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MORPHOLOGICAL AND BIOCHEMICAL STUDY OF THE SPECIES OF *Medicago lupulina* L. DISTRIBUTED IN AZERBAIJAN

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Abstract. The main objective of this research is to investigate the morphological, biochemical and bioecological features of various genotypes of *Medicago lupulina* L., a widespread forage plant of great importance in Azerbaijan. The highest coefficient of variation among the traits was for the bean diameter indicator (CV = 38.9). Principal component analysis (PCA) was used for multivariate data analysis, resulting in three principal components. These three components explain 79.66% of the total variability, indicating the high analytical value of the components. Pearson correlation analysis revealed statistically significant positive relationships between some morphological features and biochemical parameters. Plant height was positively correlated with total nitrogen ($r = 0.650$, $p < 0.01$), protein ($r = 0.687$, $p < 0.01$), tryptophan ($r = 0.499$, $p < 0.05$) and lysine ($r = 0.603$, $p < 0.01$); stem width was positively correlated with total nitrogen ($r = 0.598$, $p < 0.01$) and protein ($r = 0.581$, $p < 0.01$); leaf width was positively correlated with total nitrogen ($r = 0.733$, $p < 0.01$), protein ($r = 0.726$, $p < 0.01$), tryptophan ($r = 0.679$, $p < 0.01$) and lysine ($r = 0.582$, $p < 0.01$); thousand seed weight was positively correlated with total nitrogen ($r = 0.562$) ($p < 0.01$) and positive significant dependencies were observed with protein ($r = 0.658$, $p < 0.01$). The altitude above sea level (AASL) trait showed significant positive correlations with plant height ($r = 0.560$, $p < 0.05$), as well as with leaf width and length ($r = 0.55$-0.61, $p < 0.05$-0.01). This confirms that the level of vegetative development of plants is closely related to their nutritional value. Based on the two-factor cluster analysis, the genotypes were divided into six main clusters, between which both morphological and biochemical differences were observed. The results of similarity and dissimilarity analysis based on Euclidean distance confirmed the genetic relationships between genotypes and played an important role in identifying suitable genetic resources for breeding programs. The results of this investigation provide a scientific basis for a better understanding of the morphological, biochemical, ecological characteristics and genetic diversity of *Medicago lupulina*, to assess its potential for use in breeding and introduction programs and select more productive and adaptable forms.

Keywords: *Medicago lupulina, biochemical, morphological.*

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1. Introduction

Legumes are an important source of biological resources (protein, biologically active substances, fats, etc.) for humans worldwide and are second only to grain crops in economic importance. *Medicago* species, like other genera of the legume family, have nitrogen-fixing properties (Corby *et al*., 2011). Dry *Medicago* grass has a high nutritional value, as it contains copious protein, phosphorus, calcium and essential amino acids (valine, leucine, isoleucine, lysine, methionine, threonine, tryptophan, phenylalanine, arginine, histidine, ptyreonine, etc.) (Elamine *et al*., 2022). The leaves and stems of annual species, widely distributed in nature, are also rich in protein, vitamins and minerals. Species of this genus contain compounds that include three main groups of phytoestrogens: isoflavones, lignans and coumestan (a group of biological phenols that exhibit estrogenic activity) (Fourquin *et al*., 2013; Bandyopadhyay *et al*., 2016). In Europe and other regions of the world, *M. rigidula* is widely used in food, herbal teas and the treatment of many diseases due to its high protein content (Hanif *et al*., 2015; Çakmak *et al*., 2017).

Medicago species are considered an important source of vitamins and minerals. Research conducted on black clover revealed the presence of vitamins A, D, E, K, C, B1, B2, B, B5, B7 and B9 (Norouzi & Khademi, 2010). Biochemical analysis of *M. monantha* has shown that it contains alkaloids, anthocyanins, betacyanins, anthraquinones, coumarins, flavonoids, saponins, tannins and terpenoids. The plant has also been found to have cytotoxic properties (Kamran *et al*., 2018).

M. littoralis and *M. truncatula* are therophytic species common in sandy soils along the coasts of the Mediterranean region, extending to Portugal and western France. An investigation was conducted to determine the relationship between the phytochemical composition of these species and the environmental conditions - biotopes - in which they grow. The two species were found to have similar phytochemical composition except for isoflavones (Kökten *et al*., 2011). Analysis of the quantity of flavones and isoflavones is considered a useful method for chemosystematic identification of taxa belonging to the section *Spirocarpos*. Phytochemical properties of *M. littoralis*, in particular, are considered behavioral indicators of the ecological conditions of the sandy areas where this plant typically grows. The diversity of flavonoids and flavonoid-containing combinations isolated from *M. littoralis* has not been observed in any other coastal sandbar plant (Alessandra *et al*., 2010).

Perennial species of *Medicago* are of particular importance as forage plants. The species *Medicago sativa* contains 0.75 energy feed units per kg in the form of dry grass (Hajiev, 1969). The leaves of some annual species are used in salads, soups and sandwiches in numerous countries due to their high protein content (Çakmak *et al*., 2017). Some types of herbal tea can increase milk production during lactation, reduce cholesterol levels in the body and reduce the effects of many diseases such as certain types of cancer (malignant tumors), coronary heart disease, osteoporosis, anemia, diabetes and ulcers. Additionally, they have preventive properties associated with them (Hanif *et al*., 2015).

Medicago lupulina belongs to the genus *Medicago* of the legume family *(Fabaceae),* which includes 87 annual and perennial species distributed throughout the world. There are 17 species of the genus *Medicago* distributed in Azerbaijan (Guvendiyev *et al*., 2025). The Mediterranean region is considered the homeland of the *M. lupulina* species. The species is widespread in the temperate and subtropical climate zones of the world. One independent species and about 10 species varieties are distributed worldwide. The question of whether *M. lupulina* belongs to one or another life form has long been investigated. The monographs on the genus *Medicago* by K. Lesins and I. Lesins, as well as the descriptions of species by E. Small, show the existence of annual, biennial and perennial forms for *M. lupulina* (Lesins & Lesins, 1979; Small, 2011).

M. lupulina is used as pasture, ground cover and green manure; along roads as an erosion control measure and to stabilize embankments to prevent the spread of mining waste. Seeds sown for pasture use can survive in the soil for several years without germinating mainly because *Medicago* has hard seeds. Therefore, *M. lupulina* is considered a plant with high reproductive potential. In wild plants that remain in one place for a long time and develop by gradual germination of hard seeds, the trait of seed hardness is considered important for survival and reproduction.

Germination depends on the presence of many different elements. Increased concentrations of macronutrients in the environment result in larger and more numerous seeds, which increases concentrations of nitrogen and phosphorus. Seed germination is directly proportional to the number of microelements and other reserve organic substances accumulated in the seeds, which are necessary for the subsequent growth and development of plants (Shaikh *et al*., 2013; Kołodziejek, 2017; Alamri *et al*., 2018). Some researchers report that boron deficiency in *Medicago* species causes excess sugar to accumulate in seeds, which limits its transport to other organs and tissues, thereby negatively affecting seed yield (Liu *et al*., 2015).

Studies have been performed on the chemical composition of *Medicago* species based on individual biochemical indicators. The Georgian species *M. lupulina* was found to contain 26.87% fiber, 23.03% protein, 3.25% fat and 36.38% nitrogen-free extractive substances, while the annual and biennial species *M. lupulina* common in Western Europe contained 30.1% fiber, 15.2% protein, 3.0% fat and 28.9% nitrogen-free extractive substances (Kintl *et al*., 2023).

In another article, the amounts of protein, lysine and tryptophan were determined for the species *M. minima* and *M. polymorpha* of the genus *Medicago* in a biochemical analysis of legumes. In *M. minima*, the protein content was 37.87 ± 0.52 g, tryptophan 2.42 ± 0.12 g and lysine 4.94 ± 0.04 g; in *M. polymorpha*, the protein content was 41.29 ± 0.62 g, tryptophan 4.91 ± 0.06 g and lysine 2.31 ± 0.0 g (Elamine *et al*., 2022).

Proteins are the most important natural compounds in the living cells of plants and animals. They are high-molecular organic substances built from amino acid residues. Plant protein molecules, like animal proteins, contain C, H, O, N, P, S and almost all trace elements. Reserve proteins are stored in the seeds and used to nourish the endosperm leaves. Plants generally contain less protein than animals. The protein content of plant organs is usually 5-15% of dry weight. In plants, most of the protein is concentrated in seeds (on average 10-20% in cereals and 25-35% in legumes) (Gholami *et al*., 2014).

Plant proteins differ in composition and amino acid ratio. In some proteins, some amino acids may be absent or present in very small quantities, while in others, they may be present in large quantities. It is known that there is a shortage of food protein in the world and almost half of the world's population suffers from protein starvation. Human protein needs are met by 10-30% of animal proteins and 70-90% of plant proteins (Fourquin *et al*., 2013).

Research data are less available for annual species than for perennial species. Species of the genus *Medicago,* widely distributed in the flora of Azerbaijan, have not yet been researched in biochemical and pharmacological terms. Thus, this phytochemical and pharmacological study of species of the genus *Medicago* common in Azerbaijan offers new perspectives.

The main objective of the research is to analyze the morphological and biochemical characteristics of the genotypes of the *Medicago lupulina* species, common in various regions of Azerbaijan, to determine the relationships between these characteristics and assess their ecological compatibility. This creates a scientific basis for future selection work and agrobiological planning aimed at increasing productivity.

2. Materials and methods

Plant material

In this investigation, 20 genotypes of *M. lupulina* L. were used (Table 1). Genotypes were collected from 20 different regions of Azerbaijan (Figure 1). Table 1 lists the codes of these genotypes, the species to which they belong, the regions where they were collected, their geographic coordinates, the altitude above sea level (AASL) and the biotopes where the samples were collected. The research was carried out between 2011 and 2023.

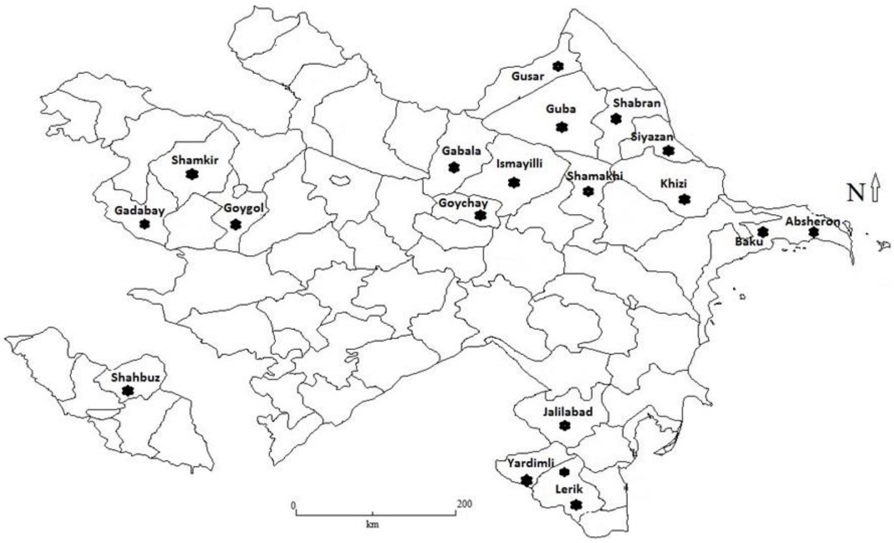

Figure 1. Areas where genotypes were collected

Table 1. Codes, names, locations, geographic coordinates, AASL and biotopes of the collection of *M. lupulina* L. specimens used in this study

Code	Taxons	Gathering place	Geographic coordinates	AASL (m)	Biotope
ML1	*M. lupulina*	Gadabay	N 40°37.705' E 045°48.923'	1406	Grassy slope
ML2	*M. lupulina*	Gabala	N 40°58.699' E 047°51.087'	756	Roadside
ML3	*M. lupulina*	Shahbuz	N 39°31.855' E 045°47.542'	2103	Pasture area
ML4	*M. lupulina*	Sheki	N 41°22.496' E 046°89.632'	262	Rocky slope
ML5	*M. lupulina*	Goygol	N 41°28.898' E 046°20.808'	1036	Hayfield
ML6	*M. lupulina*	Absheron	N 40°23.143' E 049°05.779'	12	Sandy terrain
ML7	*M. lupulina*	Baku	N 40°19.343' E 049°03.981'	19	Sandy terrain
ML8	*M. lupulina*	Guba	N 41°14.763'	1034	Pasture area

			E 048°37.564'		
ML9	*M. lupulina*	Goychay	N40°37.824' E 048°01.538'	55	Meadow
ML10	*M. lupulina*	Lerik	N 38°68.179' E 048°37.592'	1394	Rocky grassy area
ML11	*M. lupulina*	Khizy	N 40°53.122' E 049°00.100'	1288	Rocky slope
ML12	*M. lupulina*	Shamkir	N 40°42.721' E 045°55.061'	1345	Inclined slope
ML13	*M. lupulina*	Yardimli	N 38°55.053' E 048°07.043'	1304	Hayfield
ML14	*M. lupulina*	Ismayilli	N40°46.443' E 048°03.483'	675	Roadside
ML15	*M. lupulina*	Jalilabad	N39°14.954' E48°27.420'	508	Edge of the field
ML16	*M. lupulina*	Gusar	N41°38.640' E48°34.980'	847	Pasture area
ML17	*M. lupulina*	Shabran	N41°16.116' E48°53.415'	68	Rocky grassy area
ML18	*M. lupulina*	Siyazan	N41°04.224' E49°08.085'	35	Sandy terrain
ML19	*M. lupulina*	Lerik	N 38°74.378' E 048°32.787'	1706	Riverside
ML20	*M. lupulina*	Shamakhi	N 40°61.283' E 048°65.799'	581	Edge of the forest

Morphological characterization

Morphological characteristics of each collected taxon and ecological data of the collection site were recorded in special descriptors. The collected seed material was packed in special sterile paper bags and dried using silica gel under special laboratory conditions. For each of the 20 *Medicago lupulina* L. accessions, nine quantitative traits were measured according to the descriptions from Bioversity International: plant height (PH), stem width (StW), bean diameter (BD), thousand beans weight (ThBW), seed width (SW), seed length (SL), leaf width (LW), leaf length (LL), thousand seed weight (ThSW).

Biochemical characterization

Sample preparation for biochemical analysis of seeds was carried out as follows: seed material was collected from ten plants (ten biological replicates) from each collection site. The seeds were evaluated using the following biochemical parameters: the mass fraction of dry matter (%) was determined by weighing the average sample before and after drying at 105°C in a FED400 Binding thermostat (Germany); the protein content was determined according to Kjeldahl on a Velp Scientifica UDK 159 device (Italy) in (%) of dry weight; total protein was determined by the Kjeldahl method (Ermakov *et al.*, 1969); lysine was determined by the method of Museiko and Sysoev (1970) and tryptophan was determined by the method of Ermakov et al. (1969).

Statistical analysis

All measurements were performed on 30 plants from each population at the plant maturity stage and average values were calculated. IBM SPSS software (version 25.0) was used to conduct correlation analysis of morphological features. To group genotypes based on their morphological characteristics, a two-factor cluster analysis was performed using the Euclidean method in the PAST program (version 4.11) (Hammer & Harper, 2001).

3. Results and Discussion

Genetic resources provide a variety of products and the global economy, as well as the livelihoods and health of individuals, significantly rely on these resources (Hajiyeva *et al*., 2024). Species of the genus *Medicago* are found in the natural biological resources of Azerbaijan from the steppes to the middle mountain belt, in various growing conditions, especially in arid places; on grassy slopes; on rocky, gravelly, clayey soils; in gardens and plantings; among weeds and in forest clearings and meadows (Guvendiyev *et al*., 2025). The fact that species of the genus grow in different ecological conditions - on different types of soil - also affects the phytochemical composition of the plant at the end of the growing season, causing significant changes (Egesel & Kahrıman, 2012). With the accumulation of energy and nutrients as a result of synthetic processes in the leaves and plant, the development of the plant ends. At this point, the process of seed formation and maturation, as well as the supply of reserve nutrients to the tissues, is completed. As seeds mature, they become dehydrated. In an anhydrous environment, no transformation of substances is possible and the seeds enter a state of dormancy. Like other legumes, *Medicago* species can accumulate more protein through nitrogen fixation. Organic substances such as globulin, lysine, arginine, tryptophan and leucine are mainly accumulated in seeds (Bakoglu *et al*., 2010).

Plants absorb macro- and microelements, as well as inorganic substances from the atmosphere, hydrosphere and upper layers of the lithosphere, using them in their ontogenetic development. Some accumulate in their organisms as biological reserve substances (Wilson, 2005). After plants die, their remains decompose and some of the biological reserves necessary for life return to nature. In these processes, which constantly repeat in the biosphere, a circulation of microelements occurs. Substances accumulating in plant organisms form their phytochemical composition. One study noted that *Medicago* species accumulate beneficial minerals such as phosphorus, calcium, potassium, sodium, chlorine, magnesium and copper (Mezni *et al*., 2010).

Although *M. lupulina* is a promising forage and pasture plant, its various forms are poorly understood. Therefore, we compared our results with data obtained for other types of *Medicago*. An analysis of the morphological and biochemical composition of genotypes selected from areas with different environmental conditions was carried out and the influence of environmental conditions on these traits was assessed.

Statistical analysis was performed based on minimum and maximum values, mean, standard error, standard deviation, mode and coefficient of variation for each morphological and biochemical characteristic analyzed in the investigation (Table 2).

Plant height ranged from 170 to 460 mm. The average value was 301.0 mm, the standard deviation was 89.8 mm and the standard error was 20.1 mm. The absence of a mode indicates the absence of repetitions among genotypes. The coefficient of variation was 29.8%, which indicates a statistically moderate level of variability for this feature.

Medoekali and colleagues (2015) discovered that the number of seeds per bean had a standard deviation (SD) of 4.48 and a CV of 5.61, indicating similar variability in seed-related measures. Jafari et al. (2012) findings for plant height revealed a standard deviation (SD) of 5.46 and a standard error (SE) of 0.35, suggesting that data on plant height varies greatly. In addition, the trait's extensive population variability and great phenotypic diversity are confirmed by its coefficient of variation (CV) of 19 (Jafari *et al*., 2012). The width of the stem varied between 1 and 3 mm. The average value was 2.0 mm and the mode was 2 mm.

Table 2. Descriptive statistics of researched morphological and biochemical traits in *Medicago lupulina* L. genotypes

Trait	Abb	Min	Max	Mean	SE	SD	Mode	CV%
Plant height	PH	170	460	301.0	20.1	89.8	NA	29.8
Stem width	StW	1	3	2.0	0.2	0.7	2	37.1
Bean diameter	BD	1	3	2.0	0.2	0.8	2	38.9
Thousand beans weight	ThBW	5	11	7.2	0.4	1.9	6	26.6
Seed width	SW	1	2	1.5	0.1	0.5	NA	30.9
Seed length	SL	1	3	2.6	0.1	0.5	3	18.9
Leaf width	LW	5	13	9.8	0.6	2.6	11	26.4
Leaf length	LL	8	21	14.1	0.9	3.9	NA	27.8
Thousand seeds weight	ThSW	1.6	2,7	2.1	0.1	0.4	1,6	17.5
Total nitrogen	TN	3.25	4.53	4.1	0.1	0.4	NA	9.3
Protein	Pro	24.12	28.91	26.0	0.3	1.2	NA	4.5
Tryptophan	Try	269	455	348.8	10.5	47.0	NA	13.5
Lysine	Lys	604	726	649.8	7.6	34.2	NA	5.3

Abbreviations: Abb - abbreviations; SD - standard deviation; CV - coefficient of variation; Max - maximum; Min - minimum

The presence of a mode value indicates that this value is the most frequently occurring measure in the sample. The standard deviation was 0.7 mm and the standard error was 0.2 mm. The coefficient of variation was 37.1%, which indicates high statistical variability. The diameter of the bean varied from 1 to 3 mm, with an average of 2.0 mm and a mode of 2 mm. This indicates that most of the samples are concentrated in this size. The standard deviation was 0.8 mm and the standard error was 0.2 mm. The coefficient of variation was 38.9%, indicating high statistical variability for this indicator. Lesins and Lesins (1979) reported that the diameter of the bean varied between 9 and 20 mm. In other studies, the average bean diameter was recorded as 12.38 mm (Morshedi *et al*., 2022). Genchkan (1970) reported a lower average diameter of 6.18 mm. Bagheri et al. (2020) studied the species *Medicago minima* and reported an SE of 0.02, an SD of 0.52 and a CV% of 15 for the bean diameter trait. The thousand bean weight varied from 5 to 11 g, with an average of 7.2 g and a mode of 6 g. Different sources give different figures for the size and weight of beans, depending on the circumstances (Bojnansky & Fargasova, 2017). The mass is generally repeated around 6 g. The standard deviation was 1.9 g and the standard error was 0.4 g. The coefficient of variation was 26.6%, indicating a statistically moderate level of variability. Seed width and length varied from 1-2 mm to 1-3 mm, averaging 1.5-2.6 mm. The absence of a mode value indicates that no width measurement was repeated most frequently in the sample and the measurements are statistically dispersed. The length of the seed was 3 mm. The standard deviation for both features was 0.5 mm and the standard error was 0.1 mm. The coefficient of variation was 30.9% and 18.9%, indicating that the trait has statistically medium-high and medium variability. Bagheri et al. (2020) investigated the species *Medicago minima* and found an SE of 0.008, an SD of 0.16 and a CV% of 13.5 for the seed number trait. The leaf width varied from 5 to 13 mm, with an average of 9.8 mm and a mode of 11 mm. The standard deviation was 2.6 mm and the standard error was 0.6 mm. The coefficient of variation was 26.4%, which indicates moderate statistical variability of the trait. The length of the leaf varied from 8 to 21 mm. The average was 14.1 mm, the standard deviation was 3.9 mm and the standard error was 0.9 mm. The coefficient of variation was 27.8%, indicating moderate statistical variability for this feature. In their investigation of the genus *Trigonella*, Bakhtiar et al. (2022) found an SD of 5.8 and a CV% of 37.2 for leaf length, while leaf width had an SD of 7.9 and a CV% of 51.7. These parameters indicate phenotypic variability and diversity of leaf length and width. Thousand seed weight ranged from 1.6 to 2.7 g, with an average of 2.1 g. The mode was 1.6 g. The standard deviation was 0.4 g and the standard error was 0.1 g. The coefficient of variation was 17.5%, which is considered statistically stable. When Rashidi et al. (2009) researched *Medicago sativa* varieties, they found an SE of 0.04 and a CV% of 5.5 per thousand seeds. These findings suggest that while there are minor variations in seed weight between cultivars, these variations are not very noticeable.

Total nitrogen content ranged from 3.25 to 4.53%. The average was 4.1%, the standard deviation was 0.4% and the standard error was 0.1%. The coefficient of variation was 9.3%, indicating statistically low variability. In a study by Ignatiev et al. (2021), the percentage of total nitrogen and protein content in *Medicago* species varied (2.75-4.96%) depending on environmental conditions. The biochemical composition of *M. lupulina* also varies across different regions. For example, in a study by Malysheva et al. (2022), the total nitrogen content in the green mass of *M. lupulina* ranged from 4.23 to 4.51 and the protein content ranged from 25.63 to 28.91.

An important indicator of the nutritional value of forage crops is the content of crude protein. In this investigation, protein content ranged from 24.12-28.91%. The average was 26.0%, the standard deviation was 1.2% and the standard error was 0.3%. A variation coefficient of 4.5% indicates that the trait has very low statistical variability. Malysheva et al. (2022) investigated the species *Medicago lupulina* and reported a coefficient of variation of 13.6%. Different sources report the crude protein content of alfalfa during the mass flowering period as 16.82%, 17.64% and 20.1% (Malysheva *et al*., 2022). For creeping and variegated alfalfa accessions from VIR collections, this indicator's variability varies between 15% and 22% (Ignatiev *et al*., 2021).

Tryptophan content ranged from 269-455 mg. The average value was 348.8 mg, the standard deviation was 47.0 mg and the standard error was 10.5 mg. The absence of a mode value indicates that the values are distributed over a wide range and no value repeats more often than others. The coefficient of variation is 13.5%, indicating low to moderate statistical variability. Lysine content ranged from 604 to 726 mg, with an average of 649.8 mg. The standard deviation was 34.2 mg and the standard error was 7.6 mg. The coefficient of variation was 5.3%, which indicates statistical stability.

To study the importance of different traits in genotypes, the principal component analysis (PCA) method was used (Jolliffe & Cadima, 2016). Before starting such an analysis, it is necessary to check whether the data is suitable for factor analysis. For this purpose, two main tests are used: the Kaiser-Meyer-Olkin (KMO) measure of adequacy and the Bartlett test of sphericity (Ali, 2016). The obtained KMO value (0.720) and the significant result of the Bartlett test (p=0.000) indicate that the data are fully consistent with the factor analysis (Table 3). A KMO indicator in the range of 0.7-0.8 is considered a good result (Ebadi *et al*., 2022).

Based on the results of factor analysis, the variables were grouped into three main components (Table 4). These components cover different aspects of plant and seed performance and provide a deeper understanding of the data structure. Principal component analysis identified that relationships between characteristics may result from the pleiotropic effects of loci or genes that regulate these traits (Khadivi-Khub *et al*., 2015).

Table 3. KMO and Bartlett's Test

Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy		**0.720**
Bartlett's test of sphericity	Approximate chi-square	260.219
	St.V.	78
	Significance	0.000

Table 4. Results of principal component analysis for each trait investigated

Trait	PCA1	PCA2	PCA3
Plant height	0.935	0.247	-0.159
Stem width	0.898	0.306	-0.113
Bean diameter	0.896	0.285	
Thousand beans weight	0.685	-0.109	0.409
Seed width		0.777	0.278
Seed length			0.859
Leaf width	0.924	0.224	
Leaf length	0.842	0.163	-0.434
Thousand seeds weight	0.812		0.211
Total nitrogen	0.813	-0.267	
Protein	0.805	-0.191	
Tryptophan	0.715	-0.514	0.235
Lysine	0.742	-0.423	
Eigenvalue	7.548	1.490	1.317
Variability %	58.061	11.465	10.131
Cumulative %	58.061	69.526	79.657

The first component is the main factor that is influenced by most variables. The high loading values for this component indicate that morphological parameters such as plant height (0.935), stem width (0.898), bean diameter (0.896), leaf width (0.924) and leaf length (0.842) are closely related to this factor. Moreover, biochemical indicators such as thousand seed weight (0.812), total nitrogen (0.813), protein (0.805), tryptophan (0.715) and lysine (0.742) also have a strong loading in this component. The thousand bean weight (0.685) also falls into this group. This component can be characterized as a "plant development and quality" factor, since it combines both morphological and nutritional quality indicators. According to Lesins and Lesins (1979), thousand seed weight offers a trustworthy initial approximation of seed size.

The main differentiating variable in the second component is seed width, which has a high factor loading of 0.777. This means that this factor reflects the dimensional characteristics of the seed or more precisely, its width. The loadings of other variables on this component are either weak or insignificant. Therefore, the second component can be called the "seed size" factor. Graziano and colleagues assessed phenomorphological and agronomic variation among 31 natural populations of *Medicago polymorpha* collected from different environmental conditions in Sicily and analyzed the relationship of this diversity to environmental parameters. Based on two field trials and PCA, they found significant differences between the populations, with some having greater agronomic performance than commercial varieties (Graziano *et al.*, 2009).

For the third component, the main load falls on the length of the seed and the load of this indicator is 0.859. The thousand beans weight also contributed a certain amount to this component (0.409), but this figure is too low to be considered a major factor. This component can be considered a factor of "physical seed size", especially since it reflects the long size of the seed.

The conducted factor analysis found that the three components together explain 79.66% of the total variance. This means that almost 80% of the original data can be summarized using these three main factors, indicating that the factor analysis was sufficiently informative and the variables were grouped effectively. In Medoukali and collegues' study, 74.22% of the variance was explained by the first four principal components (PC1-PC4). These PCs accounted for 36.40%, 19.08%, 11.74% and 6.99% of the variance, respectively. PC1 did not exhibit a substantial link with the number of seeds in a bean or the thousand seed weight, while PC3 and PC4 did. This implies that although the weight of a thousand seeds and the quantity of seeds in a bean have less of an effect on PC1, they are more closely related to PC3 and PC4. In other words, these two characteristics have the greatest influence on PC3 and PC4 (Medoukali *et al*., 2015).

Thus, as a result of the analysis, morphological, biochemical and size indicators were clearly divided into components, which clarified the data structure. The PCA results are important for grouping multiple and interrelated morphological features and identifying the main factors (Hajiyev *et al*., 2025). Such analyses can play an important role in breeding programs and in assessing productivity and quality.

This study used Pearson correlation coefficients to analyze the relationship between plant morphological and biochemical traits and altitude above sea level (Table 5). The results show highly statistically significant positive correlations between several traits, which indicates functional or genetic relationships between them.

The correlation between the two traits was calculated. Correlation analysis can provide valuable information about the most important traits when evaluating genotypes (Norman *et al*., 2011). The strength of the linear relationship between two continuous variables is most often determined using the Pearson correlation coefficient (r), which has a value between -1 and 1 (Pearson, 1900). Identifying traits that are significantly correlated allows one trait to be predicted in advance for another, which can facilitate the selection of appropriate genotypes. Some traits we investigated showed significant interdependence, to such an extent that they could be used in selection programs. Numerous studies on populations of *M. truncatula* have also revealed notable morphological differences (Hansali *et al*., 2007; Haddioui *et al*., 2012). Plant height showed high statistically significant positive correlation with most morphological and biochemical traits. In particular, the associations with stem width ($r = 0.957$, $p < 0.01$), bean diameter ($r = 0.904$, $p < 0.01$), leaf width ($r = 0.933$, $p < 0.01$) and leaf length ($r = 0.914$, $p < 0.01$) were very strong. Among the biochemical traits, the correlations with total nitrogen ($r = 0.650$, $p < 0.01$), protein ($r = 0.687$, $p < 0.01$), tryptophan ($r = 0.499$, $p < 0.05$) and lysine ($r = 0.603$, $p < 0.01$) were also statistically significant, indicating that plant height also affects nutrient richness to some extent.

Zhang et al. (2024) observed positive and significant relationships between leaf functional traits and plant height, stem diameter and branch number. Stem width also showed a statistically significant correlation with plant height, as well as with other vegetative and generative traits. In particular, a very strong correlation was found with bean diameter ($r = 0.953$, $p < 0.01$). The associations with leaf width ($r = 0.898$, $p < 0.01$), leaf length ($r = 0.850$, $p < 0.01$), total nitrogen ($r = 0.598$, $p < 0.01$) and protein ($r = 0.581$, $p < 0.01$) were also significant. These relationships indicate that stem thickness is an important trait that determines both the vegetative development of the plant and some biochemical quality indicators. Buba (2013) emphasized a positive correlation ($r = 0.447$) between stem diameter and plant height, indicating that stem diameter is an important parameter in predicting plant height.

Table 5. Pearson correlation among studied morphological, biochemical and ecological traits

	StW	BD	ThBM	SW	SL	LW	LL	ThBM	TN	Pro	Try	Lys	AASL
PH	0.957**	0.904**	0.508*	0.103	-0.084	0.933**	0.914**	0.718**	0.650**	0.687**	0.499*	0.603**	0.560*
StW	1	0.953**	0.504*	0,134	-0.034	0.898**	0.850**	0.648**	0.598**	0.581**	0.495*	0.544*	0.475*
BD		1	0.553*	0,122	0.042	0.883**	0.783**	0.685**	0.639**	0.652**	0.491*	0.486*	0.548*
ThBM			1	0.06	0.101	0.504*	0.368	0.835**	0.496*	0.539*	0.571**	0.490*	0,093
SW				1	0,154	0.152	-0.005	0.064	-0.073	-0,148	-0,236	-0,16	-0,227
SL					1	0.051	-0.32	-0,015	-0,006	0,025	0,114	-0,147	0.018
LW						1	0.799**	0.733**	0.726**	0.679**	0.582**	0.559*	0.612**
LL							1	0.528*	0.618**	0.683**	0,412	0.556*	0.536*
ThBM								1	0.562**	0.658**	0.508*	0.548*	0,392
TN									1	0.694**	0.780**	0.676**	0.678**
Pro										1	0.552*	0.623**	0.656**
Try											1	0.772**	0.513*
Lys												1	0.493*

Note: plant height (PH), stem width (StW), bean diameter (BD), thousand beans weight (ThBW), seed width (SW), seed length (SL), leaf width (LW), leaf length (LL), thousand seed weight (ThSW), altitude above sea level (AASL)

Leaf width and length showed significant positive correlations with both vegetative and biochemical parameters. Leaf width showed highly statistically significant relationships with total nitrogen ($r = 0.733$, $p < 0.01$), protein ($r = 0.726$, $p < 0.01$), tryptophan ($r = 0.679$, $p < 0.01$) and lysine ($r = 0.582$, $p < 0.01$). In our investigation, leaf width and length were found to be positively correlated with protein content. Sangekar (2017) reported in his research that increasing leaf area increases photosynthesis, which in turn increases the overall biochemical productivity of the plant, thereby increasing protein and amino acid content. Kim et al. (2019) reported positive relationships between leaf area and levels of some amino acids, such as tryptophan and lysine, suggesting that greater leaf area results in more active metabolism and amino acid synthesis. The thousand seed weight indicator also had a statistically significant relationship with several biochemical parameters. Correlations were found with total nitrogen ($r = 0.562$, $p < 0.01$), protein ($r = 0.658$, $p < 0.01$), tryptophan ($r = 0.508$, $p < 0.05$) and lysine ($r = 0.548$, $p < 0.05$). These relationships indicate that seeds with greater mass have higher protein and amino acid content. In a study by Kulwal and Mhase (2017) a positive and statistically significant correlation was found between protein content and thousand seed weight among different chickpea (*Cicer arietinum*) genotypes. The results show that genotypes with heavier seeds have higher protein concentrations. Thousand seed weight is also an important indicator for genetic selection programs (Kulwal & Mhase, 2017). Another study assessed genetic variation and correlations between traits in the species *Lathyrus sativus* L. The results show a positive and statistically significant relationship between thousand seed weight and protein and total nitrogen content. This suggests that the mass of beans reflects their nutritional value (Mondal & Puteh, 2014). This study on soybeans showed positive and statistically significant correlations between seed size and mass and amino acids, including lysine and tryptophan. Heavier seeds tend to have higher amino acid content, which affects nutritional value (Lee *et al*., 2019).

Statistically significant correlations were also observed between biochemical parameters. The very strong correlation between total nitrogen and protein ($r = 0.694$, $p < 0.01$), as well as the links with tryptophan ($r = 0.780$, $p < 0.01$) and lysine ($r = 0.676$, $p < 0.01$), indicate that these parameters vary inversely proportionally to each other. Protein showed correlations with tryptophan ($r = 0.552$, $p < 0.05$) and lysine ($r = 0.623$, $p < 0.01$). A strong correlation ($r = 0.772$, $p < 0.01$) was also recorded between tryptophan and lysine, proving that the amino acids in the protein composition change in a dependent manner. One study found a positive correlation between total nitrogen and protein content in alfalfa plants. This suggests that increasing the total nitrogen content of a plant increases its protein content (Gjoroska *et al*., 2019). Another study found a positive correlation between the tryptophan and protein content of clover. This suggests that the amino acid composition of a plant influences protein synthesis (Wang *et al*., 2022).

The results show that altitude above sea level had statistically significant positive correlations with both morphological and biochemical parameters of the plant. Thus, significant positive relationships were found between plant height ($r = 0.560$, $p < 0.05$), stem width ($r = 0.475$, $p < 0.05$), bean diameter ($r = 0.548$, $p < 0.05$), leaf width ($r = 0.612$, $p < 0.01$) and leaf length ($r = 0.536$, $p < 0.05$). This indicates that with increasing altitude above sea level, the vegetative development of the plant improves. Significant correlations were also recorded for biochemical indicators. Statistically significant positive correlations were found between altitude and total nitrogen content ($r = 0.678$, $p < 0.01$), protein content ($r = 0.656$, $p < 0.01$), tryptophan content ($r = 0.513$, $p < 0.05$) and lysine content ($r = 0.493$, $p < 0.05$). The results indicate that altitude influences not only the morphology but also the amount of protein and amino acids in the plant. Thus, altitude can be considered an important ecological factor in terms of both the vegetative development and the nutritional value of *Medicago lupulina*.

In general, the correlation results indicate the presence of strong functional and statistical relationships between the main morphological and biochemical characteristics of the plant. The strong correlation observed between morphological parameters, especially plant height, stem thickness, leaf size and bean diameter, proves that they are closely related as general parameters of vegetative development. Strong positive correlations between these types of traits allow them to be used as complex selection criteria in selection and genetic improvement programs.

Statistically significant relationships between biochemical parameters indicate the similarity and interdependence of their mechanisms of synthesis. The fact that biochemical parameters also show statistically significant correlations with many morphological traits indicates their close connection not only with genetics but also with the general physiological and structural development of the plant.

On the other hand, the fact that some traits, such as seed width and length, show statistically insignificant relationships with other traits indicates their relative genetic stability, i.e., they are less susceptible to environmental influences and change independently. Such indicators may be useful as stable genetic markers in selection.

The fact that altitude above sea level shows a statistically significant positive correlation with several morphological and biochemical parameters indicates that environmental factors influence plant development and composition. This influence of abiotic factors such as altitude has important practical implications for the determination of selection zones.

Correlation analysis established that some morphological characteristics are closely related to each other, to biochemical composition and to environmental conditions and can be used in combination for selection. Overall, these correlations provide an important statistical basis for understanding trait covariation trends in selection and agrobiological studies and for developing effective selection strategies.

Cluster analysis is a statistical technique used to group data. The main goal of this method is to collect similar objects into one group (cluster) and separate different ones into separate clusters. A study conducted by Kosman and Leonard (2007) showed that Euclidean distance is used to assess differences between individuals.

To assess the relationships between the 20 researched genotypes, a two-factor cluster analysis was used based on nine morphological and four biochemical characteristics. The dendrogram was created using the Euclidean method in the statistical software package PAST 4.11 (Figure 2).

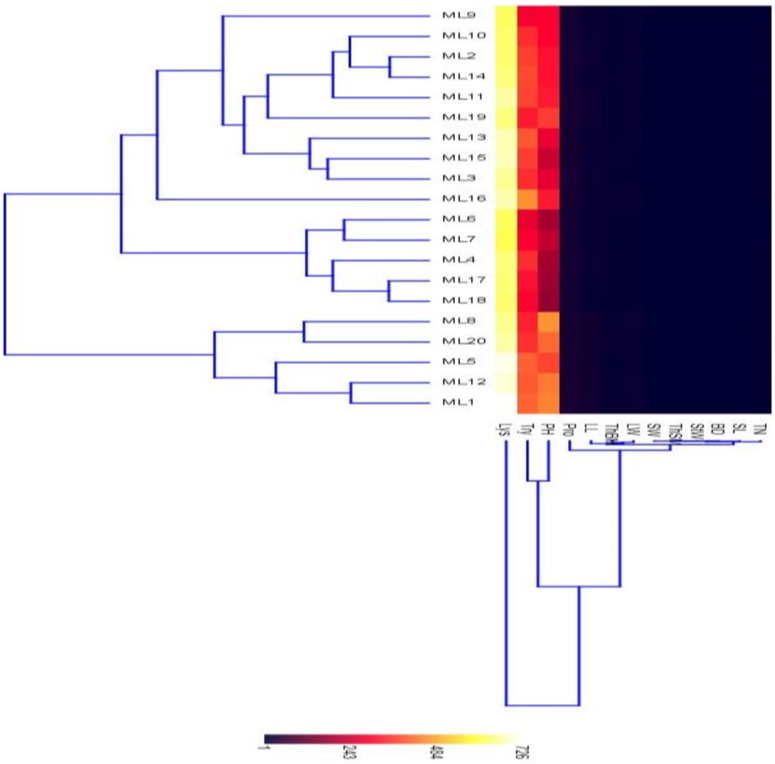

Figure 2. A dendrogram of *M. lupulina* performed using morphological and biochemical characters (for abbreviations, see Table 2)

This type of analysis facilitates the visual observation of genotype grouping (Mammadova *et al*., 2024). One of the most precise ways to express relationships between data is to use the Euclidean distance, which is the linear distance between two points (Hajiyev *et al*., 2025; Khadivi *et al*., 2025). As can be seen from the two-factor hierarchical cluster dendrogram created using Euclidean distance, the studied genotypes were grouped into six different clusters. Interestingly, all groups differed in stem width, bean diameter, thousand beans weight, seed width, seed length, leaf width, leaf length, thousand seed weight, total nitrogen and protein content and these traits spanned the entire spectrum. Cluster analysis showed that the main features influencing cluster formation are plant height, tryptophan and lysine.

Cluster 1 contains two genotypes from the Gusar and Khizi districts. It has a tall plant height and large seed sizes grouped in clusters. The plant height varies from 320 mm to 440 mm and the thousand seed weight varies from 7 g to 11 g. This indicates that the soil and climatic conditions of these territories increase productivity and are favorable for good development. Total nitrogen and protein levels are also high. Total nitrogen ranges from 4.29 to 4.53 and protein ranges from 24.78 to 28.91. The width and length of the leaves are large, indicating high photosynthetic capacity.

Cluster 2 contains five genotypes taken from the Sheki, Absheron, Baku, Shabran and Siyazan regions. The height of the plants in this cluster varies from a small 170 mm to 220 mm. The size of the seeds is also small: the weight of thousand seeds ranges from 5 g to 7 g. In this cluster, total nitrogen and protein levels are lower, with total nitrogen ranging from 3.25 to 4.32 and protein from 24.12 to 27.31. The width and length of the leaves are also small, which indicates that the plants are developing less favorably.

Cluster 3 includes three genotypes taken from the regions of Gadabay, Goygol and Shamkir. Plants in this cluster are tall (370 mm to 460 mm). The size of the seeds is also large: the thousand seed weight ranges from 8 g to 11 g. Total nitrogen and protein levels are high, with total nitrogen ranging from 4.23 to 4.51 and protein from 25.63 to 28.91. The width and length of the leaves are large, indicating high photosynthetic capacity and good development.

Cluster 4 contains four genotypes taken from the Shahbuz, Yardimli, Goychay and Jalilabad districts. Plants in this cluster are of medium height (230 mm to 290 mm). The seeds are small (5 to 7 g) and the total nitrogen and protein content is relatively low. Total nitrogen ranges from 3.86 to 4.29 and protein ranges from 24.83 to 27.31. The size of the leaves is also relatively small, indicating underdevelopment of the plants.

Cluster 5 contains two genotypes collected from the Guba and Shemakha districts. Plants in this group are tall (410 mm to 460 mm) and the seeds are large (8 g to 11 g). Total nitrogen and protein levels are high, with total nitrogen ranging from 4.23 to 4.51 and protein ranging from 25.63 to 28.91. The width and length of the leaves are large, indicating high photosynthetic capacity.

Cluster 6 includes four genotypes taken from the Gabala, Lerik and Ismailli regions. Plants in this group are small (230 mm to 310 mm), as are the seeds (from 6 g to 8 g). Nitrogen and protein levels are moderate, with total nitrogen ranging from 3.25 to 4.16 and protein ranging from 24.12 to 25.93. Leaves are medium sized (6 mm to 11 mm).

The genotypes in each cluster were collected from different areas and differed mainly in plant height, seed size and nitrogen and protein levels. The main reason for clustering is the influence of soil and climatic conditions on plant development. Genotypes in Clusters 1 and 3 produce higher yields under better soil and climate conditions. In Clusters 2 and 6, plants develop less favorably due to poorer soil and nutrient conditions.

The Euclidean distance allows for comparisons of genotypes in terms of different traits. When examining the clusters separately, the closest genotypes were ML2 and ML14 (17.17), grouped in Cluster 6 and the most distant were genotypes ML9 and ML13 (122.58) in Cluster 4. When analyzing the clusters as a whole, the most distant genotypes were ML1 and ML18 (309.64). Genotypes with small distances have similar traits, while genotypes with large distances exhibit different traits. This information enables the grouping of genotypes by traits and the making of selections appropriate for different conditions.

In the second stage of two-factor hierarchical clustering, genotypes were divided into three groups based on nine morphological and four biochemical features. Lysine content formed the first group, plant height and tryptophan content formed the second group and the remaining ten traits formed the third group. As can be seen from the color shades, the formation of all six clusters was strongly influenced by plant height, tryptophan content and lysine content.

4. Conclusion

This study found significant differences in both morphological and biochemical characteristics among the genotypes of *M. lupulina* L. Factor analysis showed that these traits could be reduced to three main components and correlation analysis revealed strong functional and statistical relationships between some traits. Cluster analysis demonstrated that genotypes have different development and quality indicators depending on soil and climatic conditions. These results are of great importance for evaluating genotypes in selection programs and increasing their adaptive potential in suitable regions.

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