

STUDIES ON THE HYBRID *LUPINUS GRAECUS* BOISS. X *LUPINUS JUGOSLAVICUS* KAZIM. ET NOW.¹⁾

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Hitherto performed breeding work proved that *Lupinus albus* hybridizes with *L. jugoslavicus* (Kazimierski, 1960), *L. termis* (Kazimierski, 1961) and *L. graecus* (Kazimierski — unpublished data). In order to reveal the evolutionary history of *L. albus* and to determine experimentally the degree of relationship between the mentioned forms, *L. graecus* and *L. jugoslavicus* were hybridized.

Present work is aimed at providing a genetic analysis of some of the hybrid properties.

MATERIAL AND METHODS

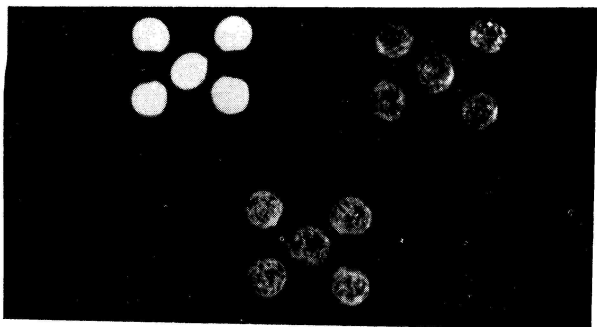
Lupinus graecus Boiss. Seed originated from Bulgaria. This primitive form of lupin, bears in Poland fruit solely under glasshouse conditions, where it remains a 10—12 leaved basal rosette; under field conditions it develops from 23 to 43 leaves. The leaves are small-sized, dark-green, nearly elliptical. Plants attain 30 cm or so in height. Dark-blue flowers with a violet shade stem from bilabiate calyxes. Dehiscent pods, averaging 6.5 cm in length (Phot. 2) and 1.1 cm in breadth are six-seeded. Small, white seeds (Phot. 1) average 150 g in the 1000 seed weight. Field-planted, it opens into flower late, and often perishes from drought. Plant raw material is bitter in taste; seed treated with a quartz lamp becomes fluorescent and is found to contain the following alkaloids: lupanine, multiflorine, hydroxylupanine, the n_7 alkaloid and lupaninic acid.

Lupinus jugoslavicus Kazim. et Now. Seed originating from Yugoslavia, was spared us by the T. A. A. in Moscow, (cf. Kazimierski and

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Nowacki, 1961). In its morphological and physiological aspect this form resembles *L. graecus*, from which it differs by the deeper coloration of dark-green leaves, dark-bluish-violet flowers and colored seeds (Photos 1 and 2). The alkaloid set of *L. jugoslavicus* is similar to that of *L. graecus*.



Phot. 1. Parent and F₁ seeds. Left: *L. graecus*; right: *L. jugoslavicus*; middle below: F₁

Parent and hybrid seeds were planted in a glasshouse, in pots containing 30 kg soil, 4 seeds per pot. Cross-fertilizing was also performed in the glasshouse. Throughout the growing season, leaf coloration, growth rate, external form, terms of flowering and ripening, and the colour of



Phot. 2. Parent and F₁ pods. Left: *L. jugoslavicus*; right: *L. graecus*; middle below: F₁

flowers were closely recorded. Following biometric measurements were taken from mature plants: entire length (height), length of side branches, distance from soil level of the lowest first-order branching, length and breadth of pods, number of seeds per pod. In addition, pod colour and dehiscence were determined. At time of bloom pollen was collected from

each flower for the performance of viability tests. Seed fluorescence was examined under a quartz lamp (L a t a w i e c, 1958) and the level and composition of alkaloids determined by means of paper chromatography (N o w a c k i, 1963).

RESULTS

L. graecus and *L. jugoslavicus* readily cross-fertilize. Five *L. graecus* flowers fertilized with *L. jugoslavicus* pollen yielded two pods including four seeds. The hybrid seeds planted in pots germinated satisfactorily into seedlings, distinguishable from parent seedlings by their vigour. This hybrid vigour persisted until maturation. Growth rate of the F_1 hybrids was the same as in parent plants. At time of blossoming of the main shoot inflorescences, there was no difference between parents and F_1 offspring (Tab. 1). First-order lateral inflorescences opened in the hybrid F_1 on the average five days earlier than in *L. jugoslavicus* (Tab. 2). *L. graecus* developed reduced lateral shoots of the first-order; they remained flowerless.

Table 1
Variability in main stem flower initiation in parents and both hybrid generations

Form, generation	Year	April					May			Number of tested plants
		16—18	19—21	22—24	25—27	28—30	1—3	4—6	7—9	
<i>L. graecus</i>	1962	2	2	2						6
<i>L. jugoslavicus</i>		3	7							10
F_1		2								2
<i>L. graecus</i>	1963			1	2	7				10
<i>L. jugoslavicus</i>			1		1	1				3
F_2			14	10	3	4			1	32

Table 2
Variability in flower initiation on 1st order lateral branches in parents and both hybrid generations

Form, generation	Year	May							Number of tested plants
		1—3	4—6	7—9	10—12	13—15	16—18	19—21	
<i>L. graecus</i>	1962								
<i>L. jugoslavicus</i>				3					3
F_1		1	1						2
<i>L. graecus</i>	1963								
<i>L. jugoslavicus</i>					1			2	3
F_2		1		1	9	6	1	4	22

Hybrid F_1 's produced dark-blue flowers with a violet shade; they blossomed abundantly and, under glasshouse conditions, set pods more efficiently than did the parent forms. Stems, leaf stalks and pods were covered with dense hairs. Dehiscent pods contained coloured seeds of an intermediate size (Tab. 3; Photos 1, 2), hard, fluorescent under a quartz lamp, high-alkaloid and similar to the parent forms in their alkaloid set.

Table 3

Length and breadth of pods in cm, no. of seeds per pod and weight of 1000 seeds in g in parents and both hybrid generations

Form, generation	Pod length in cm			Pod breadth in cm			Seeds per pod			Weight of 1000 seeds in g		
	min.	max.	ave- rage	min.	max.	ave- rage	min.	max.	ave- rage	min.	max.	ave- rage
<i>L. graecus</i>	5.8	7.7	6.6	1.0	1.3	1.1	5	6	5.8	145	155	150.6
<i>L. jugoslavicus</i>	6.0	8.5	8.0	1.1	1.2	1.1	5	6	5.8	163	172	167.5
F_1	6.8	8.0	7.3	1.2	1.4	1.3	5	6	5.5			162.0
F_2	5.3	8.8	7.1	1.2	1.4	1.2	3	7	5.7	180	201	193.7

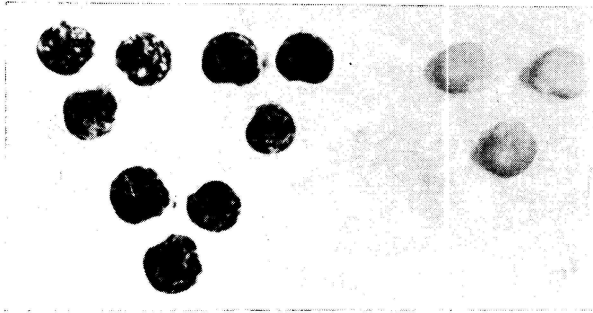
Seeds collected from the F_1 hybrids were planted in pots, in a glasshouse, on Dec. 20, 1962. Emergence took place on Jan. 2, 1963. Germination was satisfactory, seedlings possessed short hypocotyls, cotyledons spread out on the soil surface. As the parent forms and F_1 hybrids, the F_2 seedlings developed into a rosette. Leaf coloration was determined on five- or six-leaved rosettes. There were 23 dark-leaved seedlings and 9 lighter-coloured ones with a steel-grey tinge — a more or less 3 : 1 ratio. The F_2 hybrids also developed slowly, some of them surpassing the parent forms in exuberance. Save one specimen which opened as late as May 8, main stem flower initiation in the F_2 remained within the parental variability range. A certain tendency, however, to flower earlier was evident in the 43 per cent opening into bloom at the early period of parental blossoming (Tab. 1). In general, flower initiation of 1st order lateral shoots also fell in the variability range of lateral-branch-flower-initiation in *L. jugoslavicus*; *L. graecus* produced flowerless side shoots of the 1st order (Tab. 2).

The hybrid F_2 developed dark-blue flowers with a violet shade. They were assigned to three flower-colour groups: a) very deep-blue with a violet shade, b) deep-blue with a violet shade, c) blue with a violet shade, the ratio being respectively 21 : 8 : 3.

Four hybrid F_2 's developed lateral branches from the cotyledon angles. In one specimen such a particular branch opened into blossom 10 days

earlier than the 1st order lateral shoot grown from the upper stem, and 9 days later than the main-stem inflorescence.

Stems, leaf stalks and pods of the F_2 hybrids were covered with dense hair. Pod and seed setting was satisfactory. Dehiscent pods resembled in size the parent pods (Tab. 3). Parent plants and the F_1 and F_2 hybrid offspring were similar in the number of seeds per pod (Tab. 3). Twenty seven plants set coloured seeds, while five only — white seeds (Phot. 3).



Phot. 3. F_2 seeds

In the 1000 seed weight, the F_2 's slightly exceeded the parent F_1 forms (Tab. 3). The morphological aspect of the parent plants and offspring was very similar. Both hybrid generations grew into rosettes and their foliage was abundant (Tab. 4).

Table 4
Variability in the no. of leaves per main stem in parents and both hybrid generations

Form, generation	Year	No of leaves				No. of tested plants
		18—20	21—23	24—26	27—29	
<i>L. graecus</i>		1	3	2		6
<i>L. jugoslavicus</i>	1962	3	4	3		10
F_1				2		2
<i>L. graecus</i>			5	5		10
<i>L. jugoslavicus</i>	1963	1	1	1		3
F_2		7	17	7	1	32

Analyses of pollen grains in the F_1 's and F_2 's were performed with the use of a mixture of acetocarmine and glycerin. It was found that the proportion of abortive pollen was low, and very much like that in the parent forms (Tab. 5).

Table 5

Percentage pollen viability in parents and both hybrid generations

Form, generation	Viable pollen grains %		
	min.	max.	average
<i>L. graecus</i>	96.2	99.8	98.3
<i>L. jugoslavicus</i>	99.5	99.6	99.5
F_1			99.4
F_2	96.9	100.0	99.3

DISCUSSION OF RESULTS

Several morphological and physiological characters are very similar in *L. graecus* and *L. jugoslavicus*. Their alkaloid sets are identical. Multiflorine, identified by Wiewiórowski et al. (1961, 1961a), is the most noteworthy alkaloid, termed hitherto LA₄ (Kazimierski, 1960; Kazimierski a. Nowacki, 1961). It is the principal alkaloid in the section of coarse lupins and may be regarded as evidential in the chemical affinity between the tested forms and the section of coarse lupins.

Differences between the parent forms can be reduced to divergencies in flower, leaf and seed pigmentation and to small disparities in the seed size. In *L. jugoslavicus*, leaves and flowers are slightly darker than in *L. graecus*; seeds of the former are coloured and slightly larger.

First generation hybrids displayed a greater vigour than the parents; their pod and seed setting was more satisfactory. These phenomena might be due to heterosis. Features of the second hybrid generation remained within the parental variability range. In the first generation, flower, leaf and seed pigmentation of *L. jugoslavicus* were found to be dominant. On closer examination it was found that specifically the more intense green coloration of leaves and the marble pattern of seeds were the dominant element; flower colour was a resultant of a cumulative action of both parental genes. The ready crossability of the examined forms, nearly entire pollen viability, high fertility of the F_1 and F_2 hybrid offspring indicate a close relationship between them.

CONCLUSIONS

1. All the studied forms interbreed readily and produce fertile offspring.
2. Variability range of both hybrid generations remains within the parental limits.
3. A nearly complete (100%) pollen viability in the hybrid progeny,

large similarity of morphological features in the parental forms and their identical alkaloid sets indicate a close relationship between *L. graecus* and *L. jugoslavicus*.

Streszczenie

BADANIA NAD MIESZAŃCEM *LUPINUS GRAECUS* BOISS. × *LUPINUS JUGOSLAVICUS* KAZIM. ET NOW.

1. Badane formy krzyżują się łatwo ze sobą i dają płodne mieszańce.
2. Zakres zmienności cech mieszańców pierwszego i drugiego pokolenia mieścił się w granicach zmienności form rodzicielskich.
3. Prawie 100% żywotność pyłku u mieszańców, duże podobieństwo cech morfologicznych u form rodzicielskich i identyczny garnitur alkaloidowy wskazują na bliskie pokrewieństwo *L. graecus* i *L. jugoslavicus*.

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