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GENETICAL CONTROL OF ALKALOIDS IN *LUPINUS ALBUS*

JILL E. M. HARRISON and WATKIN WILLIAMS

Department of Agricultural Botany, University of Reading, England

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INDEX WORDS

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SUMMARY

The pattern and concentration of component alkaloids in wildtype and mutant genotypes homozygous for the alleles *pauper*, *exiguus* and *nutricius* in *L. albus* have been determined. Alkaloid production in genotypes homozygous for identical and complementary alleles have been determined. The study has revealed at least two alleles possessing different effectiveness in reducing alkaloid levels at the *pauper* locus, and the frequency distribution of alkaloid concentration in F_2 indicates dominance of the high-alkaloid allele is incomplete. Seven alkaloids were identified in the wild-type genotype, of which four were found in all homozygous mutant genotypes in approximately similar proportions. The mutations identified by means of the Dragendorff reaction appear to affect a common substrate without affecting the amount of the different component alkaloids that are produced.

INTRODUCTION

Genetically-determined, low-alkaloid phenotypes in the genus *Lupinus* were first reported in *Lupinus luteus* by VON SENGBUSCH (1930). Low-alkaloid mutations have since been reported in *L. albus* and *L. angustifolius* (GLADSTONES, 1970; WALLER & NOWACKI, 1978). Of the 10 recessive mutations that have been listed by various authors, it has been established that *pauper, mitis, reductus, exiguus* and *nutricius* are alleles at different loci. Tests on the alleles *suavis* and *minutus* are incomplete and their independence cannot be authenticated, while *primus* and *tertius* have been clearly identified as synonyms of *pauper*. *Minimus*, listed in WALLER & NOWACKI (1978), is probably a misprint of *minutus* (HACKBARTH, 1961; PORSCHE, 1964).

None of the mutations completely eliminates alkaloids which in the wild-type genotypes may attain 1.5–2.2 per cent of the dry matter. Of those studied in detail, *pauper*, now represented in many 'sweet' cultivars, is the most effective in reducing alkaloid levels: homozygous genotypes of *pauper* possess 0.02–0.05 per cent alkaloid while homozygotes for other mutant alleles e.g. *nutricius*, contain up to 0.1 per cent.

Wild-type genotypes of *L. albus* contain up to 10 different quinolizidine alkaloids of which lupanine is predominant (SCHWARZE & HACKBARTH, 1957; HUDSON & ZAND-MOGHADDAM, personal communication). Data on the levels of the other alkaloids are inconsistent, with different authors reporting widely different values. The alkaloid profile in homozygotes for *pauper* (e.g. cv. Gela) and *exiguus* (e.g. cv. Neuland) were reported by SCHWARZE & HACKBARTH (1957) to resemble the wild-type in that lupan-

ine was the predominant component with lower concentrations of oxalupanine and sparteine, whereas HUDSON & ZAND-MOGHADDAM (pers.comm.) recorded only lupanine in *pauper* genotypes.

This study was undertaken to establish the pattern and concentration of alkaloids in the wild-type and in genotypes homozygous for the three mutant alleles, *pauper, exiguus* and *nutricius*. Alkaloid levels in genotypes which have hitherto been assumed to be homozygous for identical alleles were also determined.

MATERIALS AND METHODS

Low-alkaloid phenotypes which show no reaction with the Dragenhoff reagent are described as 'sweet' and high-alkaloid forms which are Dragendroff positive as 'bitter'. This separation also reflects the human taste reaction. The cultivars used, their origin and genotype in respect of alkaloid-controlling genes were as follows:

Cultivar	Origin	Allele
Sweet		
Kievskij Mutant	Kiev, USSR	*pauper*
Kievskij Skorospely	Kiev, USSR	*pauper*
Astra	Chile	*pauper*
SSK 79	Georgia, USA	*pauper*
Ultra	FDR	*pauper*
Neuland	FDR	*exiguus*
Nahrquell	DDR	*nutricius*
Shinfied	Reading, UK	?
Gyulantania	Hungary	?
Bitter		
Lupini Bean	Italy	Wild type

The material was grown under glass in pots under standardised cultivation conditions but plants belonging to different generations were not grown concurrently.

Extraction procedure. The following method developed by A. B. BECK (private communication) was used. One g of finely chopped seed was strirred into 50 ml of boiling 50% ethanol and left to cool overnight at 4°C. After decanting, the seed tissue was extracted with four sequential amounts of 70% ethanol, which were also left overnight at 4°C. The combined extracts were evaporated under vacuum at 40°C to about 2 ml, the pH adjusted to 4–4.5 with sulphuric acid, and left overnight at room temperature when the pH was lowered to 2.5 and solid material separated by centrifugation. Lipids were removed with two lots of dichloromethane in a separating funnel and pH again adjusted to 9–9.5 with sodium hydroxide. The alkaloids were then extracted with three lots of chloroform and after adjusting the pH to 10.5–11, two further chloroform extractions performed. After drying on anhydrous sodium sulphate the combined extracts were evaporated to dryness under vacuum at 40°C and residual alkaloids transfered into 2 ml of chloroform which was evaporated in a stream of warm air. The residue was finally dissolved in 1 ml of chloroform per g of original seed

tissue.

The method was scaled down so that one seed or even half a seed could be tested, but with dried plant materials greater quantities of tissue and of solvents were employed.

Separation of component alkaloids. The alkaloids were initially separated by thin layer chromatography but this method was discontinued after satisfactory techniques of gas-liquid chromatography had been developed. G.L.C. methods had the advantage for this study of detecting small amounts of alkaloids and of enabling direct quantitative estimates of concentrations.

The G.L.C. method was developed from techniques described by CRANMER & MABRY (1966) and LLOYD et al., (1960). Seven-foot glass columns with 4 mm internal diameters using three phases – 3% S.E. 30, 3% X.E. 60 and 5% D.C. 560, all on 80–100 mesh, acid washed Chromosorb W gave satisfactory results and each was employed at different times. A temperature of 223°C was suitable with all three phases with a flow rate of approximately 40 ml/min for the nitrogen carrier. The instruments were Pye 104 and 204 with a flame ionisation detector and standard samples of sparteine, lupanine, isolupanine, oxalupanine and 13-hyroxylupanine were used as reference.

Relative concentrations calculated from the peak areas proved to be the most reliable of several estimation methods tried. Standard samples of alkaloids were not available in sufficient quantities to establish absolute amounts from peak areas, which need not indicate equivalence in weight because not all alkaloids produce equal responses in the detector.

Determination of alkaloid content. Although G.L.C. gave a fairly accurate estimation of the proportion of the alkaloids in extracts, titration proved more satisfactory for determining total alkaloid in high-alkaloid phenotypes. This method was not sufficiently sensitive to measure alkaloids in low-alkaloid seed for which relative concentrations were calculated from areas of G.L.C. traces.

For titration, alkaloid dissolved in methanol was added to a known volume of 0.005 M sulphuric acid and titrated with 0.01 M sodium hydroxide, using methyl red as indicator: blank titrations were also carried out with acid alone. Percentage alkaloid was calculated from:

$$\frac{\text{Volume of 0.01 M NaOH}}{\text{Weight of seed (g)}} \times \frac{248}{1000}$$

where the volume of NaOH is the differences in ml between the blank titration and the sample, and 248 the molecular weight of lupanine, the prevalent alkaloid.

For high-alkaloid extracts, alkaloid concentration was calculated as percentage of seed dry weight, this being a more reliable measure than total alkaloid per seed when seed size is variable.

The Dragendorff test. The Dragendorff reagent is an orange solution which gives a brown reaction with liquids containing alkaloids in excess of 0.5 per cent. In this study tests were made using the reagent dried on filter paper onto which sap from test plants was expressed from the base of young flowers. Seeds to be tested were soaked in water for two hours and a cut surface pressed against reagent paper.

Table 1. Component alkaloids (per cent of total) in wild-type and mutant genotypes.

Cultivar	Genotype	Alkaloids						
		lupanine	13-hydroxy-lupanine	dehydro-angusti-foline	dehydro-lupanine	oxa-lupanine	oxa-sparteine	iso-spart-eine
Lupini Bean	Wild type	63.2 (3.5)	12.6 (1.5)	12.5 (2.5)	2.4 (0.4)	4.2 (0.8)	4.5 (0.5)	0.6 (0.1)
Kievskij Mutant	*pauper*	28.7 (4.3)	49.0 (3.1)	11.1 (1.0)	11.3 (1.7)	–	–	–
Kievskij Skorospely	*pauper*	40.9 (4.2)	48.0 (5.6)	6.5 (1.3)	4.7 (0.7)	–	–	–
SSK 79	*pauper*	42.6 (4.0)	35.1 (3.7)	12.2 (2.3)	10.1 (1.5)	–	–	–
Astra	*pauper*	40.2 (4.3)	35.9 (3.4)	13.3 (1.5)	10.6 (1.7)	–	–	–
Shinfield	?	51.6 (2.7)	31.8 (2.5)	10.2 (3.7)	6.4 (1.5)	–	–	–
Gyulatania	?	37.4 (6.8)	39.9 (5.8)	9.4 (1.9)	13.3 (2.5)	–	–	–

( ) SE, n = 8.

Table 2. Alkaloid concentration in parents, F_1 and F_2 seed.

Cultivar name	Relative alkaloid			% Alkaloid F_2 wild-type seed[2]
	cultivar	F_1 mean[1]	F_2 low-alkaloid seed[2]	
Kievskij Mutant	1.60 ± 0.12	1.56 ± 0.25	1.92 ± 0.12	0.83 ± 0.04
Kievskij Skorospely	1.62 ± 0.23	1.64 ± 0.13	2.39 ± 0.17[3]	0.79 ± 0.03
SSK 79	2.94 ± 0.12	–	2.88 ± 0.15	0.92 ± 0.04
Astra	3.24 ± 0.27	3.33 ± 0.11	2.34 ± 0.17	0.95 ± 0.04
Shinfield	3.71 ± 0.07	3.75 ± 0.14	–	0.84 ± 0.02
Gyulatania	4.36 ± 0.23	4.30 ± 0.17	–	1.05 ± 0.03

$\pm$ = SE, n = 8.
[1] Female parent as in col. 1.
[2] Mean of all hybrids involving cv. in col. 1.
[3] Kievskij Skorospely gave high F_2 value with SSK 79.

RESULTS

The alkaloids of wild type and mutant cultivars. In the wild type Lupini Bean seven alkaloids were present in quantities sufficient to yield measurable peaks. Lupanine was the predominant alkaloid (see Table 1): only two others, dehydroangustifoline and 13-hydroxylupanine, reached 12 per cent concentration. The records for oxaspartine, isosparteine and oxalupanine which were detected only in the wild type differ from results obtained in the two cultivars Lupini Bean and VLS (HUDSON & ZAND-MOGHADDAM, pers.comm.). The alkaloid profile of the mutant genotypes also differ from publised data in that 4 of the 7 alkaloids in the wild type were also detected in the mutant, and in approximately similar proportions whereas other reports have

recorded only lupanine in *pauper* genotypes. The proportion of lupanine in the mutants is significantly less, and of 13-hydroxylupanine correspondingly more than in the wild type and there is indication that the allele *pauper* in Kievskij Mutant produces substantially less lupanine than in the other three *pauper* cultivars. This needs further substantiation, however, since F_1 seed derived from crossing Kievskij Mutant ♀ with either wild-type or low-alkaloid ♂ parents possessed 34–51 per cent lupanine concentration which resembled comparable progeny from other *pauper* genotypes. Since the level and profile of alkaloids are maternally-inherited, consistent differences in lupanine concentration would be detectable in F_1 seed.

The alkaloid content of F_1 and F_2 seed. The relative alkaloid content in F_1 and F_2 seed of families from all possible crosses involving the low-alkaloid cultivars is given Table 2. F_1 seed from all these parents was 'sweet' irrespective of the parental genotypes, but the relative total alkaloid concentration of different parents and of crosses of homozygotes for the allele *pauper* differed significantly. The two Kievskij cultivars possess identical alkaloid values and were the 'sweetest' of the genotypes tested, while Astra, also homozygous for *pauper*, and Gyulatania, homozygous for an unknown allele, had significantly higher values. It has not been established whether differences in alkaloid in the cultivars is due to the effect of gene background or to differences in function of the low-alkaloid alleles. Even the *pauper* genotypes which have been characterised on complementarity tests as genetically identical, could reflect a multiple allelic series at the *pauper* locus.

The range and differences in alkaloid levels among the cultivars were confirmed in F_1 seed while the concentration in wild-type and low-alkaloid F_2 seed fully reflect the genotype of F_1 plants.

The mean values of low-alkaloid and bitter F_2 seed do not establish clearly that the range of alkaloid values in the *pauper* genotypes is controlled by different alleles at the *pauper* locus. However, reference to Table 3 where crosses involving *pauper* genotypes are set out separately show that the high-alkaloid cultivars SSK 79 and Astra consistently yield higher alkaloid levels in F_2 seed than the two Kievskij cultivars, suggesting that there may be at least two *pauper* alleles represented among the 4 parents which do not show genetic complementarity in F_1. Similarly, Gyulatania and Shinfield and the F_2 bitter segregates in their crosses with Lupini Bean (Table 4) indicate that the also contain alleles which differ in activity from each other and from that of *pauper*.

Segregation in the F_2 generation. All segregating F_2 families gave good agreement either with monogenic segregation, or where parents having complementary alleles were involved, with 9:7 ratios. There is no evidence that more than one major mutant allele which might have accounted for differences in alkaloid concentration between the cultivars is operating in any of the parents.

The segregation pattern of alkaloid concentration in 101 plants derived from crosses between wild-type and mutant cultivars can be seen from Figs. 1a, b and c. The data are from F_3 seed resulting from selfing F_2 plants and therefore reflect relative alkaloid status of F_2 genotypes. Interest in the data centres on the two frequency peaks present in all the families and in the consistent tendency for the higher peak to be associated with the lower alkaloid concentrations which agrees with the assumption that dominance of alleles determining high-alkaloid is incomplete. Further evidence from

Table 3. Relative alkaloid concentration in F_2 seed derived from crosses between *pauper* genotypes (mean of reciprocal crosses).

	Kievskij Mutant	Kievskij Skorospely	Astra	SSK 79
Kievskij Mutant	1.60 a[+]			
Kievskij Skorospely	1.81 ab	1.62 a		
Astra	2.07 bc	2.28 c	3.24 d	
SSK 79	1.83 ab	3.80 c	3.00 d	2.94 d

[+]Different lettered group = differences at p = 0.05.

Table 4. Alkaloid concentration in F_1 and F_2 of reciprocal crosses of mutant genotypes with wild type cultivar, Lupini Bean.

Parents	Lupini Bean	Kievskij Mutant	Kievskij Skor.	Astra	SSK 79	Gyula-tania	Shin-field
F_1 seed							
Lupini Bean ♀[1]	0.76	0.74	0.72	0.73	0.75	0.78	0.76
Lupini Bean ♂[2]	–	2.02	1.69	–	–	4.44	3.40
F_2 (high-alkaloid segregates)							
Lupini Bean ♀[1]	–	0.84	0.78	0.89	0.79	1.26	1.04
Lupini Bean ♂[2]	–	0.84	0.74	–	–	1.42	1.02

[1]Percentage alkaloid.
[2]Relative alkaloid concentration.

crosses within the species *L. angustifolius* and from the function of other low-alkaloid alleles in *L. albus* (HARRISON, 1980) indicates that incomplete dominance is a common characteristic of alleles controlling alkaloid content in *Lupinus*.

CONCLUSION

The work has revealed wide variation in alkaloid concentration between 'sweet' cultivars carrying alleles at a single locus as well as between those homozygous for mutant alleles at independent, complementary loci. The study has also revealed that at least two different alleles probably exist at the *pauper* locus, one in the two Kievskij cultivars and at least one in the cultivars Astra and SSK 79; the genotypes Shinfield and Gyulatania possess two other alleles which are complementary to each other as well as to *pauper*.

The frequency distribution of alkaloid level in the high-alkaloid segregates in F_2 families have indicated that dominance for alkaloid level is commonly incomplete.

Apart from minor differences, the alkaloid profiles in homozygous, mutant genotypes resembled closely those in the wild type. This supports the view that the mutations for low alkaloid production detected so far, act to reduce the level of substrates which are common to all the alkaloids rather than an intermediate substrate at a relatively late stage in biosynthesis when chemical differences between the various alkaloids are being finally specified. Since the mutants available have been exclusively detected

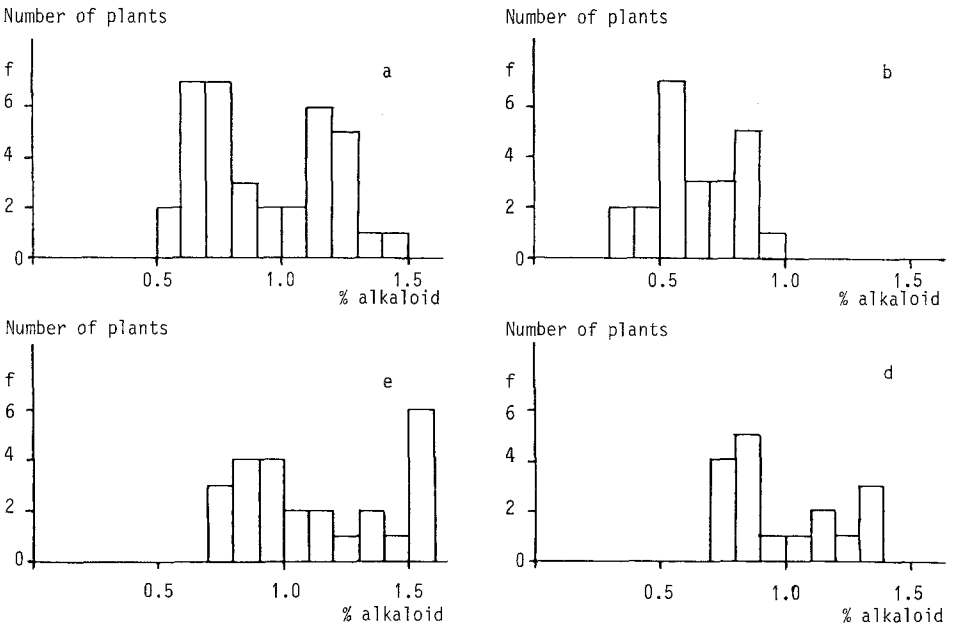

Fig. 1. Percentage alkaloid in bitter F_2 plants from crosses involving Lupini Bean. (Data from analyses of F_3 seed).
a) Lupini Bean × Kievskij Mutant (*pauper*); b) Lupini Bean × Kievskij Skorospely (*pauper*); c) Lupini Bean × Gyulatania; d) Lupini Bean × Shinfield.

by the Dragendorff test which relates only to total alkaloid content, mutations affecting the biosynthesis of individual alkaloids, especially those other than lupanine, would not necessarily be detected.

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