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Assessment of Genetic Variability, Heritability and Economic Productivity in White Lupin (*Lupinus albus* L.) Lines

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ABSTRACT

An annual legume, white lupin is traditionally grown throughout the Mediterranean and the Nile valley for use as fodder, green manuring, and human food. White lupin can be directly used on farms in self-sustaining systems due to the lack of anti-nutritional elements. Commercial varieties, bred by pedigree selection, are pure lines that are primarily self-pollinating. Lupun species are grain legumes that may be a good substitute for soybeans as a source of protein in meals. In Europe, lupines have been proposed as a potential substitute crop and traceable protein supply. The object of study in this research was the genetic variability of 9 white lupin lines concerning 9 quantitative traits by calculating genetic parameters and applying multivariate analysis methods. The experimental activity was carried out in the period 2022-2024 at the Institute of Forage Crops (Pleven). For all studied traits (except for the number of branches), phenotypic coefficients of variation dominated over genotypic coefficients of variation, with values from 2.25 to 22.15% and from 0.79 to 12.52%, respectively. The difference between phenotypic and genotypic coefficients of variation ranged from 1.39 for 1st pod height to 11.94 for seed weight per plant and seed yield. The variation of the traits of seeds number per plant (22.15%), 1st pod height (13.91%), pods number (18.63%), seed weight per plant (18.37%) and seed yield (18.37%) was determined to a large extent by the environmental conditions. Moderate to high values of heritability and genetic advance were found for 1st pod height (70.82%; 9.68), plant height (68.08%; 12.26) and seed yield (27.18%; 12.27), which reveal the possibility of achieving rapid selection progress in improving these traits. For pods and seeds number per plant, a relatively high coefficient of inheritance (48.03%; 40.27%) was also found, but a lower genetic advance (2.00; 6.72), which may complicate the selection for these traits. The cluster analysis divided the white lupin lines into two main groups and several subgroups depending on their biological manifestations. According to the complex of studied traits, L120, L103 and L118 were genetically distant from L101, L111 and L108.

Keywords: *Lupinus albus* L., genetic parameters, variability, economic production, yield.

INTRODUCTION

The genus Lupinus includes more than 280 species of annual and perennial herbaceous and shrubby forms. Only four species are domesticated and have agricultural importance. One of them is the white lupine (*Lupinus albus* L.). It is an annual species originating in the Mediterranean (Kurlovich, 2002). It has a variety of uses as fodder, in the food and cosmetic industries. Also, its beautiful colors define it as an ornamental plant (Lakić et al., 2019, 2022; Petrova, 2022). White lupin is a high-productive crop, rich in protein, and relatively tolerant to water deficit, soil acidity, and salinity. It improves soil fertility and contributes to the sustainability of agricultural production and food security related to climate change (Beyene, 2020). Interest in its cultivation as a food or feed crop in Europe is also determined by the seed's high protein content, reaching up to 40%, as well as other favorable quality indicators that respond to the growing demand for healthy plant-based food (Pilorgé

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and Muel, 2016; Fehér et al., 2020). White lupin cultivation is characterized by high energy efficiency (Muraveev et al., 2012), and define it as a crop suitable for organic production. However, its breeding is limited and started relatively late - in the 1950s and 1960s. Modern white lupin varieties have improved agrotechnical and quality traits. One of the main traits is low alkaloid content (Nalle et al., 2012; Abraham et al., 2019; Pecetti et al., 2023). The main objectives of breeding programs include stability of yield and seed quality characteristics, resistance to biotic and abiotic stress, and early maturation (Annicchiarico and Alami, 2012; Popović et al., 2013, 2022; Lucas et al., 2015; Atnaf et al., 2018; Bojović et al., 2019; Rakašćan et al., 2021). The success of selection in improving plant traits is largely determined by the genetic variability of breeding materials and the transmissibility of a given trait. Variation coefficients at phenotypic, genotypic and environmental levels provide information about crop variability and the relative significance of genetic and environmental components. The presence of high variability in breeding materials determines a greater opportunity for improvement through selection, but, on the other hand, the presence of high transmissibility is important for realizing such possibility. Heritability assessment is essential in determining the transmission index of a trait from parents to offspring (Singh et al., 2019). Heritability, combined with genetic advancement, provides reliable information on the transmissibility of traits, which helps in developing appropriate selection strategies (Singh et al., 2019). The genetic diversity of *L. albus* germplasm, including commercial and local cultivars, has been studied by a number of researchers (Atnaf et al., 2017; Beyene, 2020; Kroc et al., 2021; Zafeiriou et al., 2021). Characterization of resources by various quantitative and qualitative characters using appropriate methods and approaches is a basis for effective management of the genetic resources in lupin and an essential step in any breeding program (Bellucci et al., 2021; Mavromatis et al., 2023).

This study aimed to assess the genetic variability in white lupin lines with a view to their use in the breeding process.

MATERIAL AND METHODS

Experimental Area and Methods

This study was conducted during the period 2022-2024 at the Institute of Forage Crops (Pleven). The objects of experimental activity were nine lines of white lupin (L101, L116, L120, L103, L118, L111, L108, L125, L104), which were created through artificial sexual hybridization. The long plot design method was used, in three replications, with a plot size of 5 m^2. Sowing was done manually, with a row spacing of 50 cm and an intra-row spacing of 10 cm. The white lupine was grown under organic production conditions, without using fertilizers and pesticides. The biometric characteristics of the plants included an assessment of 9 traits: plant height (cm), 1st pod height (cm), number of main branches, pods number per plant, seeds number per plant, seed weight per plant (g), 1000 seeds mass (g), pod length (cm), seed yield (kg da^{-1}).

Statistical analysis

Principal component analysis (Chahal and Gosal, 2002), biplot analysis (Yan et al., 2000) and cluster analysis (Ward, 1963) were performed to assess genetic variability. Data processing was carried out using analysis of variance, through which the following parameters were calculated: phenotypic coefficient of variation (PCV, %), genotypic coefficient of variation (GCV, %) (Burton, 1952), genotypic (σ2g) and phenotypic variance (σ2p), heritability in broad sense (H2, %) (Mahmud and Kramer, 1951), genetic advance (GA) and genetic gain (GG) (Johnson et al., 1995). All data were statistically processed with the software product Statgraphics Plus for Windows Version 2.1 and Microsoft Excel 2016.

RESULTS AND DISCUSSION

Genetic diversity is a fundamental resource in developing new varieties, especially in changing climate conditions. The evaluation of genetic diversity and its inclusion in breeding programs is essential for achieving positive results in improving the varietal composition of crops. Knowledge of the expression of genotype quantitative traits can facilitate selection and accelerate the breeding process (Mavromatis et al., 2023). Many researchers have found the presence of a great genetic diversity regarding agronomic characteristics in *L. albus* (Mülayim et al., 2002; Jansen, 2006; Petrova, 2021). Under the experimental conditions, considerable differences were found between the white lupin lines regarding the nine quantitative traits studied, except for the number of main branches and pod length. This provides grounds for the study to focus on evaluating the created lines by applying some phenotypic and genotypic parameters. Lakic et al. (2022) reported significant differences in the assessment of genetic diversity in white lupin accessions for traits such as plant height, number of main branches, etc. Mulugeta et al. (2015) also reported significant differences between white lupin genotypes concerning their morphological characteristics.

Phenotypic and genotypic coefficients of variation. Phenotypic and genotypic coefficients of variation ranged between 2.25 to 22.15% and 0.79 to 12.52%, respectively (Table 1). According to Sivasubramaniah and Meron (1973), PCV and GCV values can be defined as low (<10%), moderate (10-20%) and high (>20%). For all the studied traits (except for the number of branches), the phenotypic coefficients calculated were higher than the genotypic coefficients. High PCV was found for seeds number (22.15%), medium PCV (<20%) - for 1st pod height, pods number, seeds weight per plant and seed yield, and low PCV - for 1000 seeds mass (9.63%) and plant height (9.44%). Data on the genotypic coefficient of variation showed a medium level of variability (10.41-12.52%) for pod number, seed number and 1st height pod. Low variability was reported for the remaining traits and it was best manifested for pod length and 1000 seed mass. The differences between PCV and GCV were established in the conditions of the environment in which the lines were grown. The level of difference between PCV and GCV indicates the degree of environmental influence on the traits. This difference ranged from 1.39 for 1st pod height to 11.94 for seed weight per plant and seed yield.

The superiority of PCV for the last two traits and seed numbers indicates a considerable environmental influence on the manifestations of these traits and a good opportunity for their improvement through phenotypic breeding. Atnaf et al. (2017) reported similar results for the main quantitative traits in white lupin accessions. The authors pointed out that the variability of the traits, expressed through the genotypic coefficient of variation, ranged from 3.87% to 17.66%, with the highest value for seed yield. When evaluating 25 white lupin genotypes for 22 traits, Beyene (2020) indicated that genotypic and phenotypic coefficients ranged from 4.39% to 29.54% and 3.41% to 28%, respectively. The author reported low values of GCV and PCV for pod length and medium values for 1st pod height and pods number, which is also found in the present experiment. The low estimates for pod length were also reported by Hibstu (2016). Regarding seed yield in white lupin, a number of researchers (Mera et al., 2006; Annicchiarico et al., 2010; Beyene, 2020) found high GCV and PCV. At present conditions, a moderate PCV (18.37%) and low GCV (6.43%) were reported for the same indicator, probably due to the relatively small number of lupin lines studied.

Phenotypic and genotypic variance. The values of genotypic variance for all analyzed traits were lower than the corresponding phenotypic variances, which is also found in previous studies in white lupin (Gudeta, 2019; Petrova, 2022). The differences recorded for 1st pod height, plant height, pods number and seeds number were smaller than the differences in the other indicators. The values of genotypic variance ranged from

0.003 (number of main branches) to 285.23 (seed yield). The genotypic variance was relatively low for the traits of pod length, seed weight per plant and pods number, with numerical values close to unity. The highest phenotypic variance was observed in seed yield (2292.84) and 1000 seeds mass (681.06), and the lowest one was observed in number of branches and pod length. A relatively high phenotypic variance was also found for seeds number (210.95), indicating that these traits are influenced to a greater extent by the environment. Regarding both indicators, phenotypic and genotypic variance, the highest values were calculated for seed yield, followed by 1000 seeds mass and seeds number. When evaluating a collection of 36 lupin accessions regarding 18 traits, Gudeta (2019) found the same dependence.

Heritability in broad sense, genetic advance, genetic gain. Heritability values are determined as low (<30%), moderate (30-60%) and high (>60%), and genetic advance as low (<10%), moderate (10-20%) and high (>20%) (Johnson et al., 1995). The knowing heritability of a trait is important to the breeder, as it shows the possibility and extent to which improvement through breeding is possible. It is a measure of genetic relationship between parents and offspring and is widely used in determining the extent to which a given characteristic can be transmitted from parents to off spring (Robinson et al., 1965). The heritability in broad sense of studied lupin lines ranged from 5.86% (number of branches) to 70.82% (1^{st} pod height). Plant height (68.08%), pods number (48.03%), and seeds number (40.27%) showed higher heritability than the other traits. For 1000 seed mass, seed weight per plant and seed yield, heritability was low (>21%), which is an expression of environmental influence on the manifestation of these traits. The trait of pod length (8.17%) was inherited similarly to the number of branches. A number of researchers (Annicchiarico et al., 2010; Hefny, 2013; Mulugeta et al., 2015) reported high values of heritability in more than the main quantitative traits in white lupine, which, according to the authors, indicates a predominance of the genetic effects of the trait over the environmental effects. High heritability shows a high proportion of genetic variation that can be inherited and used in the selection of genotypes. According to Singh et al. (2019), high heritability alone is not sufficient to ensure efficient breeding in advanced generations unless it is combined with a substantial genetic advance. Genetic advance is another important parameter that measures the differences between the average genotypic values of the selected lines and the average genotypic values of the source population from which they were selected. Higher estimates of genetic advance and genetic gain can be referred to seeds number (6.72; 42.83), seed yield (12.27; 37.90) and 1^{st} pod height (19.68; 29.59). The number of branches, seed weight per plant and pod length had the lowest genetic advance.

In their studies with a collection of white lupins, Atnaf et al. (2017) also pointed out that high heritability does not always guarantee a high genetic gain in the breeding process. Heritability should be viewed in combination with genetic advance when predicting individual selection of better genotypes. High assesments of heritability together with high genetic advance are a good opportunity for further improvement in advanced generations (Panse, 1957). In our study, a high heritability coefficient and moderate genetic advance were found for plant height and 1^{st} pod height, which is consistent with the results obtained by Beyene (2020). This indicates a predominance of additive gene action in their expression and suggests good genetic gain in the breeding process. From the moderate values for seed yield, it can be expected that this trait is influenced by non-additive gene actions or that there is a significant interaction of genotype × environment.

Cluster analysis. The cluster analysis (Figure 1) of the white lupin lines was based on structural components and seed yield. The dendrogram was formed by 2 main clusters. The first cluster, located in the upper part of the dendrogram, is larger and consists of two subclusters, including seven lines. In the first subcluster, L20, L103 and L118 have low level of remoteness. Their genetic similarity is due to the manifestations of traits such as seeds number and pod number (Table 2).

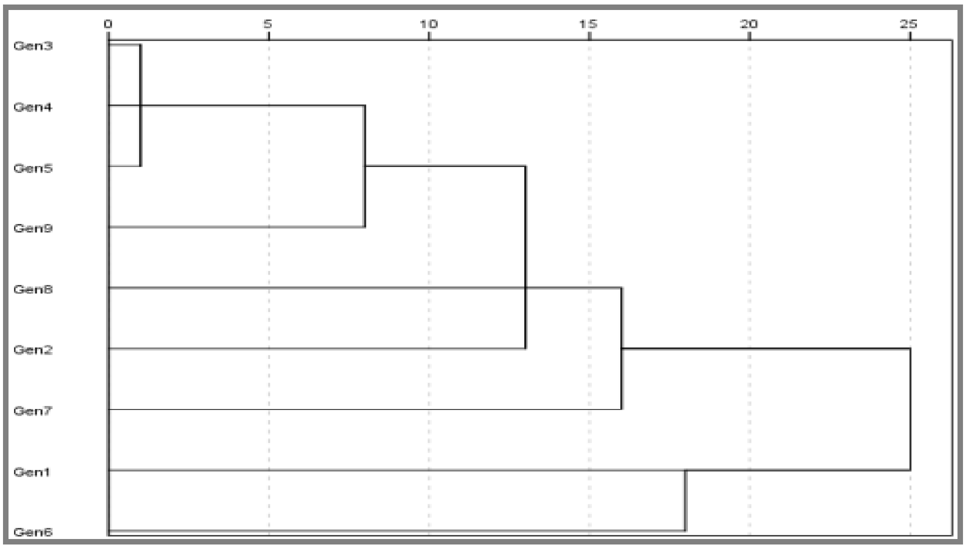

Figure 1. Dendrogram of main quantitative traits in white lupin lines.
*Gen1-L101, Gen2-L116, Gen3-L120, Gen4-103, Gen5-L118, Gen6-L111, Gen7-L108, Gen8-L125, Gen9-L104

These lines can be characterized as high-productive and large-seeded. L104 also belongs to this group. It is similar to the previous lines in terms of number of branches, pod length, and 1st pod height, but in terms of other traits (seed productivity, seeds, and pod number), it demonstrates genetic distance. In the lower part of the first cluster, the position of L116 and L125 shows the similarity between them (especially regarding plant height, 1st pod height, seed weight per plant and number of branches) compared to line L108, which has longer stems and develops a smaller number of branches. The second cluster is smaller and represented by only two lines: L101 and L111.

They are characterized by having a mass of 1000 seeds below the average for the studied group and forming a great number of pods and seeds per plant. Their genetic similarity is due to their high productivity. A similar grouping of white lupin accessions regarding main traits has been reported in previous investigations (Harty et al., 2016; Zafeiriou et al., 2021), in which genotypes are clustered depending on the expression of individual characters.

Principal component analysis. A projection of yield and related characteristics on principal components is presented in Figure 2. The yield vector is in the right half of the coordinate system but in the negative plane of the ordinate axis, which indicates that the environmental conditions have a strong influence on its value. In the quadrant bounded by the positive part of the abscissa and ordinate axes, the vectors of plant height, 1st pod height and pods number are located. They form sharp angles with each other (especially the first two), which shows a strong positive relationship among them. Their location creates an opportunity for selecting genotypes with tall stems and more pods per plant, which indirectly leads to increased yield. The traits number of branches, seeds number, seed weight per plant and 1000 seeds mass conclude sharp angles with yield, and they are also influenced by environmental conditions. The projection of pod length in the opposite quadrant and the angle (near the origin of the coordinate system) that it forms with the seed yield vector clearly shows that the very strong ability to form seeds in pods (through pod length) could not lead to an increase in yield.

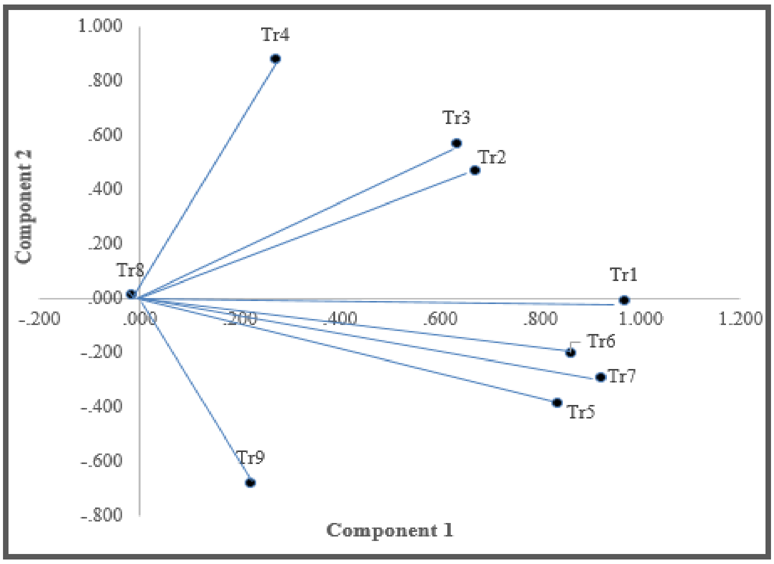

Figure 2. Projection of yield and related characteristics on principal components in white lupin lines. Tr1-number of branches, Tr2-plant height, Tr3-1st pod height, Tr4-pods number, Tr5-seeds number, Tr6-seed weight per plant, Tr7-1000 seeds mass, Tr8-pod length (cm), Tr9-seed yield

The applied principal component analysis provides a more detailed explanation of the trait variability in the studied lupin lines. Two principal components above unity are extracted (Table 3), and they determine the main directions of variation.

Table 1. Variance of components and heritability

Parameters	NBM	PH	FPH	PN	SN	SW	TSM	PL	SY
Min	1.20	67.60	18.20	5.75	21.75	7.25	209.44	7.32	193.27
Max	5.75	122.40	63.17	38.00	142.80	34.00	340.05	9.52	288.60
GCV (%)	9.92	7.93	12.52	10.43	10.41	6.43	2.89	0.79	6.43
PCV (%)	6.84	9.44	13.91	18.63	22.15	18.37	9.63	2.25	18.37
σ2p	0.179	70.09	33.77	9.96	210.95	10.18	681.06	0.19	2292.84
σ2g	0.003	49.82	27.32	3.07	47.41	1.26	62.59	0.02	285.23
GA	0.01	12.26	19.68	2.00	6.72	0.81	4.94	0.09	12.27
GC	25.26	20.88	29.59	35.15	42.83	37.88	19.49	10.89	37.90
H^2	5.86	68.08	70.82	48.03	40.27	27.19	21.61	8.17	27.18

PCV-phenotypic coefficient of variation, GCV-genotypic coefficient of variation, σ2g-genotypic variance, σ2p-phenotypic variance, H2-heritability in broad sense, GA-genetic advance, GG-genetic grain; NMB-number of main branches per plant, PH-plant height (cm), FPH-1st pod height (cm), PN-pods number per plant, SN-seeds number per plant, SW-seed weight per plant (g), TSM-1000 seeds mass (g), PL-pod length (cm), SY-seed yield (kg da^{-1}).

Table 2. Variance of components and heritability

Lines	NBM	PH	FPH	PN	SN	SW	TSM	PL	SY
L101	3.26	93.19	49.58f	18.51	81.39	18.84	255.75	8.40	245.00
L116	3.10	94.13	40.80.	16.87	69.10	19.60	289.15	8.60	255.00
L120	3.34	104.04	53.77	16.29	65.97	18.47	284.48	8.36	240.00
L103	2.98	87.93	40.28	16.58	65.72	18.88	291.64	8.63	245.30
L118	2.96	91.83	34.70	19.20	69.13	19.66	290.71	8.53	255.67
L111	3.13	92.57	40.47	21.50	85.63	20.67	249.55	8.53	269.00
L108	2.65	93.64	42.87	16.61	61.98	17.30	281.32	8.61	225.00
L125	2.97	80.83	38.00	13.90	57.13	15.03	261.67	8.51	195.33
L104	2.75	85.96	42.53	15.75	64.48	15.63	255.20	8.25	203.33
$LSD_{0.05}$	0.14	2.48	2.13	1.44	6.27	2.02	15.18	0.31	27.00

NMB-number of main branches per plant, PH-plant height (cm), FPH-1st pod height (cm), PN-pods number per plant, SN-seeds number per plant, SW-seed weight per plant (g), TSM-1000 seeds mass (g), PL-pod length (cm), SY-seed yield (kg da^{-1}).

Table 3. Principal component analysis in white lupin lines

Components	Initial Eigenvalues		
	Total	% of variance	Cumulative
1	5.252	58.36	58.36
2	1.625	18.05	76.41
3	0.878	9.75	86.16
4	0.679	7.54	93.69
5	0.293	3.26	96.96

Table 4. Influence of factors PC1 and PC2 on main quantitative traits

Traits	Initial Eigenvalues	
	1	2
Number of main branches per plant	0.935	-0.236
1st pod height	0.935	-0.236
Pods number per plant	0.928	-0.168
Seeds number per plant	0.869	-0.368
Seed weight per plant	0.788	0.372
1000 seeds mass	0.495	-0.250
Pod length	0.421	0.878
Plant height	0.535	0.767
Seed yield	-0.313	0.055

Table 5. Influence of factors PC1 and PC2 on white lupin lines

Lines	Component 1	Component 2
L101	0.968	-0.013
L116	0.966	-0.060
L120	0.949	-0.001
L103	0.809	-0.061
L118	-0.763	-0.054
L111	0.762	0.573
L108	-0.567	0.278
L125	0.151	0.924
L104	0.569	-0.596

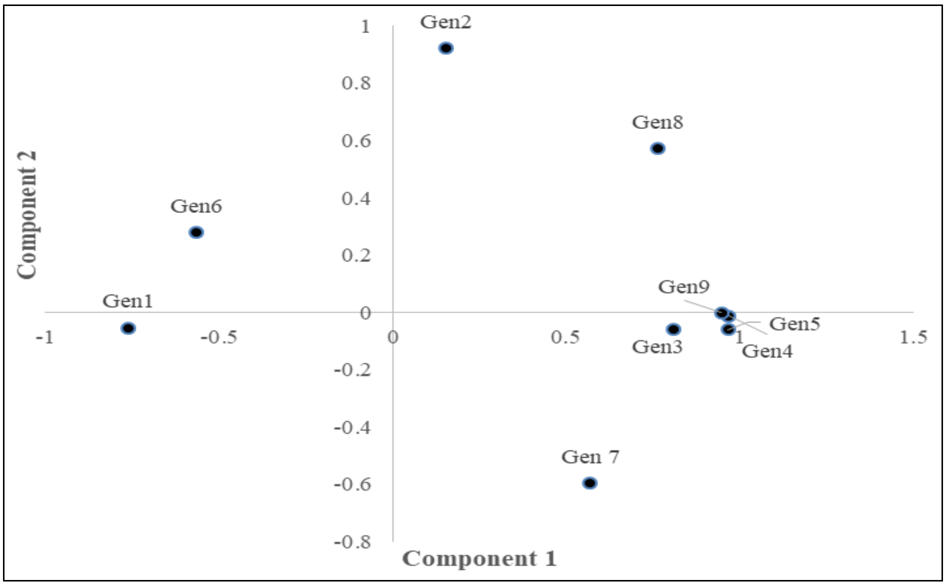

Figure 3. Placement of white lupine lines in factorial plane;
*Gen1-L101, Gen2-L116, Gen3-L120, Gen4-103, Gen5-L118, Gen6-L111, Gen7-L108, Gen8-L125, Gen9-L104

The first component (PC1) determines 58.36% of the total variation, and the share of the second component (PC2) is 18.05%. The remaining components explain a much smaller part of the variability, which is considered as residual variation. The weighting coefficients of traits (Table 4) show that the manifestations of traits, such as the number of branches, 1st pod height, pods number, seeds number, and seed weight per plant, are determined primarily by the first principle component. The second principal component mainly affects pod length and plant height. Both components have a weak effect on seed yield, with PC2 having a stronger effect.

Of the lines studied, L101, L116, L120, L103 and L104 are significantly affected by the first principal component (Table 5). The second principal component is decisive in the behavior of L118, L108 and L125. For L111, it can be said that the influence of the two factors is almost equal.

Figure 3 presents a projection of the lupine lines according to the manifestation of their characteristics. In the upper right quadrant of the coordinate system are located L116 and L125.

Their location is mostly based on the manifestations of plant height, 1st pod height, seed weight, and number of branches. Line L116 has the highest values for the first principal component, which is related to pods number and seeds number per plant. Unlike L125, L116 has larger seeds and a higher yield. Lines L103 and L118 are also located in the positive part of the coordinate system and almost on the abscissa axis itself. This particular positioning suggests that in some traits they will be similar to L116 and L125 and in others - to the lines occupying a place in the quadrant below the abscissa. In the right negative quadrant of the coordinate system, lines L120 and L108 are located. They form a smaller number of pods and seeds, and the variation in their yield depends largely on environmental conditions. Lines L101 and L111 also have highly variable yields over the years. Their location in the left negative part of the abscissa proves their strong dependence on environmental conditions. Other researchers (Hefny, 2013; Ahmed et al., 2023) have also used this method to explain the ranking and identification of different lupin genotypes based on the manifestations of their quantitative and qualitative traits and parameters.

Lupin is an excellent source of protein since it contains far more essential amino acids than soybean. Without affecting the calibre or volume of animal products produced, its seeds can be fed to animals in place of soybeans (Tadele, 2017). Even if their oil content is relatively low in comparison to oilseeds, their high concentration of polyunsaturated fatty acids (Rizzo et al., 2023) is more important from a nutritional standpoint than their oil content (Suchy et al., 2008). These nutritive characteristics make lupin ideal for animal feed and human consumption (Sawicka-Sienkiewicz et al., 2008).

CONCLUSIONS

Knowing a trait's heritability is crucial for breeders since it indicates the potential and degree of improvement through breeding. The results of the research using multivariate analysis methods to examine the genetic diversity of nine white lupin lines with respect to nine quantitative traits. are as follows:

The examined lupin lines' heritability, varied from 5.86% (number of branches) to 70.82% (1st pod height). Heritability was higher for plant height (68.08%), pod number (48.03%), and seed number (40.27%) than for the other characteristics. Heritability was low (>21%) for 1000 seed mass, seed weight per plant, and seed yield, indicating the impact of the environment on the development of these characteristics.

Most traits are environmentally influenced, except a few like 1st pod height and plant height.

Selection for 1st pod height, plant height, and seed yield can achieve rapid genetic progress.

Selection for pods and seeds number per plant requires careful strategy due to low genetic advance. Cluster analysis identifies genetically distant lines, useful for breeding and hybridization. Distant lines (L120, L103, L118 vs. L101, L111, L108**)** could be used in breeding programs to maximize heterosis or generate novel variation.

To address sustainability issues and employ it in the livestock industry, more research on the effects of present genotypes and lines on agronomic traits, biological activities, and low anti-nutritional factors would be essential.

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