banks.

The third class of investigated accessions (CV), a total alkaloid content in % of seed DW ranged from 0.0022 (Wt 96,143) to 2.1562 (Wt 95,937). Statistical analyzes identified three significantly different groups. The biggest was the third group (57 accessions in both years) with the lowest alkaloid content: 0.0022 % of the seeds DW (Wt 96,143) in the first year and 0.0055 % (Wt 96,208) in the second year. Among accessions of this group, Australian and Polish

Table 2 Significantly different groups within individual classes of accession origin (for total alkaloid content and content of major alkaloids (% of the seed DW) of *L. angustifolius*

Alkaloid	Group	CO		XD		CV	
		2011	2012	2011	2012	2011	2012
Total	1						
	Number of accessions	26	10	8	24	5	5
	Content (% of the seed DW)	1.8190–2.8752	2.2016–2.5024	1.7194–2.4402	1.7920–2.3991	1.7148–2.0550	1.8042–2.1562
	2						
	Number of accessions	60 (Bc)	39 (Bc)	9 (Bc)	22 (Bc)	5 (Bc)	7 (Bc)
	Content (% of the seed DW)	0.9164–1.7361	1.3296–1.9192	0.9265–1.6297	1.2855–1.7426	1.0876–1.4635	1.4033–1.7025
	3						
	Number of accessions	5 (Sc)	7 (Sc)	16 (Sc)	33 (Sc)	24 (Sc)	34 (Sc)
	Content (% of the seed DW)	0.0163–0.4592	0.0384–1.1523	0.0005–0.0351	0.0146–0.1539	0.0022–0.1321	0.0055–0.4494
Lupanine	1						
	Number of accessions	26	13	6	24	6	2
	Content (% of the seed DW)	0.8261–1.4777	0.9781–1.2987	0.9354–1.3361	0.9546–1.2368	0.8443–1.0852	1.1372–1.3778
	2						
	Number of accessions	41 (Bc)	23 (Bc)	9 (Bc)	22 (Bc)	4 (Bc)	9 (Bc)
	Content (% of the seed DW)	0.3918–0.8103	0.6145–0.9244	0.5301–0.8741	0.5320–0.9339	0.5039–0.6722	0.8028–1.0745
	3						
	Number of accessions	24 (Sc)	20 (Sc)	18 (Sc)	33 (Sc)	24 (Sc)	35 (Sc)
	Content (% of the seed DW)	0.0087–0.3666	0.0220–0.5121	0.0001–0.3142	0.0082–0.1037	0.0009–0.0417	0.0039–0.4303
13-hydroxylupanine	1						
	Number of accessions	3	5	1	2	5	5
	Content (% of the seed DW)	1.4678–1.9104	1.1643–1.6273	0.9820	0.7477–1.1090	0.5359–0.6536	0.4945–0.6645
	2						
	Number of accessions	22	25	16 (Bc)	44 (Bc)	5 (Bc)	8 (Bc)
	Content (% of the seed DW)	0.7971–1.2485	0.5640–0.9435	0.3793–0.6604	0.2716–0.6427	0.3120–0.4758	0.2262–0.4534
	3						
	Number of accessions	66 (Sc, Bc)	26 (Sc, Bc)	16 (Sc)	33 (Sc)	24 (Sc)	33 (Sc)
	Content (% of the seed DW)	0.0051–0.6922	0.0124–0.5424	0.0003–0.0110	0.0038–0.0687	0.0009–0.0492	0.0016–0.1436

Table 2 continued

Alkaloid	Group	CO		XD		CV	
		2011	2012	2011	2012	2011	2012
Angustifoline	1						
	Number of accessions	23	6	8	25	5	9
	Content (% of the seed DW)	0.3715–0.7085	0.4990–0.6318	0.3190–0.4202	0.2964–0.4748	0.3119–0.3536	0.2625–0.3313
	2						
	Number of accessions	54 (Bc)	42 (Bc)	9 (Bc)	21 (Bc)	5 (Bc)	4 (Bc)
	Content (% of the seed DW)	0.1812–0.3609	0.1944–0.4178	0.2246–0.2734	0.1516–0.2892	0.1813–0.2333	0.0896–0.2067
	3						
	Number of accessions	14 (Sc)	8 (Sc)	16 (Sc)	33 (Sc)	24 (Sc)	33 (Sc)
	Content (% of the seed DW)	0.0019–0.1703	0.0017–0.1725	0.0001–0.0038	0–0.0317	0.0003–0.0221	0–0.0370
Isolupanine	1						
	Number of accessions	8	7	6	15	4	11
	Content (% of the seed DW)	0.0388–0.0704	0.0354–0.0592	0.0272–0.0459	0.0325–0.0515	0.0324–0.0564	0.0255–0.0441
	2						
	Number of accessions	24	19	10	28	7	8
	Content (% of the seed DW)	0.0179–0.0367	0.0155–0.0328	0.0132–0.0248	0.0169–0.0303	0.0122–0.0248	0.0083–0.0191
	3						
	Number of accessions	59	30	17	36	23	27
	Content (% of the seed DW)	0–0.0153	0.0023–0.0122	0–0.0032	0.0003–0.0072	0–0.0042	0–0.0064

Table 3 Selected examples of the total alkaloid content and qualitative composition of the major alkaloids in seeds of the *L. angustifolius* L. collection

Accession catalogue no.	Total alkaloid content (% DW)	Major alkaloids (%)			
		Lupanine	13-hydroxylupanine	Angustifoline	Isolupanine
Wt 95 788	1.45	48.8	32.2	17.3	1.9
Wt 95 740	1.48	6.7	70.1	20.7	2.5
Wt 95 846	1.39	71.4	26.9	1.4	0.4
Wt 95 755	1.83	22.9	45.4	31.5	0.2
Wt 96 143	0.002	45.0	40.1	14.6	0.0
Wt 96 108	0.05	27.3	28.6	10.0	34.2

Table 4 Mean alkaloid contents, their share in the total content for two classes of origin (CO and CV) and three significantly different groups, together with substantial correlations between individual alkaloids and the total alkaloid content in *L. angustifolius* L.

	First group		Second group		Third group	
	Mean content (% DW)	Share in total (%)	Mean content (% DW)	Share in total (%)	Mean content (% DW)	Share in total (%)
CO						
Lupanine	0.8483	39.15	0.6144	41.92	0.2165	34.12
13-hydroxylupanine	0.8925	41.19	0.5594	38.17	0.3147	49.60
Isolupanine	0.0174	0.80	0.0156	1.06	0.0099	1.56
Angustifoline	0.4084	18.85	0.2762	18.85	0.0934	14.72
Total	2.1666		1.4656		0.6345	
Correlations	Lupanine		Lupanine	0.4582**	Lupanine	0.8342**
	13-hydroxylupanine	0.6152**	13-hydroxylupanine	0.2663**	13-hydroxylupanine	0.8978**
	Isolupanine		Isolupanine		Isolupanine	
	Angustifoline	0.6915**	Angustifoline	0.3946**	Angustifoline	0.7136**
CV						
Lupanine	1.0261	53.51	0.7508	52.89	0.0282	43.25
13-hydroxylupanine	0.5479	28.57	0.4198	29.57	0.0239	36.66
Isolupanine	0.0276	1.44	0.0298	2.10	0.0038	5.83
Angustifoline	0.3159	16.47	0.2191	15.44	0.0092	14.11
Total	1.9176		1.4195		0.0652	
Correlations	Lupanine	0.8220**	Lupanine	0.6815**	Lupanine	0.9312**
	13-hydroxylupanine		13-hydroxylupanine		13-hydroxylupanine	0.9687**
	Isolupanine		Isolupanine		Isolupanine	0.5560**
	Angustifoline		Angustifoline		Angustifoline	0.9777**

** Significant level $P = 0.01$

cultivars predominate as a result of the large narrow-leafed lupin acreage and advanced breeding programs including gas chromatography for alkaloid content estimation (Cowling et al. 1998; Swiecicki et al. 2015). In the first and second groups, there are some high alkaloid accessions, sometimes more bitter than Bc (cv. Karo). This is understandable as in this self-pollinated lupin bitter cultivars are used on a limited scale for specific, non-food aims.

In each of the analyzed classes (CO, XD, CV), three significantly different groups were created, but for obvious reasons the most numerous in the CO class were high alkaloid accessions while in the CV class these were low alkaloid accessions. Bc appeared in the middle alkaloid group, while Sc was in the lowest alkaloid group.

Three significantly different groups were also isolated for the four investigated alkaloids and classes of origin. Taking into account lupanine and angustifoline in the CO class, the most numerous was the second group (average content) while for 13-hydroxylupanine and isolupanine the most numerous were accessions with the lowest content (third group). Among the XD class (lines improved by man), a certain tendency directed to increasing the number of accessions in the second and third group (decreased content of individual alkaloids) can be observed.

In the CV class, the most numerous was the third group, with the lowest content of the four investigated alkaloids and the total alkaloid content. This suggests, that most frequently, the decreasing of total alkaloid content was a result of a decreasing of the four major alkaloids. An analysis of contrasts confirms the above (Table 1). For the total alkaloid content as well as lupanine, 13-hydroxylupanine, angustifoline and isolupanine show substantial differences between mean results while comparing classes CO-XD, CO-CV (strongest differences) and XD-CV (CO-Sc, additionally, as mentioned earlier), as a result of breeding improvement aimed towards decreasing alkaloid content.

Very interesting is an analysis of the share (in %) of individual alkaloids in relation to total alkaloid content (http://www.igr.poznan.pl/uploads/resources/Lupinus%20angustifolius.pdf, Table 3). The average share was as follows: lupanine—46.4 % (0.98–73.0 %), 13-hydroxylupanine—35.6 % (15.6–71.1 %), angustifoline—15.5 % (0–49.8 %) and isolupanine—2.5 % (0–34.0 %). This variation is much more broad than hitherto met in the literature (Petterson 1998). It seems that a qualitative content (a share of individual alkaloids in relation to total alkaloid content) can be used in characterization of narrow-leafed lupin accessions and cultivars. It can be also assumed that above average values are typical for *L. angustifolius* L., particularly for high alkaloid wild lines, although 13-hydroxylupanine quite frequently dominates over lupanine. Among investigated material, accessions were selected with almost exclusively (90–100 %) lupanine and 13-hydroxylupanine. Their substantial presence in seed DW can be considered to be species specific. The third major alkaloid is angustifoline. In exceptional cases, its content was equal to lupanine and/or 13-hydroxylupanine. An average content of isolupanine (2.5 %) allows it to be consider a major alkaloid—according to Wink (1995)—abundance >1 % of total alkaloids—although its content below 1 % was stated quite frequently (in some cases it was not revealed). In comparison to the average share of individual alkaloids, the following exceptions were observed—when the proportion of lupanine or 13-hydroxylupanine is lower, the proportion of angustifoline or isolupanine is increased.

Additionally, the influence of the content of individual alkaloids on the total content in seeds DW was investigated in two classes of accession origin (CO and CV). For individual pairs "content of an individual alkaloid—total alkaloid content"—the Pearson correlation coefficient was estimated. Table 4 gives the mean total and individual alkaloid content as a % of the seed DW, and their share as a % of total alkaloid content. Also included is the correlation coefficient of those alkaloids with the biggest positive (substantial at the level 0.01) influence on total alkaloid content. For wild lines (CO class), the biggest influence on total alkaloid content was shown by the content of 13-hydroxylupanine and angustifoline, and additionally lupanine, in the second and third groups (decreased alkaloid content). In the CV class, the biggest positive influence on total alkaloid content was demonstrated by lupanine and in the third group (sweet accessions) additionally by 13-hydroxylupanine and angustifoline. In both CO and CV classes in the third group (the lowest alkaloid content), the total alkaloid content was substantially, positively correlated with lupanine, 13-hydroxylupanine and angustifoline. Isolupanine at very low levels in the narrow-leafed lupin seeds had no substantial influence on total alkaloid content in any of the classes or groups.

The above analyzes of the total content and qualitative composition of alkaloids in narrow-leafed lupin seeds show a much broader variation than that hitherto described in the literature. They show also, that a total alkaloid content in some narrow-leafed lupin accessions is lower than in modern, improved cultivars. A question can be suggested also: is it possible to decrease total alkaloid content via the complete elimination of one major alkaloid?

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