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GENETIC ANALYSIS OF ALKALOID ACCUMULATION IN SEEDS OF WHITE LUPIN (*Lupinus albus* L.)

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In the white lupin (*Lupinus albus* L.), the approach to breed 'sweet' cultivars with a reduced content of alkaloids in seeds and forage is challenging. The study was conducted to advance the limited knowledge on genetic causes controlling the accumulation/composition of alkaloids in seeds of the white lupin. Using a diallel cross design among the chosen 'sweet' and 'bitter' parents, all the range of genetic variation, inheritance mode, type of gene action, and combining abilities were examined among parents and their F_1 and F_2 progenies. Total alkaloid content and individual composition of alkaloids were evaluated by gas chromatography. In the study, a relatively complex mode of alkaloid inheritance was observed. For the proportion of some alkaloids (e.g. sparteine and ammodendrine), the simple additive-dominance model of analysis was less adequate suggesting an incidence of non-allelic interaction and dependent distribution of genes in parents. Both additive and non-additive gene effects were significant for most alkaloids, but the preponderance of gene dominance prevailed. Overdominance (mainly in F_1) and partial or complete dominance (in F_2) of genes was noticed for properties of most alkaloids. Directional dominance was evident and variable, depending on the examined alkaloids. Contribution of non-additive gene effects and the degree of dominance tended

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to decrease among F_2-progenies, suggesting a relatively rapid fixation of additivity in successive hybrid generations. However, selection for the content and composition of alkaloids should be more effective if the still lasting, masking non-additive gene causes would be eliminated in subsequent hybrid generations.

Keywords: Lupinus albus L., seed alkaloids, inheritance, gene action, heritabilities.

INTRODUCTION

Four *Lupinus* species, including the white lupin (*L. albus* L.), are usually grown for animal feeding, human consumption, or as a green manure crops. Useful features of the species are appreciated especially by farmers who are searching for alternative and/or further sources of protein, energy (oil, carbohydrates), lysine and vitamins in end products and/or by those who are advocating the rotational incomes of the crops in more sustainable production technologies (n-fixation by root nodule) (ABRAHAM *et al*., 2019; PETTERSON, 1998; BRUMMUND and ŚWIĘCICKI, 2011; ŚWIĘCICKI *et al.*, 2007, 2015; GAWŁOWSKA and ŚWIĘCICKI, 2007). Although the *Lupinus* species are becoming progressively attractive for the European growers, enhanced concentrations and less acceptable properties of quinolizidine and piperidine alkaloids (ALKs) in the final products (seed meals and forage) appear to be the major anti-nutritional issues that limit their wider use (WINK, 2011; WINK *et al.*, 1995; ANISZEWSKI, 2007; LEE *et al.*, 2007; RESTA *et al.*, 2008; BOSCHIN and RESTA, 2013; ANNICCHIARICO *et al.*, 2014; KROC *et al.*, 2017; FRICK *et al.*, 2017). Hence, the strategy to breed lupin cultivars of a reduced concentration and/or rearranged composition of ALKs in seeds has become exceptionally important.

A broad genotypic variation in the seed accumulation of ALKs was already reported. According to KROC *et al.* (2017) total alkaloid content for 367 accesions in *L. albus* varies from 0.02 to 12.73% of the seed dry weight. For wild lines and primitive populations (CO) the alkaloid content was between 0.06 and 12.73%, for present and old cultivars (CV) between 0.02 and 4.91%. According to HARRISON and WILLIAMS (1982) the level of quinolizidine alkaloids in seeds of *L. albus* varies from 2.2% of the seed DW in the wild forms to 0.02–0.05% of the seed DW cultivars with the gene reducing alkaloid content: *pauper*.

The potential application of this knowledge for lupin improvements was recommended, relatively few innovative efforts have been hitherto made to breed the species for the reduction of harmful seed feature (WINK, 2011; KAMEL *et al.*, 2016). Until now, progress in breeding programs for the reduced ALKs content in lupin seeds may be limited as mainly grounded with results of earlier studies on spontaneous or induced mutants in which decreases in the seed accumulation of ALKs were identified to be associated with simply inherited recessive mutations of various genes/alleles (NOWACKI, 1964; HARRISON and WILLIAMS, 1982; WILLIAMS *et al.*, 1984). In respective lupin crops, from one to few genes were found of which recessive alleles control a decreased total content of ALKs (ŚWIĘCICKI *et al.*, 2015). As pointed by numerous experts, however, less studies have been hitherto directed toward better understanding of the likely more complex genetic scenario of all aspects of alkaloid metabolism in lupines, including biosynthesis pathways and their regulation as well ALKs' transportation and storage within generative and vegetative plant tissues (e.g.WINK, 2011; LEE *et al.*, 2007; BUNSUPA *et al.*, 2012; BOSCHIN and RESTA, 2013; FRICK *et al.*, 2017, KROC *et al.*, 2019a,b). This debate, however, seems to suggest a polygenic nature of the accumulation of ALKs in seeds of the species.

Since the limited knowledge on white lupin genetics may have an impact on the current breeding methodology and goals, attempts for achieving more information on the manner in which ALK seed contents are inherited is essential if the expected nutritional benefits from the reduced contents and/or rearranged compositions of ALKs are to be realized in the species. Hence, following are the two major objectives of this study: (*i*) to assess how the ALK properties in seeds of white lupin are inherited, and (*ii*) to conduct preliminary identification of parental forms and hybrid combinations that are most promising for future breeding initiatives. For that purpose, progenies of diallel crosses done among a collection of accessions representative for a domestic germplasm, were evaluated.

MATERIALS AND METHODS

Plant materials and experimental designs

All crossings excluding reciprocals were made in a diallel fashion among five inbred lines being representative for a domestic collection of white lupin (*Lupinus albus* L.); these parents were previously known to exhibit differences in seed properties and yielding capacity (ŚWIĘCICKI 2008, unpubl.). Among them, the cvs. Kalina and Butan are classified as the so-called 'sweet' forms, the cv. Mustal and the accession of Portuguese origin, WT95264, correspond to the 'bitter' ones, while the cv. Wat represents an intermediary type (Table 1). Seeds of parents and cross-progenies (F_1 and F_2) were examined.

Table 1. Alkaloid accumulation in seeds of parental forms (general means pooled over both experiments)

Parents	Alkaloids [#]									
	ALKs *mg/g* d.w.	SPA *μg/g* d.w.	AMM *μg/g* d.w.	ALB *μg/g* d.w.	ISO *μg/g* d.w.	LUP *mg/g* d.w.	ANG *mg/g* d.w.	FLO *mg/g* d.w.	DEH *mg/g* d.w.	13OH *mg/g* d.w.
WT95	37.51	13.90	324	908	159	27.17	1.26	1.33	3.08	3.27
Kalina	0.49	0.00[*]	25	37	21	0.24	0.02	0.03	0.01	0.12
Mustal	61.60	0.06	523	576	187	47.89	1.34	2.10	4.68	4.31
Wat	10.74	0.01	151	88	49	8.16	0.37	0.44	0.41	1.08
Butan	0.64	0.00[*]	41	2	6	0.40	0.03	0.03	0.02	0.10
$LSD_{0.05}$	*4.15*	*9.06*	*48*	*191*	*13*	*3.81*	*0.11*	*0.12*	*0.42*	*0.34*

[#] - alkaloid abbreviations: ALKs, total amount of all alkaloids; SPA, sparteine; AMM, ammodendrine; ALB, albine; ISO, α-isolupanine; LUP, lupanine; ANG, angustifoline; FLO, 11,12-seco-didehydro-multiflorine; DEH, dehydrooxy-sparteine; 13OH, 13-hydroxy-lupanine;

[*] - trace amounts, ≤ 0.001-0.004 *μg/g*.

In the initial season (2014), the parental lines were reproduced under uniform field conditions and crossed to obtain F_1 seeds. In 2015 and 2016 seasons, all materials were cultivated in two separate experiments. In experiment 1, parents were grown in randomised blocks with five plot-replications. Randomly chosen parental plants (ten plants/replication/cross-combination) were manually crossed, and the harvested parental and hybrid F_1-seeds consisted the 1st part of materials examined for ALKs' quantities. In experiment 2, parents and F_1-hybrids were grown in randomised blocks with three plot-replication, and the harvested parental and F_2-

seeds (up to ten plants/replication/cross-combination) comprised the 2nd part of materials examined for the ALKs.

The field experiments were performed in the experimental farm of the Poznań Plant Breeders Ltd. at Wiatrowo (Poland) using a procedure described by KROC *et al.* (2017). In each season, replicated plots with three 1m-long rows spaced at 25 cm and 15 cm distance between plants were adopted. There were favourable climatic conditions in both seasons. To promote the plant growth and development, standard/optimal agricultural practices (fertilization, weed control, and plant protection) typical for lupin cultivation in central regions of Poland were used.

Data collection

Seed samples for further chemical analyses were collected from individual plants of all generations by hand harvest at the full plant maturity. Total content and compositions of alkaloids in the harvested parental and hybrid seeds (from individual plants of parents and hybrids) were evaluated by gas chromatography following the procedure described by KAMEL *et al.* (2016).

Data analysis

Standard statistic methods were used for data evaluations. The analysis of variance was performed based on individual plot observations using the MSTAT-C package (Michigan Univ. 1993). Means were compared by Duncan's multiple range tests. Using the SAS DGH v.1.0 package (Agric. Univ., Poznań; KALA *et al.*, 1994), variance components, general combining abilities (GCA) and specific combining abilities (SCA), and Mather's genetic parameters were estimated according to the concepts of Hayman's, Griffing's, and Mather and Jinks's for diallel mating, as detailed by KALA *et al.* (1994) and SINGH and CHAUDHARY (2004).

RESULTS and DISCUSSION

Range of genetic variation

In both experiments, parental lines and their hybrid progenies exhibited significant differences in the total content and composition of ALKs in seeds (Table 1, Table 2). The parents exhibited specific variations in the accumulation of ALKs even after they were classified to the same category. For instance, even the most bitter cv. Mustal showed an ability to accumulate largest amounts of alkaloids in seeds, but it accumulated much less sparteine (SPA) and albine (ALB) than the second bitter line WT95264. In turn, the major sweet cv. Kalina usually tended to accumulate smaller amounts of most ALKs in seeds than the other sweet cv. Butan, but the contents of ALB and α-isolupanine (ISO) in seeds of the former were distinctly greater than in seeds of the latter.

In general, the scope of the genotypic variation in most of the ALK quantities among F_2 progenies was within the range of parents. However, markedly more alkaloids were accumulated in F_1 seeds than in parental seeds, which indicate the considerable influence of heterotic effects. On an average, in seeds of the F_1 generation, we found from 32–45% [for dehydrooxy-sparteine (DEH) and ALB] through 69–93% [for all ALKs, lupanine (LUP), ammodendrine (AMM), ISO, 13-OH-lupanine (13OH) and angustifoline (ANG)] to 140–170% [for 11,12-seco-didehydromultiflorine (FLO) and SPA] more alkaloids than in the parental forms. Although the extent of genotypic variation for the contents of all ALKs and those of AMM, LUP, FLO, DEH (and ALB) were found to be partly limited among the F_2 progenies, some positive (but less

attractive here) transgressive effects were observed in the accumulation of SPA and ISO. In both examined generations we were unable to identify transgressive segregations in a negative direction, except for ALB content among F_2 hybrids.

Though parental forms were consciously chosen for this examination, the range of potentially fixable genetic variation, as measured by narrow-sense heritabilities, was found to be relatively narrow (h^2_{NS} = 0.11–0.37, only) in experiment 1 with Ps and F_1s. However, the range of this variation has considerably increased among Ps and F_2s in experiment 2, attaining even h^2_{NS} = 0.73–0.78 for the content of DEH, ANG, LUP, and all ALKs.

Table 2. General means and results of analysis of variance (mean squares) for combining ability effects in Exp. 1 (Ps+F_1s) and Exp. 2 (Ps+F_2s) for the content of alkaloids in seeds of white lupine

Alkaloids [#]	Exp.	Means		Analysis of variance (Ps + F_1s, F_2s)				
		Ps	F_1s F_2s	Entries	GCA	SCA	Error	Baker index [(b]
ALKs (*mg/g* d.w.)	Exp. 1 Exp. 2	22.33 22.06	37.93 26.02	2465.0** 1167.3**	3115.5** 3248.4**	2204.8** 334.9**	81.1 45.0	0.11 0.74
SPA (*µg/g* d.w.)	Exp. 1 Exp. 2 [(a]	3.43 2.16	9.26 77.84	383.3** 24.4**	630.2** 11.3	284.5* 29.6**	111.0 8.6	0.36 -0.33
AMM (*µg/g* d.w.)	Exp. 1 [(a] Exp. 2 [(a]	215.1 209.9	371.2 229.5	178.0** 49.4**	139.8** 131.7**	193.2** 16.6*	7.9 7.3	-0.09 0.78
ALB (*µg/g* d.w.)	Exp. 1 [(a] Exp. 2 [(a]	299.1 345.1	434.2 65.5	478.5** 250.3**	651.6** 519.3**	409.3** 142.6**	27.8 22.7	0.15 0.47
ISO (*µg/g* d.w.)	Exp. 1 [(a] Exp. 2 [(a]	83.8 84.5	146.1 145.3	30.48** 19.67**	25.30** 37.19**	32.56** 12.65**	1.06 2.81	-0.07 0.42
LUP (*mg/g* d.w.)	Exp. 1 Exp. 2	16.96 16.59	28.68 19.88	1470.8** 729.7**	1968.7** 1981.5**	1271.7** 228.9**	49.1 25.2	0.14 0.71
ANG (*mg/g* d.w.)	Exp. 1 Exp. 2	0.602 0.605	1.161 0.664	1.927** 0.716**	1.455** 2.087**	2.116** 0.167*	0.098 0.065	-0.09 0.84
FLO (*mg/g* d.w.)	Exp. 1 Exp. 2	0.797 0.775	1.911 1.010	7.239** 1.342**	3.450** 3.354**	8.754** 0.537*	0.436 0.217	-0.22 0.72
DEH (*mg/g* d.w.)	Exp. 1 Exp. 2	1.614 1.663	2.130 1.451	12.304** 6.346**	20.120** 18.154**	9.177** 1.623**	0.588 0.310	0.27 0.78
13OH (*mg/g* d.w.)	Exp. 1 Exp. 2	1.757 1.794	3.105 2.493	13.331** 6.416**	9.337** 15.533**	14.929** 2.769**	0.635 0.826	-0.13 0.65

[#] - for the alkaloid abbreviations see explanations in Table 1;
[(a] – for demonstrative purposes, MS-values (Anova) for chosen alkaloids were multiplied by (10^{-3});
[(b] – proportions of additive/dominance variance were estimated according to Baker (1978) as $2\sigma^2_g/(2\sigma^2_g + \sigma^2_s)$ ratios;
*, ** – significant at P≤0.05 and P≤0.01 levels, respectively.

Combining abilities

Analysis of variance for the combining abilities was performed for each experiment separately to assess the potential influence of the examined seed generations on noticed effects

of these variance sources. As summarized in Table 2, both GCA and SCA were significant for the content of all alkaloids (except for SPA in experiment 2), independently of the generation evaluated. Hence, both additive and non-additive gene actions were involved in the control of ALKs content and their composition in seeds of the examined white lupin accessions.

Table 3. GCA and SCA effects for the total content of alkaloids (ALKs) and that of particular alkaloids in seeds of F_2-progenies (Exp. 2); parents excluded from the analysis

Parents/Crosses	Alkaloids [#]									
	ALKs *mg/g* d.w.	SPA *μg/g* d.w.	AMM *μg/g* d.w.	ALB *μg/g* d.w.	ISO *μg/g* d.w.	LUP *mg/g* d.w.	ANG *mg/*g d.w.	FLO *mg/g* d.w.	DEH *mg/g* d.w.	13OH *mg/g* d.w.
GCA effects:										
WT95264	17.46**	84.7*	42.08	126.3**	91.43**	13.51**	0.563**	0.621**	1.100**	1.314**
KALina	-15.02**	3.7	-69.40*	22.2	-18.31	-12.12**	-0.262*	-0.564**	-1.022**	-0.997**
MUStal	7.33**	-18.2	47.04	6.3	-8.98	6.60**	0.038	0.076	0.431*	0.159
WAT	-6.09*	-19.0	-39.78	-68.8*	-43.35*	-5.17**	-0.193*	-0.127	0.111	-0.541
BUTan	-3.68	-51.2	20.06	-86.0**	-20.80	-2.83	-0.147	-0.007	-0.620**	0.065
SCA effects:										
WT95 × KAL	6.20 [+]	147.1**	68.07	104.4**	19.56	5.74*	-0.017	-0.032	-0.025	0.192
WT95 × MUS	-5.91 [+]	-90.6 [+]	-43.45	72.1[+]	-61.19*	-4.50 [+]	-0.159	-0.226	-0.242	-0.660
WT95 × WAT	1.94	34.1	-14.38	-70.8[+]	-24.62	2.27	0.007	-0.042	0.293	-0.510
WT95 × BUT	-2.23	-90.6 [+]	-10.24	-105.7**	66.26*	-3.51	0.170	0.300	-0.026	0.978*
KAL × MUS	-11.13**	-63.3	-31.35	-83.9*	-6.81	-9.83**	-0.117	-0.161	-0.555*	-0.277
KAL × WAT	3.52	-60.0	-9.15	-18.9	29.54	3.02	0.111	0.153	0.064	0.230
KAL × BUT	1.41	-23.8	-27.57	-1.6	-42.29	1.07	0.023	0.040	0.516*	-0.144
MUS × WAT	5.38	32.7	30.26	-3.0	43.52	3.30	0.175	0.308	0.466 [+]	1.026*
MUS × BUT	11.66**	121.2*	44.54	14.7	24.48	11.03**	0.101	0.080	0.332	-0.089
WAT × BUT	-10.84**	-6.8	-6.74	92.6*	-48.44[+]	-8.59**	-0.293*	-0.420 [+]	-0.823**	-0.745

[#] - for the alkaloid abbreviations see explanations in Table 1;
[+], *, ** - significant at P≤0.10, P≤0.05 and P≤0.01 levels, respectively.

However, the relative contribution of the GCA and SCA effects for the whole genotypic variation was strongly dependent either upon the particular alkaloid examined or the experiment, i.e. hybrid generation evaluated. Among F_1 progenies, the genetic variance in the content of all ALKs, SPA, LUP, and DEH was mainly associated with the GCA effects, while for the content of AMM, ANG, ISO, FLO and 13OH – the SCA effects were more essential. In contrast, the variance in the content of all alkaloids assessed among F_2-progenies was primarily related with the GCA effects. As evidenced by Baker's indices, the magnitude of GCA-dependent additive variance in F_2 generation usually attained 65–84%, while among F_1s this proportion was not larger than 27–36%. Such generation-dependent alterations in the significance of combining abilities appear to be comparable with those reported by KORUBIN-ALEKSOSKA *et al.* (2014) for nicotine content in tobacco.

General and specific combining abilities when established among F_2s are presented in Table 3. As far as the most expected decrease in ALKs content in seeds is concerned, the most

promising combiner was the sweet cv. Kalina. Noticeably, the reduced ALKs content in seeds of its F_2 progenies was accomplished by similar declines in the accumulation of LUP, FLO, DEH, and 13OH (mainly) and that of AMM and ANG (to a lesser extent). The second attractive combiner was the cv. Wat; in seeds of its progenies essential reductions in the content of ALKs, LUP, ALB, ANG, and ISO were noticed. In turn, the collection line WT95264 was the less accepted combiner contributing to the highest alkaloids contents in its cross progenies.

Considering the SCA effects, the most attractive specific combinations were the F_2 progenies of the crosses Kalina × Mustal and Wat × Butan. No distinct relations between SCAs for some characteristics were observed in hybrid progenies of the second sweet cv. Butan. In the progeny of the cross Mustal × Butan marked increases in ALKs content were noticed, whereas in the progeny of the cross between the sweet cv. Kalina and the bitter Mustal (in contrast to the former cross), critical reductions in the accumulation of ALKs were found. All the observations imply either different alleles acting in parents and/or the effects of recombination between the likely specific parental factors in the examined offsprings.

Genetic components of variance

Analysis of genetic components of variance indicated that majority of them were highly significant for the quantitative properties of all alkaloids in both experiments (Table 4). The component *D*, which measures the influence of additive gene action, was significant for the properties of almost all ALKs in both generations (with some exception for SPA); however, the function of this component was markedly greater among F_2 progenies. The role of gene dominance was found to be prevalent among the F_1 generations as shown by the highly significant H_1 and H_2 estimations for most characters in experiment 1. In turn, the relative contribution of the dominance of genes markedly decreased in experiment 2 with F_2s being less or insignificant mainly for the content of AMM, ANG and FLO. In this generation, the value of H_2 for the last-mentioned alkaloids was not significant suggesting symmetry in the distribution of genes with positive and negative effects in parents, whereas an asymmetrical distribution of these genes was evident for the content of residual alkaloids. Independently on the generation evaluated, the sum of dominance effects over all the heterozygous loci (h_2) was significant for the content of most alkaloids, except for that of AMM, ANG, LUP, FLO, and DEH in the F_2 generation.

The mean degrees of dominance, as measured among F_1- and F_2-seeds by the respective $(H_1/D)^{½}$ and $(0.25H_1/D)^{½}$ estimates, were relatively high in experiment 1 (approximate values: 1.3–3.0) indicating overdominance of genes responsible for the content of seed alkaloids among F_1 progenies. However, with an exception for ISO, the dominance degrees critically decreased (values 0.3–0.7) among F_2 progenies, which has been indicative for the partial dominance of genes governing the alkaloid properties in seeds of this generation. Such a reduction in the significance of the dominance effects in F_2s is rather expected in self-pollinated species due to possible linkages of some loci or gene interactions (e.g. LI and CHANG, 1970). Except for SPA (in F_1) and ISO (in F_2) the *F* values were positive and highly significant, showing more frequent dominant than recessive alleles in parents, irrespective of whether or not these have increasing or decreasing effects on the examined alkaloids contents. Nevertheless, the negative *F* found for SPA (in F_1) suggests enhanced frequency of recessive alleles that were responsible for the sparteine accumulation in F_1-seeds. In both experiments, the contribution of H_1 was frequently larger than H_2, suggesting that positive and negative alleles at loci responsible for most

characters were unequally distributed in parents. The ($H_2/4H_1$) ratios – which are further proofs for average proportions of negative (v) and positive (u) alleles of genes showing dominance in parents – were usually lower than 0.25, indicating their unequal frequencies at all the loci involved. General accordance between the F, ($H_2/4H_1$) and H_2 estimates was noticeable here.

Table 4. Genetic components of variance and chosen genetic parameters for the content of alkaloids in seeds of white lupine in Exp. 1 (Ps + F_1s) and Exp. 2 (Ps + F_2s); coefficients of regressions (b) for Wr/Vr, correlations (r) between (Wr+Vr) and parental means (Yr), and the order of dominance in parents for ALKs' contents in Exp. 2 are included (ALKs, all alkaloids; SPA, sparteine; AMM, ammodendrine; ALB, albine; ISO, α-isolupanine; LUP, lupanine; ANG, angustifoline; FLO, seco-didehydromultiflorine; DEH, dehydrooxosparteine; 13OH, 13-OH-lupanine)

Genetic components (parameters)	ALKs *mg/g* d.w.	SPA *µg/g* d.w.	AMM *µg/g* d.w.	ALB *µg/g* d.w.	ISO *µg/g* d.w.	LUP *mg/g* d.w.	ANG *mg/*g d.w.	FLO *mg/g* d.w.	DEH *mg/g* d.w.	13OH *mg/g* d.w.
Exp. 1 (Ps + F_1s)										
			($\times 10^{-3}$)#	($\times 10^{-3}$)#	($\times 10^{-3}$)#					
D	533.0**	28.8+	32.7**	101.9**	4.95**	317.5**	0.303**	0.563**	3.306**	2.649**
F	529.7**	-32.9+	40.0**	83.7**	5.65**	301.3**	0.354**	0.935**	2.979**	3.645**
H_1	1360.5**	95.3+	115.1**	249.6**	19.62**	786.7**	1.223**	5.125**	5.909**	9.019**
H_2	1119.9**	104.9*	99.5**	220.3**	17.06**	643.1**	1.079**	4.379**	4.718**	7.549**
h_2	623.4**	86.9*	62.3**	46.7**	9.95**	351.5**	0.799**	3.176**	0.682*	4.655**
$(H_1/D)^{1/2}$	1.60	1.82	1.88	1.57	1.99	1.57	2.01	3.02	1.34	1.85
$H_2/4H_1$	0.206	0.275	0.216	0.221	0.217	0.204	0.221	0.214	0.200	0.209
h^2_{NS}	0.292	0.350	0.135	0.281	0.172	0.319	0.139	0.137	0.369	0.105
Exp. 2 (Ps + F_2s)										
			($\times 10^{-3}$)#	($\times 10^{-3}$)	($\times 10^{-3}$)#					
D	585.8**	n.e.##	35.1**	148.6**	5.16**	345.7**	0.339**	0.628**	3.763**	2.926**
F	196.9**	n.e.	26.6**	118.4**	1.02	103.8**	0.073*	0.311**	1.836**	1.325**
H_1	340.6**	n.e.	12.3	105.3**	9.73**	236.1**	0.129*	0.387*	1.619**	2.119**
H_2	256.3**	n.e.	6.9	82.5**	8.14**	184.0**	0.086	0.246	1.095**	1.607**
h_2	40.0	n.e.	1.0	200.1**	9.46**	27.7	0.009	0.142	0.116	1.254*
$(0.25\ H_1/D)^{1/2}$	0.381	n.e.	0.296	0.421	0.687	0.413	0.308	0.393	0.328	0.425
$H_2/4H_1$	0.188	n.e.	0.139	0.196	0.209	0.195	0.168	0.159	0.169	0.190
h^2_{NS}	0.750	n.e.	0.628	0.485	0.491	0.730	0.781	0.632	0.765	0.610
b (Wr/Vr)	1.21	0.01(bb	1.58(b	1.20	0.62	1.14	0.97	1.21	1.27	0.83
r (Wr+Vr)/Yr	0.18	0.47	0.31	0.97**	- 0.84+	0.22	- 0.37	0.09	0.58	- 0.45
Dominance order(a	1 2 4 5 3	3 5 4 1 2	1 4 2 5 3	5 4 2 3 1	1 3 4 2 5	1 2 4 5 3	1 2 4 3 5	1 2 4 3 5	2 1 5 4 3	1 2 4 3 5

– for demonstrative purposes, estimated values of genetic components (i.e. D, F, H_1, H_2, h_2) for AMM, ALB and ISO were multiplied by (10^{-3});
– not estimated since error variance > parental variance; (a – parents: 1 - WT95; 2 - Kalina; 3 - Mustal; 4 - Wat; 5 - Butan;
(b, (bb - different from zero at P≤0.05 and P≤0.01 levels, respectively; +, *, ** – significant at P≤0.10, P≤0.05 and P≤0.01 levels, respectively.

Direction and the order of dominance

As far as data from the experiment 2 (Ps + F_2s) are concerned, coefficients of regression (b) of array covariances (Wr) on array variances (Vr) did not differ from unity for the content of most ALKs (Table 4). But, these coefficients were significantly different from unity for SPA and

AMM contents, which indicate the potential failure to satisfy some assumptions of a diallel analysis. This effect could be associated either with non-allelic/epistatic interactions, correlated gene distributions, and/or reduced variation range among the F_2s (e.g. for SPA).

Table 4 also shows correlations (*r*) between the order of dominance of parents (Wr+Vr) and parental measurements (Yr) in this material. The *r*-values were found to be different for various alkaloids, although significant mostly for ALB and ISO accumulations in seeds. The positive correlations indicated that the direction of dominance was towards a reduced content of ALB (mainly) and likely also that of DEH, AMM and LUP (to a lesser extend). As unexpected rather, genes that increase the accumulation of these ALKs were more often recessive and the parents WT95264 and Mustal appeared to possess most of them. In turn, the correlations between (Wr+Vr) and Yr for ISO (mainly), 13OH and ANG contents were negative, showing dominance in the direction of an increased accumulation of these alkaloids in seeds. Thus, particularly the parental cvs. Butan and Kalina with reduced ISO contents had the most recessive genes responsible for a lesser accumulation of this alkaloid in seeds.

The final interpretations that have to be drawn on the manner of gene actions governing the alkaloid properties in white lupin seeds are dependent on the answer whether the additive-dominance model of our analysis was reasonable to explain the observed variation. The present study was performed with a supposition that at least major assumptions for an adequacy (or partial adequacy) of the model (like homozygous parents, diploid segregation, no epistasis, linkage and/or lethal genes, etc.) were satisfied and this was also confirmed by the used statistic DGH-package for most, but not for all characteristics of ALKs examined. The model was found to be less or inadequate for some ALKs suggesting non-allelic interactions, linkage of genes, or reciprocal effects important for the variation in the characteristics. However, some of the assumptions could only be partly verified following an analysis of a complete diallel. Since a half diallel was evaluated in our study, there was no opportunity to identify the reciprocal causes. This appears to be an important question, especially when the alkaloid properties in hybrid seeds of lupin are evaluated.

In the present study, the measured seeds of F_1 generation were harvested on maternal plants, while those of F_2 generation were formed and harvested on F_1 plants. Lupin alkaloids are translocated into seeds from the photosynthesising tissues where they are synthesized (i.e. in chloroplasts). Hence, the level of alkaloids and their transportation could also be affected either by nuclear or chloroplastic genes that modulate the photosynthetic system. Since alkaloids in lupin seeds are originating from green parts of a female plant, effects of relevant genes that control ALK concentration in seeds could be substantially influenced by the maternal genotype/phenotype, as frequently evidenced in *Lupinus* species in earlier study of a research team from the University of Reading in UK (e.g. HARRISON and WILLIAMS 1982; WILLIAMS *et al.*, 1984).

Given the potential reciprocal influences on seed ALKs in lupin, we must consider that in some cases of the present study still only a partially adequate model could be used in the analysis. Therefore, a caution is needed when interpreting some data. Further understanding of how all those factors are interacting may be valuable for breeding practice that deals with alterations of ALK properties in white lupin.

The studied parental forms of white lupin and their hybrid progenies were found to exhibit distinct differences in the content and composition of alkaloids in seeds. Obtained data showed that the accumulation of alkaloids in seeds of white lupin is likely controlled by a relatively

complex polygenic system. This observation seems to be in accordance with the data previously reported for alkaloids in *Papaver* and *Nicotiana* (e.g. KORUBIN-ALEKSOSKA *et al.*, 2014; YADAV *et al.*, 2009; DEWEY and XIE, 2013; WANG and BENNETZEN, 2015).

CONCLUSIONS

The studied parental forms of white lupin and their hybrid progenies were found to exhibit distinct differences in the content and composition of alkaloids in seeds. Obtained data showed that the accumulation of alkaloids in seeds of white lupin is likely controlled by a relatively complex polygenic system.Although the 'sweet' cv. Kalina appears to be a promising source for lupin breeding that deals with the reduced concentration of ALKs in seeds, the magnitude of additive variation in local collections is still relatively narrow. Hence, search for innovative 'sweet' accessions of white lupin is substantiated if novel sources of 'sweet' genes, diversification of genetic base and faster breeding progress are challenging. Expression of the second examined 'sweet' cv. Butan and its progenies seem to corroborate this postulation.

From the obtained results, apparently the contribution of the dominance of genes for the variation in total ALKs content and their composition is, likely, still substantial in local early hybrid populations. But, the importance of the non-additive gene effects and the degree of dominance tended to decrease in F_2, suggesting a relatively rapid fixation of additivity in successive hybrid generations, especially for ALKs content. The promising results indicated a possibility to reduce the content and/or alter the composition of ALKs in white lupin seeds. The dominance effects still operate in the examined F_2 generations, suggesting that those effects cannot be ignored, and to eliminate their masking outcomes such selection, especially for the ALKs composition, should be prolonged for the possibly late hybrid generations.

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GENETIČKA ANALIZA AKUMULACIJE ALKALOIDA U SEMENU BELE DETELINE (*Lupinus albus* L.)

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Izvod

U beloj lupini, pristup da se oplemenjuju „slatke“ sorte sa smajnim sadržajem alkaloida u semenu i krmi je izazov. Studija je pokrenuta da bi se unapredilo ograničeno znanje o genetičkom uzroku koji kontroliše akumuliranje / kompoziciju alkaloida u semenu bele lupine. Korišćenje dialenog ukrštanja odbranih „slatkih“ i „gorkih“ roditelja, sav opseg genetičkog variranja, modul nasleđivanja, tip akcije gena i kombinaciona sposobnost su ispitani između roditelja i njihovog F_1 i F_2 potomstva. Ukupan sadržaj alkaloida i pojedinačna kompozicija alkaloida su ispitani pomoću gasne hromatorafije.

U ovom radu relativno kompleksni način nasleđivanja alkaloida je primećen. Za deo istih alkaloida (spartein i ammodendrine), jednostavan aditivno-domitatan model analize je manje adekvtan ukazujući na pojavu nealelne interakcije i zavisne distribcije gena u roditeljima. Oba aditivni i ne aditivni genski efekat je značajan za mnoge alkaloide ali je preovladala dominatnost. Overdomantnost (uglavnom F_1) i delimična ili kompletna dominatnost (u F_2) gena je zapažena za svojstva većine alkaloida. Usmerena dominatnost je očigledna i varirajuča, zavisno od ispitanog alkaloida. Doprinos ne aditivnom efektu gena i stepen dominatnosti skloni su smanjenju između F_2 potomstva, ukazujući na relativno brzu fiksaciju adativnosti u uspešnoj hibridnoj generaciji. Selekcija za sadržaj i kompoziciju alkaloida morala bi da bude efikasnija ukoliko još traje, trebalo bi eliminisat u sledećoj hibridnoj genraciji uzrok neaditivnih gena u sledećoj hibridnoj generaciji.

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