

PHENO-MORPHOLOGICAL AND AGRONOMIC DIVERSITY IN A COLLECTION OF WILD AND DOMESTICATED SPECIES OF THE GENUS *LUPINUS*

Dariusz ZALEWSKI, Renata GALEK*, Bartosz KOZAK, Ewa SAWICKA-SIENKIEWICZ

Wroclaw University of Environmental and Life Sciences, Plant Breeding and Seed Production,
Department of Genetics, Wroclaw, POLAND
Corresponding author: renata.galek@up.wroc.pl

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ABSTRACT

The aim of this study was evaluated variability in collection of 20 genotypes represented eight domesticated and wild species of genus *Lupinus*. Analyses were made using multi-dimensional assessment based on twenty simultaneously considered characters from three years fields experiment. The cluster analysis (CA) and principal component analysis (PCA) were performed for illustrated diversity among investigated genotypes. The first three principal components contributed for 75.8% of the overall multi-trait variability in the means of all analyzed characters. The first principal component was found to account for 38.4% of the variability. The phenological phases and the height of a plant had the greatest contribution to this particular component. The second principal component accounted for 23.3% of the diversity regarding the number of pods from a lateral stem and emergence. On the other hand, the number of pods from the main stem and 1000-seed weight constituted traits the strongest linked with the third principal component, which accounted for 14.1% of the diversity. The obtained results will be helpful to define a strategy in the future lupin breeding programs.

Keywords: pheno-morphological traits, agronomic traits, cluster analysis, genus *Lupinus*, principal component analysis

INTRODUCTION

Genus *Lupinus* is a large group of interesting and usefulness species. Lupins are an excellent source of protein and oil, and therefore they have been applicate in animal nutrition and the human diet (as a supplement to low-calories food especially for diabetics). Furthermore, alkaloid compounds, mostly quinolizidine group contained in the lupine seeds may be useful for the manufacture of blood pressure lowering drugs, and analgesics (Heistinger and Pistrick, 2007; Fychan et al., 2008; Sipsas, 2008; Sweetingham and Kingwell, 2008).

Discovering on the "new" lupin is associated with the current global trends in agronomical practices, aimed towards promoting organic farming, designed to protect the environment. Lupin belongs to desirable elements in cropping system, especially as a plant rotary with cereals and oilseeds, due to the beneficial effect on soil fertility and increasing plant health. Lupins have a significant impact on the growth yield of succeeding plant crops (Evans et al., 2001).

The extraordinary adaptability value of lupins species was resulted in spreading them on several continents. The

genus *Lupinus* has two centers of natural distribution. One of them is situated in the Mediterranean region, which is the so-called Old World, with ecological niches reaching as far as Central Africa. The other covers the western coast of Americas, stretching through Alaska (in the north) up to Argentina, with a large subcentre in Brazil – the so-called New World. Although New World lupins are represented by about 300 species only one – *Lupinus mutabilis* was domesticated (Römer et al., 1999). In the region of the Mediterranean Sea basin and Africa there are 13 annual species, which have been divided into groups and taxonomic sections based on serological reactions of their storage proteins, morphological similarity and results of experiments aimed at acquisition of interspecific hybrids. With regard to the built of the seed coat, two groups have been distinguished, i.e. smooth- and rough-seeded species. The group of smooth-seeded lupins is formed by the following sections: *Albus*, *Luteus*, *Angustifolius* and *Micranthus*, whereas in the rough-seeded there are *Pilosus*, *Atlanticus*, *Princei* and *Somaliensis*. The Mediterranean region constitutes the natural distribution area of three cultivated species with smooth-seeded – *L. albus*, *L. luteus*, *L. angustifolius* (Gladstones et al., 1998).

The lupin breeding progress is caused by crossing varieties or breeding lines, spontaneous and induced mutants and by carrying out the selection pressure (Buirchel, 1994). Opportunities to increase genetic variation using interspecific hybrids in the genus *Lupinus* are difficult because of differences in the number of chromosomes between species of crops belonging to different taxonomic sections and developed cytogenetic barriers during of evolution, although possible (Clements et al., 2008; Sawicka-Sienkiewicz et al., 2008). In view of this situation, there is need for conducting a separate breeding of each cultivated species of lupine (white, yellow, narrow-leafed and Andean lupin). In order to practical use of natural and induced variability and increase the efficiency of breeding programs it is necessary to create an appropriate genetic base and it is very important to know the range of traits variation in specific climatic conditions.

The purpose of the undertaken investigations was to arrive at multi-dimensional assessment of diversity of the

lupin collection gathered at the Department of Genetics, Plant Breeding and Seed Production.

MATERIALS AND METHODS

The collection consisted of specimens that represented eight species from the genus *Lupinus*: *Lupinus albus* L. (cv. Butan), *L. angustifolius* L. (cvs. Elf, Emir, Graf), *L. mutabilis* (cvs. Potosi, selected lines from populations - POP.15, XM.5, LM.34), *L. hispanicus subsp. hispanicus* (Plant Introduction No. - PI 385134), *L. pilosus* (PI 11424, 491183, 249758), *L. palaestinus* (PI 15616), *L. cosentinii* (PI 32535, 32533, 32534, 32530) and *L. atlanticus* (PI 384613, 386413). Three years' field experiments (2003–2005) were established by the method of randomized blocks and carried out in three replications. Seeds of twenty genotypes were sown at the spacing of 10 cm x 20 cm, on experimental plots covering 1 m². The measurements were taken from ten randomly chosen plants growing in a given plot. The characters of subsequent plants were recorded, including morphological and related to the yield structure (Table 1) were evaluated.

Table 1. Eigenvectors of the twenty pheno-morphological and agronomic traits for first third principal components (PCs) in 20 genotypes of genus *Lupinus*'

Traits	PC1 (38.4%)	PC2 (23.3%)	PC3 (14.1%)
	Eigen vector		
Height of plant (cm)	-0.75	0.50	-0.02
Height of main stem (cm)	-0.70	0.31	-0.16
Lateral branches no.	-0.34	-0.67	0.24
Inflorescence length of main stem (cm)	-0.39	0.68	-0.47
Pods no. on main stem	-0.06	0.30	-0.87
Inflorescence length of lateral branches (cm)	-0.56	0.59	-0.12
Pods no. on lateral branches	-0.12	-0.45	-0.46
Pods no. from lateral branch	-0.43	-0.81	-0.01
Pods no. per plant	-0.48	-0.66	-0.48
Harvest index – main stem (%)	0.56	-0.18	-0.48
Harvest index – lateral branch (%)	0.54	-0.65	-0.04
Weight of 1000 seeds (g)	0.18	0.59	0.70
Emergence	-0.31	0.70	-0.10
1st pair of leaves	-0.68	-0.18	-0.49
Budding of the main stem	-0.68	-0.40	0.31
Flowering on the main stem	-0.88	-0.31	0.16
Flowering on lateral branches	-0.93	-0.09	0.07
Pods setting on the main stem	-0.90	-0.26	0.16
Pods setting on lateral branches	-0.91	0.09	-0.07
Vegetation period	-0.81	0.07	0.42

Note. Values in bold indicate values ≥ 0.7 for each principal components.

Means of each character were standardized prior to analysis in order to avoid the effect due to differences in scale. The standardization of mean was made to provide a multivariate analysis.

A multivariate analysis was performed to calculate the average Euclidian distances between each pair of accessions, and then compiled into a matrix. The data from this matrix were next used both for Cluster Analysis (CA) and Principal Component Analysis (PCA). In the CA, the accessions were clustered into hierarchical groups represented in a dendrogram according to the Unweighed

Pair Group Method using Arithmetic Averages (UPGMA). In the PCA, a multidimensional data set of 20 characters, 20 accessions, were analyzed. According to Kaiser's criterion (Kaiser, 1960), only those principal components analysis (PCA) that showed the eigen value ≥ 1 were taken into account. Correlation coefficients were calculated between characters, compiled into a matrix and submitted to a PCA. The calculations for CA were made with the application of the STATISTICA 10.0 software. For division of the obtained clusters, Grabiński's criterion (1992) was applied, which assumes

“cutting off” of a dendrogram at the point where the quotient of neighbouring distances represents the highest value. Graphically, location of particular genotypes was shown in the space of the first three principal components with the use of the NTsys PC 2.21.

RESULTS AND DISCUSSION

The first three principal components accounted for 75.8% of the overall multi-trait variability in the means of all characters analyzed in twenty genotypes under evaluation. The first principal component was found to account for 38.4% of the variability. The phenological phases and the height of a plant had the greatest contribution to this particular component. The second principal component accounted for 23.3% of the diversity regarding the number of pods from a lateral stem and emergence. On the other hand, the number of pods from the main stem and 1000-seed weight constituted traits the strongest linked with the third principal component, which accounted for 14.1% of the diversity (Table 1).

The performed analysis allowed to divide the studied genotypes into six clusters using CA and five cluster based on PCA (Fig. 1, 2). *L. hispanicus ssp. hispanicus* was the species that was clearly distinguishable from the

others and formed separately cluster I based on CA and PCA. This is an effect of pressure selection in different than in other species climatic and soil conditions selection. This species, however, cannot be directly used as initial material for breeding due to a number of disadvantageous properties, i.e. too long the vegetation period, production of a large number of lateral branches and too low index of harvest from the main stem and from the lateral ones (Table 2). The three studied cultivars of the narrow-leaved lupin fell within one group – cluster IV (Fig. 1, 2; Table 1). *L. angustifolius* is the widest cultivated species from the genus *Lupinus*. This group of lupins was characterized by an array of favorable traits, short vegetation period and a reduced number of lateral branches in particular. Its failure, however – as in the majority of lupins – are abortion of flowers and juvenile pods, which negatively affected the index of harvest from the main and lateral branches (Table 2). Genotypes representing *L. cosentinii* grouped in cluster V. They characterized by lower abscission of flowers and pods from the main stem and the lateral branches (Table 2). Besides, they didn't produce numerous lateral branches, were short. Investigated genotypes of *L. cosentinii* could be used in breeding aimed at improvement of yield structure traits and shorted vegetation period.

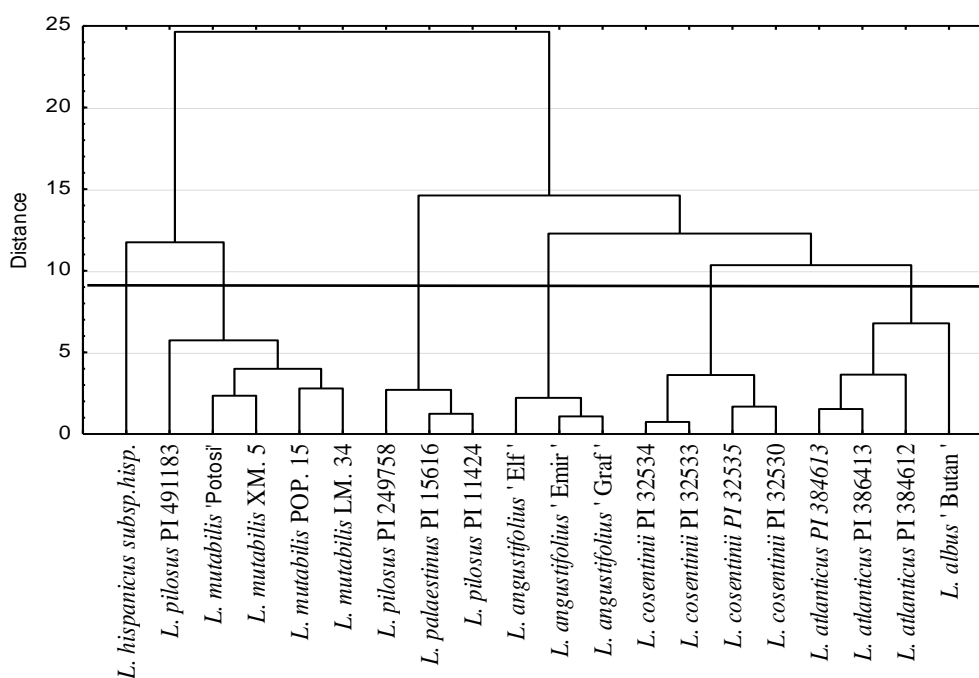


Figure 1. Dendrogram based on twenty analyzed traits in collection of *Lupinus* genotypes

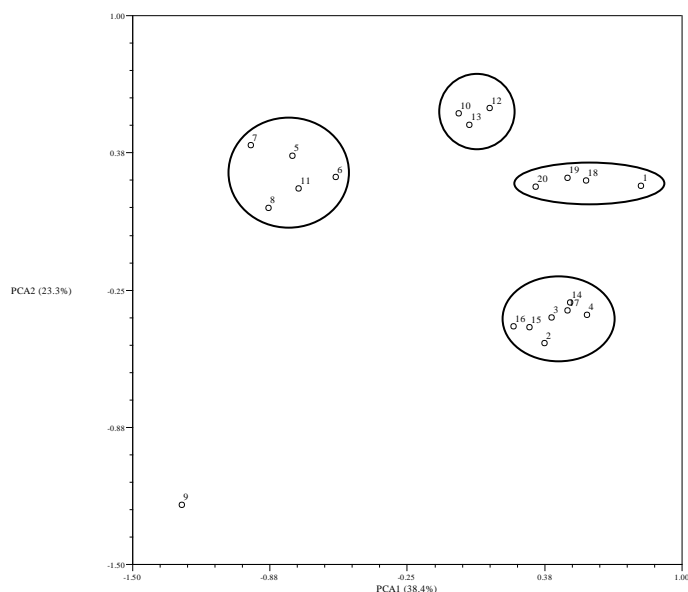


Figure 2. PCA biplot of 20 traits in collection *Lupinus* genotypes

1 - *L. albus* 'Butan'; 2 - *L. angustifolius* 'Graf'; 3 - *L. angustifolius* 'Emir'; 4 - *L. angustifolius* 'Elf'; 5 - *L. mutabilis* LM. 34; 6 - *L. mutabilis* XM. 5; 7 - *L. mutabilis* POP. 15; 8 - *L. mutabilis* 'Potossi'; 9 - *L. hispanicus subsp. hisp.*; 10 - *L. pilosus* PI 11424; 11 - *L. pilosus* PI 491183; 12 - *L. pilosus* PI 249758; 13 - *L. palaestinus* PI 15616; 14 - *L. cosentinii* PI 32530; 15 - *L. cosentinii* PI 32533; 16 - *L. cosentinii* PI 32534; 17 - *L. cosentinii* PI 32535; 18 - *L. atlanticus* PI 384612; 19 - *L. atlanticus* PI 386413; 20 - *L. atlanticus* PI 384613

Table 2. Mean values of pheno-morphological and agronomical characters in the groups obtained by clusters analysis

Traits	Mean						Mean
	Clusters*						
	I	II	III	IV	V	VI	
Height of plant (cm)	49.4	75.2	63.0	49.1	38.0	40.6	52.6
Height of main stem (cm)	57.1	86.4	69.0	55.3	63.9	45.6	62.9
Lateral branches no.	6.6	3.5	3.2	5.1	3.0	2.5	4.0
Inflorescence length of main stem (cm)	9.8	24.0	18.2	17.6	13.6	17.4	16.8
Pods no. on main stem	6.9	13.8	7.6	8.6	11.7	13.3	10.3
Inflorescence length of lateral branches (cm)	5.9	12.0	10.2	7.7	5.8	7.4	8.2
Pods no. on lateral branches	5.8	5.4	4.1	4.4	6.7	4.3	5.1
Pods no. from lateral branch	36.9	19.8	14.1	23.3	20.3	10.6	20.8
Pods no. per plant	43.9	33.5	21.9	31.8	31.8	23.8	31.1
Harvest index – main stem (%)	40.2	36.5	33.3	40.7	54.4	56.8	43.7
Harvest index – lateral branch	40.0	26.0	28.7	45.3	60.2	32.4	38.8
Weight of 1000 seeds (g)	62.8	186.9	571.3	148.0	218.8	252.8	240.1
Emergence	14.0	17.1	17.1	12.4	15.7	17.0	15.6
1st pair of leaves	7.8	7.7	4.4	6.2	5.5	5.5	6.2
Budding of the main stem	73.6	44.0	39.8	33.7	38.4	37.4	44.5
Flowering on the main stem	81.2	58.7	48.2	44.3	46.6	44.5	53.9
Flowering on lateral branches	87.3	71.1	58.6	48.0	55.6	53.4	62.3
Pods setting on the main stem	89.0	65.0	52.9	45.9	50.7	48.2	58.6
Pods setting on lateral branches	91.0	83.1	66.0	50.9	62.7	62.0	69.3
Vegetation period	142.3	127.6	129.7	102.0	112.8	106.8	120.2

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Cluster I *L. hispanicus ssp. hispanicus*
Cluster II *L. pilosus* PI 491183, *L. mutabilis* 'Potosi', *L. mutabilis* POP. 15, *L. mutabilis* XM. 5, *L. mutabilis* LM. 34
Cluster III *L. palaestinus* PI 15616, *L. pilosus* PI 11424, PI 249758
Cluster IV *L. angustifolius* 'Elf', 'Emir', 'Graf'
Cluster V *L. cosentinii* PI 32535, PI 32533, PI 32534, PI 32530
Cluster VI *L. atlanticus* PI 384613, PI 386413, PI 384612, *L. albus* 'Butan'

The similarity of the white lupin cultivar Butan to *L. atlanticus*, recorded in the case of many properties (Table 2). Genotypes from those species belonged to cluster VI (Fig. 1). This fact is interesting because of *L. albus* originates from the territories within the Mediterranean Sea basin and is commonly cultivated in many countries, whereas the wild species *L. atlanticus* occurs in areas at higher altitudes – in the Atlas Mountains. Species belong to cluster VI characterized by a high 1000-seed weight, and favorable harvest index for the main stem but a less favorable index of harvest from lateral branches (Table 2). Taking into consideration their large 1000-seed weight, high index of harvest from the main stem, production of insubstantial number of lateral branches and a relatively short vegetation period, these forms are particularly promising and can find use in the pertinent research and plant breeding. Simultaneously, they did not produce biomass, plants were short, had a small number of lateral branches and matured relatively early (Table 2). All the evaluated genotypes of *L. mutabilis* and *L. pilosus* (PI 491183) were classified in one group – cluster II (Table 2, Fig1). These genotypes were high with long inflorescences, but they did not produce a favorable number of pods and their harvest index was low. What's more their disadvantage was a long vegetation period. A similarly long vegetation period was typical of the genotypes of *L. palaestinus* as well as *L. pilosus* PI 249758 and PI 11424, which make cluster III (Table 2, Fig 1). The character that distinguished this group was a very high weight of 1000 seeds, a property rather unfavorable in agricultural practice. Disadvantages included also the tendency to produce a large biomass and a low harvest index.

When attempting to obtain new varieties, it is advisable to conduct a comprehensive assessment of the initial material. Breeders verify their materials based on evaluation of quantitative or qualitative traits. For evaluation of breeding material various statistical methods are employed – both uni- and multi-dimensional ones (Ghafoor and Arshad, 2008; Galek, 2010; Ilker et al., 2009; Mitrović et al., 2012).

Since the environmental impact is of high importance in the evaluation of plants' phenotype, it is justifiable – when the genotype-environment interaction has been found significant – to perform analyses not only of the averages for several years but also of the results for particular years of research separately. Many authors studied two-year or three-year results for each year separately in the case of such species as the mungo bean or alfalfa (Ghafoor et al., 2002; Ghafoor and Ahmad, 2003; Ghafoor and Arshad, 2008) and thus they did not carry out analyses of the data averaged for several years. However, Ghafoor et al. (2008) evaluated a very large number of mungo bean genotypes (484) based on the annual means. The research by Di Giorgio et al. (2009) also pertained to the results of annuals but samples were taken from 36 locations of natural populations of the *Scorpiurus muricatus*. In our study we used analyses based on means from three years period.

Application in our study of principal component and cluster analyses allows to specify which attributes among all the 20 analyzed are of importance in the overall variability. It also gives us the ability to designate groups of similar genotypes for all the characteristics and to determine which genotypes are distinguishable in terms of several features together. This allows to choose an appropriate strategy for the breeding program.

The multidimensional analysis of principal components for wild and domesticated species of the lupin, which has for the first time been undertaken, made it possible to define which characters are of utmost importance in shaping the observed diversity. The simultaneously applied cluster analysis enabled to group the genotypes in respect of their similarity based on twenty traits in total, an outcome which can be very helpful in selection-breeding practice.

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