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Research Article

Specific features of the biochemical composition of life forms of black medic (*Medicago lupulina* L.)



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BACKGROUND: Black medic is used as a pasture, cover, siderate, anti-erosion culture for fixing embankments along roads, on mining dumps and for phytoremediation. Mono-, bi- and polycarpic forms are distinguished within the species, differing in the number of fruitions during the plant life cycle. The presence of polymorphism of morphological features and features of ontogenesis suggests the presence of differences in biochemical parameters in the selected groups.

AIM: The aim of the study was to study the polymorphism of biochemical parameters of various life forms of black medic.

MATERIALS AND METHODS: The material for the study was 20 accessions of black medic of various origins from groups of mono-, bi- and polycarpic plants of the VIR collection. For the study, a freshly harvested green mass of plants was used. The assessment was carried out according to the following biochemical parameters: the content of dry matter, protein, sugars, ascorbic acid, total acidity, chlorophylls, carotenoids, carotenes, β -carotene, anthocyanins. Statistical processing of the results included calculation of the main parameters of variation, analysis of variance and discriminant analysis.

RESULTS: The nutritional value of black medic was characterized by the content of crude protein and sugars. The range of protein variability ranged from 11,94 to 19,69 mg / 100 g of raw matter, sugars — from 0,44 to 2,67%. Differences in the sugar content of plants of different groups were revealed. The content of ascorbic acid was the highest in five varieties from the polycarpic plant group presented in the study in comparison with wild-growing accessions and the monocarpic Bereginia variety. Monocarps are distinguished by the content of anthocyanin ($19,5 \pm 1,41$ mg / 100 g); no significant differences were found in the content of other pigments.

CONCLUSIONS: The results of in-depth biochemical analysis of accessions from the collection of plant genetic resources demonstrate the high variability of biologically active substances in the green mass of plants and will significantly improve the choice of the initial material for selection for forage usage.

Keywords: genetic resources; biochemical composition; polymorphism of traits; monocarps; bicarps; polycarps.

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Научная статья

Особенности биохимического состава жизненных форм люцерны хмелевидной (*Medicago lupulina* L.)

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Актуальность. Люцерну хмелевидную используют как пастбищную, покровную, сидератную, противозероизионную культуру для закрепления насыпей вдоль дорог и на отвалах горнодобывающей промышленности для фиторе-медиации. В пределах вида были выделены группы с моно-, би- и поликарпическими формами, имеющими отличия морфологических признаков и особенностей онтогенеза, что предполагает возможное различие биохимических показателей в выделенных группах.

Цель исследования — изучение полиморфизма биохимических показателей разных жизненных форм люцерны хмелевидной.

Материалы и методы. Материалом для исследования послужили 20 образцов люцерны хмелевидной различного происхождения из групп моно-, би- и поликарпических растений коллекции ВИР. Для исследования использовали свежесобранную зеленую массу растений. Оценку проводили по следующим биохимическим показателям: содержанию массы сухих веществ, белка, сахаров, аскорбиновой кислоты, общей кислотности, хлорофиллов, каротиноидов, каротинов, β -каротина, антоцианов. Статистическая обработка результатов включала вычисление основных параметров варьирования, дисперсионный анализ и дискриминантный анализ.

Результаты. Питательную ценность люцерны хмелевидной характеризовало содержание сырого белка и сахаров. Диапазон изменчивости белка составил от 11,94 до 19,69 мг на 100 г сырого вещества, сахаров — от 0,44 до 2,67 %. Выявлены различия у растений разных групп по содержанию сахаров. Содержание аскорбиновой кислоты оказалось наивысшим у пяти представленных сортов из группы поликарпических растений в сравнении с дикорастущими образцами и монокарпическим сортом Берегиня. Монокарпики выделяются по содержанию антоциана ($19,5 \pm 1,41$ мг/100 г); по содержанию остальных пигментов достоверных различий не обнаружено.

Заключение. Результаты углубленного биохимического анализа образцов зеленой массы *Medicago lupulina* из коллекции генетических ресурсов растений ВИР демонстрируют высокую изменчивость биологически активных веществ и соединений — показателей устойчивости к стрессовым факторам среды, что помогает отбору исходного материала для селекции кормового направления.

Ключевые слова: генетические ресурсы; биохимический состав; полиморфизм признаков; монокарпики; бикарпики; поликарпики.

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BACKGROUND

The black medic (*Medicago lupulina* L.) is a species of the family Fabaceae. The genus *Medicago* includes 63 annual and perennial species [1]. The black medic is widely distributed in the temperate and subtropical zones of the Old and New Worlds. It is considered to be originated in the Mediterranean [2]. The black medic is used as a pasture, cover, green manure, and anti-erosion crop for fixing embankments along the roads and on dumps of the mining industry for phytoremediation [3–8]. It is used as a pasture crop because it has high potential for self-reproduction due to the shedding of seeds and their long-term preservation in the soil without germination for several years [4]. About ten varieties are represented in world practice, including one domestic.

The question of whether a species belongs to a particular life form has been raised for a long time. It is generally accepted that within a species there are annual, biennial, and perennial forms [1, 2, 9–13]. Grossheim [9] considers *M. lupulina* as including both annual (var. *vulgaris* Koch and var. *Willdenowii* Boenn.) and perennial species (var. *perennans* Grossh.). Monographs focused on the genus *Medicago*, have also indicated the presence of annual, biennial, and perennial forms in the genus [1, 2].

Recently, groups of annuals, biennials, and perennials, distinguished by life expectancy, have been considered as mono-, bi-, and polycarpic; representation in each of the groups is based on the number of fruiting during the life cycle of the plants. The life cycle of the monocarpic group is one season, and in case of fruiting failure, the life span increases until the next year when fruiting is completed. Bicarpics and polycarpics form vegetative organs, a rosette of shortened leafy shoots, and a few generative shoots in the first year of life. In the second year of life, plants from both groups bear fruit. Bicarpics from group 2 die off after fruiting, whereas polycarpics from group 3 continue to bear fruit the following year [14]. Monocarpic and bicarpic black medic were found in the Oryol region during the study of the black medic ontogeny [15]. It is thought that mono-, bi- and polycarpics are present in varying proportions in different populations [16].

In the N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) collection were identified mono-, bi-, and polycarpic accessions of various geographic origins. Black medic monocarpics with fast growth after germination and fruiting in the year of sowing or delayed fruiting the following year are suitable for creating long-term pastures, remediation of disturbed grasslands, and used as a cover crop for cereals and green manure. Polycarpics and bicarpics can be used as components of the lower horizon in multicomponent herbal mixtures for livestock feed.

The chemical composition of black medic in terms of individual parameters has been known for a long time. *Medicago lupulina* from Georgia has a fiber content of 26.87%, crude protein of 23.03%, crude fat of 3.25%, nitrogen-free extractives of 36.38%, and ash of 10.57%. One-biennial plants from Western Europe contained 30.1% fiber, 15.2% crude protein, 3.0% crude fat, 28.9% nitrogen-free extractives, and 6.1% ash. A perennial *M. lupulina* from Georgia had a fiber content of 21.0%–23.77%, crude protein of 19.1%–22.33%, crude fat of 2.50%–2.87%, nitrogen-free extractives of 32.06%–45.2%, and ash of 9.34%–12.2%. The annual form of *M. lupulina* had 30.1% crude fiber, 15.2% crude protein, 3.0% crude fat, 28.9% nitrogen-free extractives, and 6.1% ash [9]. Pavlov [17] recorded a fiber content of 23.8%–30.1%, crude protein of 15.2%–20.4%, fat of 2.8%–3.0%, nitrogen-free extractives of 28.9%–32.1%, and ash of 8.2%–9.3%. The black medic is recommended as a rich source of protein, minerals, and phytoestrogens [7]. Among biochemical indicators, the content of protein ($22.9\% \pm 0.7\%$), fats ($5.3\% \pm 0.0\%$), soluble sugars ($7.41\% \pm 0.1\%$), starch ($10.42\% \pm 0.7\%$), and ash ($9.92\% \pm 0.0\%$) is indicated. In south-east Lithuania, the average content of carotenes in the above-ground mass of the black medic was 190 mg/kg of dry matter, and the average content of ascorbic acid was 513.7 mg/kg of dry matter [18]. The content of the black medic is 22.3%–23.1% protein, 3.2%–3.5% fat, 21.7%–22.9% fiber, 41.2%–41.8% nitrogen-free extractives, and 9.8% ash [13].

Differences in biochemical parameters have been recorded in previously identified life forms based on morphological traits and ontogeny aspects.

As mentioned above, the main indicators of the quality of *M. lupulina* green mass have been studied for a long time. In this study, the groups of *M. lupulina* with different developmental cycles (mono-, bi-, and polycarpics) are characterized for the first time.

This study aimed to characterize the nutritional value of mono-, bi-, and polycarpics and identify possible regularity in the distribution of the main biochemical parameters within each group. The data obtained will enable a more complete use of the potential of the *M. lupulina* species in breeding work to improve the chemical composition of fodder varieties.

MATERIALS AND METHODS

Twenty lines of monocarpic, bicarpic, and polycarpic black medic were chosen from 60 accessions of black medic from VIR collection, that have been studied in 2018–19, and were sown in 2020 (Table 1). Parts of the plants with generative shoots and bottom leaves were selected for biochemical analysis. Generative shoots were in the flowering phase and the beginning of fruiting with green beans. The monocarpic accessions were

collected on August 17 2020, bicarpic and polycarpic accessions were collected on August 24, 2020.

Agrotechnics, collection, and preparation of plant material for biochemical studies were conducted according to the VIR methods [19].

Sample preparation was carried out as follows. Fresh material of five plants (five biological replicates) of each accession was taken for analysis. The material was analyzed according to VIR methods [19]. The assessment was performed using the biochemical indicators of the content of dry matter mass, protein, sugars, ascorbic acid, total acidity, chlorophylls, carotenoids, carotenes, β -carotene, and anthocyanins. The content of dry matter mass (%) was determined by weighing the sample before and after drying in an FED400 Binder thermostat (Germany) at 105°C. The protein content (% dry weight) was determined according to Kjeldahl on the Velp Scientifica UDK 159 device (Italy). Sugar (mono- and total) (%) was determined according to the Bertrand method. Ascorbic acid was detected by direct extraction from plant tissue with 1% hydrochloric acid followed by titration with Tillmans's stain of 2,6-dichloroindophenol (mg/100 g). The total (titratable) acidity was determined by titration of the extract with 0.1 N alkali in the presence of an indicator, converted to malic acid (in % per malic acid) [19].

Chlorophylls and carotenoids were isolated with 80% acetone, and their absorption was measured on an Ultrospec II spectrophotometer at various wavelengths, namely 662 nm, 645 nm for chlorophylls a and b, respectively; 440 nm for carotenoids, with subsequent calculation of the concentration of pigments according to the Wetshtein and Holm equations; 454 nm for the total carotenes; 447 nm for lutein; 443 nm for violoxanthin, and 440 nm for xanthophyll. The total carotenes, lutein, violoxanthin, and xanthophyll were studied by the paper chromatography method developed by Sapozhnikov with subsequent determination of optical density on a spectrophotometer [19]. To correct for the content of green pigments, the optical density of the obtained extracts was simultaneously calculated at 750 nm. The calculation of pigment content is given in mg/100 g. The content of the total anthocyanins was determined by the spectrometric method, which involved isolating them with a 1% hydrochloric acid solution, followed by spectrophotometric analysis at a wavelength of 510 nm, converted to cyanidin 3,5-diglycoside (in mg/100 g dry weight) [20].

The reliability of the influence of the "life form" factor was assessed by the method of analysis of variance using the Fisher criterion at a confidence level of 0.05. Statistical analyzes of the results were performed using

Table 1. List of accessions of the black medic (*M. lupulina* L.) (Pushkin laboratories VIR, 2020)

VIR Catalog No.	Life form	Name	Origin
k-3664	Monocarpic	Wild-growing	Ukraine
k-15407	Monocarpic	Wild-growing	Omsk region
k-15414	Monocarpic	Wild-growing	Ukraine
k-25376	Monocarpic	Wild-growing	Kyrgyzstan
k-25734	Monocarpic	Wild-growing	Kazakhstan
k-46521	Monocarpic	Wild-growing	Krasnodar Territory
k-48568	Monocarpic	Bereginya	Lithuania
k-51458	Monocarpic	Wild-growing	France
k-48504	Bicarpic	Wild-growing	Pskov region
k-48509	Bicarpic	Wild-growing	Pskov region
k-52555	Bicarpic	Mira	Moscow region
k-52749	Bicarpic	Wild-growing	Leningrad region
k-52772	Bicarpic	Wild-growing	Novgorod region
k-22169	Polycarpic	Wild-growing	Germany
k-31076	Polycarpic	Wild-growing	Lithuania
k-38396	Polycarpic	Wild-growing	UK
k-41610	Polycarpic	Virgo Pajbjerg	Germany
k-43251	Polycarpic	Nordol	Denmark
k-48497	Polycarpic	Virgo	Denmark
k-48662	Polycarpic	Wild-growing	Czechoslovakia

the software package Statistica 12.0 and included the calculation of the main parameters of variation, analysis of variance with "life form" as the source of variation to assess the significance of differences between groups, and discriminant analysis to assess the information value of individual traits for distinguishing life forms of the black medic.

RESULTS AND DISCUSSION

There are a few studies on different forms of the black medic although this crop belongs to a group of promising fodder and pasture crops. We, therefore, compared our indicators with data on other taxa of alfalfa. Based on the analysis of the biochemical composition of the green mass of 20 accessions of the black medic, significant differences were noted in the content of the studied substances among the black medic species of various life forms.

Dry substances. Analysis of the green mass of the black medic showed that the accumulation of dry matter mass in the accessions ranged from 19.44% to 27.64% with a mean content of 23.02% (Table 2). Monocarpic accessions had the highest dry matter mass

content, with two wild-growing accessions, k-15407 from the Omsk region (26.76%) and k-25376 from Kyrgyzstan having dry matter mass of 26.76% and 27.64%, respectively.

An important indicator of the nutritional value of fodder crops is the content of crude protein. In this study, the protein content in the green mass of the black medic accessions ranged, depending on origin, from 11.94% to 19.69% per absolutely dry matter with a mean of 14.40% (Table 2).

Black medic is a highly nutritious crop, not inferior in quality to perennial creeping alfalfa and variegated alfalfa species widely used in agriculture (*M. sativa* L. and *M. varia* Mart.). According to different sources, during the mass flowering period the crude protein content of alfalfa is 16.82% [21], 17.64% [22], and 20.1% [23]. The variability of the indicator for accessions of creeping and variegated alfalfa from VIR collections ranges from 15% to 22% [24]. The highest protein content was recorded in the black medic bicarpics (19.7%) with two accessions having protein content of more than 17%, namely wild-growing k-52772 from the Novgorod region (17.6%) and k-52555 variety Mira from the Moscow region (19.69%).

Table 2. Mean content of essential nutrients in the black medic (fresh weight)

Indicator	Mean value	Minimum value	Maximum value	Coefficient of variation
Dry matter, %	23.02 ± 0.52*	19.44	27.64	11.16
Protein, % dry weight	14.40 ± 0.39	11.94	19.69	13.16
Ascorbic acid, mg/100 g	66.70 ± 6.94	24.80	148.80	50.96
Monosaccharides, %	1.16 ± 0.12	0.23	2.33	52.15
Disaccharides, %	0.64 ± 0.11	0.00	1.69	86.72
Sugar amount, %	1.80 ± 0.09	0.44	2.67	24.97
Total acidity, %	0.31 ± 0.01	0.14	0.41	17.71
Chlorophyll a, mg/100 g	74.31 ± 3.83	34.33	115.74	25.24
Chlorophyll b, mg/100 g	33.07 ± 1.62	14.76	49.20	23.95
Total chlorophylls, mg/100 g	107.38 ± 5.26	49.09	164.94	24.01
Carotenoids, mg/100 g	29.42 ± 1.52	11.88	43.74	25.34
Carotenes, mg/100 g	6.79 ± 0.30	3.14	10.54	21.45
β-Carotene, mg/100 g	5.00 ± 0.24	2.12	7.63	24.01
Anthocyanins, mg/100 g dry weight	16.06 ± 1.03	8.50	25.50	31.50

* Statistically significant at $p < 0.05$.

Ascorbic acid

Ascorbic acid (AA) is a vital metabolite necessary for the regulation of the main physiological and biochemical processes in plants and is also one of the most important antioxidants [25]. The content of AA in the mixture of forage grasses of pasture lands in Yakutia [26] ranged from 25.85 to 45.65 mg/100 g. In this study, a high AA content was recorded in the black medic plants ranging from 24.80 to 148.80 mg/100 g with a mean value of 66.70 mg/100 g and a strong variation (CV = 50.96) (Table 2). The high variability in the AA content in individual accessions enables breeding to improve the chemical composition of plants. The highest content of AA (more than 99 mg/100 g) was recorded in four varieties, including one bicarpic k-52555 Mira from Moscow region (124.0 mg/100 g), three polycarpics: k-43251 Nordol from Denmark (99.2 mg/100 g), k-41610 Virgo Pajbjerg from Germany (114.1 mg/100 g), and k-48497 Virgo from Denmark (124.0 mg/100 g). These accessions with high content of AA are recommended for use in practical breeding and fodder production.

Sugars

The dry matter of the green mass of black medic consists mainly of carbohydrates. Sugar is the first product produced by a plant during photosynthesis and is always present in the vegetative mass. The accumulation of sugar, a source of energy, in various organs of plants contributes to their preservation during periods of stress. A certain level of sugar in the grasses is desirable for grazing purposes as it is the available energy for the animals and contributes to the palatability of the feed. Carbohydrates also affect the metabolism, milk production, and reproductive capacity of animals [27]. When harvesting silage, sugar is also required for fermentation at about 15% of dry weight in fresh herbs and 3%–4% in haylage after fermentation [28].

In the studied group of accessions (Table 2), the highest variability was recorded in the content of disaccharides (CV = 86.72), followed by that of monosaccharides (CV = 52.15), and the least in total sugars (CV = 24.97), showing that they were the most stable values. Variation in total sugars in the green mass of the black medic ranged from 0.44% to 2.67% (average 1.8%), and from 0.23% to 2.33% in monosaccharides (1.16%), and from trace amounts up to 1.69% (0.64%) in disaccharides. Kosolapova and Mussie [23] reported a sugar content of 1.3% in the green mass of alfalfa from the Volga region. In this study, the black medic was characterized by a low sugar content of 7.82% (dry weight) at a rate of at least 10%. We identified three accessions with the highest content of total sugars (more than 2.3%), namely one wild monocarpic k-25734 from Kazakhstan (2.5%) and two polycarpic accessions: wild k-31076 from Lithuania (2.33%) and k-43251 Nordol from Denmark (2.67%).

However, when converted to dry matter, only selected polycarpic accessions corresponded the optimal rate (10.29% and 13.71%, respectively).

Organic acids

Organic acids are one of the most common products of plant metabolism. Many organic acids are water-soluble acids that determine the acidity of the plant object and give a peculiar taste to plant food. The main pharmacological value of plant organic acids is normalization of the activity of the gastrointestinal tract by lowering the pH of the medium. When using alfalfa as feed, high values of organic acids are necessary for silage formation. Titratable (total) acidity is used as an important assessment of a plant material.

In this study, the titratable acidity was low and averaged 0.31% with reference to malic acid and the variability ranged from 0.14% to 0.41% (Table 2). In the literature, we could not find data about the content of organic acids in alfalfa. Pobednov et al. [29] present data on individual organic acids (lactic, malic and citric) in alfalfa, with the total organic acids of 0.55% in terms of dry weight. In this study, the value of titratable acidity relative to dry weight was 1.35%. The highest content was recorded in the wild-growing polycarpic k-48662 from Czechoslovakia (0.41%).

Pigments

Photosynthesis is the leading factor in crop formation, during which up to 95% of dry matter is created [30]. Pigments are the most important components of the photosynthesis apparatus. All green tissues of higher plants contain chlorophylls a and b, as well as carotenoids, which are localized in chloroplasts. These include β -carotene, lutein, violaxanthin, and neoxanthin, which are often accompanied by smaller amounts of α -carotene, zeaxanthin, β -cryptoxanthin, and antheraxanthin [31] and play a direct role in the process of photosynthesis.

Chlorophylls a and b. The degree of adaptation of plants to the climatic conditions of a particular region, as well as the stability and quality of the crop are largely determined by the state of the leaf apparatus, the main organ that provides photosynthesis [32]. In our experiments, the contents of chlorophylls a and b in plants varied greatly and depended on the origin of the plant (Table 2). The chlorophyll content ranged from 49.09 mg/100 g to 164.94 mg/100 g (mean value 107.38). Three wild-growing plants had a high content of total chlorophylls of more than 143 mg/100 g, namely one bicarpic k-48504 from the Pskov region (143.65) and two polycarpics, k-38396 from Great Britain (157.28) and k-31076 from Lithuania (164.94).

Carotenoids. Carotenoids are yellow pigments that are extremely widespread in plants and consist of a mixture of xanthophylls (60%) and carotenes (40%). The color of

carotenoids is often masked by chlorophyll molecules. Osipova [31] recorded the total content of carotenoids in the green mass of yellow alfalfa and variegated alfalfa ranging from 30.16 mg/100 g to 42.62 mg/100 g, which is comparable to that we found in the black medic in this study. We recorded a carotenoid content of the green mass of the black medic ranging from 11.88 mg/100 g to 43.74 mg/100 g (average 29.42, Table 2). The accessions with high content of carotenoids also had a high content of chlorophyll, namely one bicarpic k-48504 from the Pskov region (38.49 mg/100 g) and two polycarpics, k-38396 from the UK (43.74) and k-31076 from Lithuania (42.47). Previous study shows that lutein and zeaxanthin are the dominant carotenoids in the leaves of yellow and variegated alfalfa varieties, followed by violoaxanthin and β -carotene, while neoxanthin is the least common [31].

Total carotenes. In this study, the total content of carotenes in the black medic also showed significant variability (Table 2), ranging from 3.14 mg/100 g to 10.54 mg/100 g (average 6.79). The highest content of the total carotenes of 10.54 mg/100 g was recorded in the bicarpic variety Mira from the Moscow region.

β -carotene. In plants, carotenes are a mixture of isomers, of which the most active is β -carotene, which accounted for 53%–96% of the total carotenes recorded in this study, and its variability ranged from 2.12 mg/100 g to 7.63 mg/100 g (average 5.0 mg/100 g) (Table 2). The remaining fraction of the total carotenes is accounted for by α -carotene and the rest of the carotenes are less than 1% of the total. The green mass of the yellow alfalfa and variegated alfalfa varieties is distinguished by

a high content of β -carotene (from 9.63 to 13.8; average 11.18 mg/100 g) [31]. In this study, the content of β -carotene in the black medic was two times lower than that in the yellow and variegated alfalfa varieties. The highest content of β -carotene was found in the same wild-growing accessions with a high content of chlorophylls and carotenoids, namely one bicarpic k-48504 from the Pskov region (6.63 mg/100 g) and two polycarpics, k-38396 from the UK (7.37) and k-31076 from Lithuania (7.63).

Anthocyanins. The next group of pigments is anthocyanins, which have strong antioxidant properties [33]. These are coloring, flavonoid-like substances, responsible for the red color of the plant and having a number of positive biological effects [34]. The level of anthocyanins (Table 2) in the leaves of the black medic was low and ranged from 8.50 to 25.50 mg/100 g of dry matter (average 16.06). There are no data on the content of anthocyanins in the alfalfa species in the scientific literature for comparison. The highest content of anthocyanins (more than 20 mg/100 g) was recorded in four accessions, three of which were monocarpics, namely one variety k-48568 Bereginya from Lithuania (21.02 mg/100 g), two wild varieties: k-25376 from Kyrgyzstan (24.20 mg/100 g) and k-15414 from Ukraine (25.34 mg/100 g), and one was a bicarpic, the Mira variety from the Moscow region (20.58 mg/100 g).

For comparison of the indicators obtained for accessions from the three groups of monocarpics, bicarpics, and polycarpics of the black medic, the biochemical characteristics of each of the groups of accessions are presented in Table 3.

Table 3. Content of main nutrients in different groups of the black medic (fresh weight)

Indicator	Life form			
	Monocarpic	Bicarpic	Polycarpic	Average
Dry matter, %	25.4 \pm 0.46	20.8 \pm 0.37	21.1 \pm 0.51	22.7 \pm 0.56
Protein, % dry weight	13.6 \pm 0.28	16.6 \pm 1.00	14.3 \pm 0.65	14.6 \pm 0.43
Ascorbic acid, mg/100 g	49.8 \pm 4.72	66.5 \pm 16.85	76.3 \pm 14.03	63.2 \pm 6.86
Monosaccharides, %	0.7 \pm 0.13	1.3 \pm 0.12	1.7 \pm 0.14	1.2 \pm 0.13
Disaccharides, %	1.3 \pm 0.09	0.3 \pm 0.03	0.2 \pm 0.12	0.7 \pm 0.13
Sugar amount, %	2.0 \pm 0.09	1.6 \pm 0.10	1.9 \pm 0.16	1.9 \pm 0.08
Total acidity, %	0.34 \pm 0.01	0.30 \pm 0.02	0.30 \pm 0.02	0.31 \pm 0.01
Chlorophyll a, mg/100 g	73.4 \pm 3.81	79.4 \pm 6.89	84.1 \pm 8.52	78.6 \pm 3.71
Chlorophyll b, mg/100 g	33.8 \pm 1.80	33.7 \pm 2.75	35.4 \pm 3.64	34.3 \pm 1.54
Total chlorophylls, mg/100 g	107.1 \pm 4.68	113.0 \pm 9.64	119.5 \pm 12.15	112.9 \pm 5.09
Carotenoids, mg/100 g	31.4 \pm 1.51	28.1 \pm 3.18	31.3 \pm 3.31	30.5 \pm 1.48
Carotenes, mg/100 g	6.9 \pm 0.44	6.1 \pm 0.29	7.8 \pm 0.52	7.0 \pm 0.30
β -Carotene, mg/100 g	5.2 \pm 0.19	5.0 \pm 0.47	5.5 \pm 0.57	5.2 \pm 0.23
Anthocyanins, mg/100 g dry weight	19.5 \pm 1.41	15.8 \pm 1.86	13.0 \pm 1.28	16.3 \pm 1.04

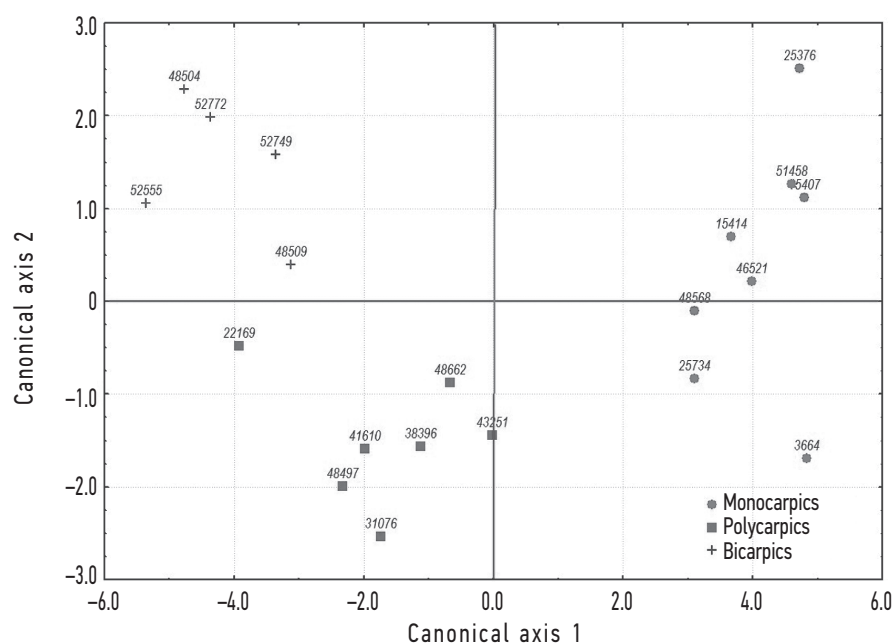


Fig. 1. Distribution of the black medic accessions from the VIR collection in the space of canonical axes

The green mass of monocarps differed significantly from that of the accessions of other groups in having a high content of dry matter (25.61%) and low content of protein (13.68%); bicarps were characterized by low values of dry matter (20.79%) and high values of protein content (16.31%); polycarps were characterized by low content of dry matter (21.13%) and protein content (14.48%). The content of titratable acids, as well as AA, in the accessions of green mass of monocarpic, bi-, and polycarpic forms of the black medic had no significant differences. Sugar content differed significantly among the black medic forms. Accessions of monocarps had the minimum content of monosaccharides and the highest content of disaccharides, whereas the green mass of bicarps and polycarps had a high content of monosaccharides and a low content of disaccharides. The total sugar content of the studied forms did not differ significantly. No significant differences were revealed between the studied groups in chlorophyll content although the values of chlorophyll were slightly higher in the polycarps group of black medic. The content of carotenes, including β -carotene, in the green mass of the black medic polycarpic forms was significantly higher than in other groups, the monocarpic forms were characterized by average values, and the lowest values were recorded in the bicarpic accessions. Anthocyanin content significantly differed among all three groups. Monocarps had the highest values, bicarps had the medium values, and lowest values were recorded in polycarps.

Discriminant analysis was used to evaluate the informational value of the studied characters in the division of life forms of *M. lupulina*. The classification function included the indicators of the content of dry matter, protein, AA, total acidity ($p \leq 0.05$), and total sugar content ($p > 0.05$).

The number of correct solutions, taking into account the influence of the above indicators, was 95%. The canonical discriminant analysis of the results obtained enabled to clarify the differentiation of monocarpic, bicarpic, and polycarpic accessions of the black medic taken in the study. Two "informatively valuable" variables Root 1 and Root 2 were identified. In the structure of the first variable (Root 1), the main role is played by disaccharides and protein, in the second variable (Root 2), the main role is played by protein content and AA content (89.8% and 10.2% dispersion, respectively). The first canonical variable separated the monocarps and the second variable, separated the bicarpic and polycarpic accessions. The polycarpic accession k-22169 (Germany) is closer to the bicarpic forms in terms of the complexity of traits (Figure 1).

The analysis results and subsequent statistical processing revealed that accessions of the green mass of mono-, bi-, and polycarps of the black medic share similar characteristics of biochemical composition but differed in indicators characterizing the nutritional value. The monocarps group was characterized by a high content of dry matter in the green mass of disaccharides and anthocyanins, the bicarps group was characterized by high contents of protein and monosaccharides, and polycarps group were characterized by high contents of monosaccharides, chlorophyll a, carotenes, and β -carotene.

CONCLUSION

The black medic is rich in nutrients that are very important in the diet of ruminants. The accessions of the studied alfalfa combining high contents of protein,

AA, sugars, and pigments are of the highest quality. The significant variability in the content of substances in the accessions of black medic enables breeding work to improve the chemical composition of *M. lupulina* varieties of fodder value. The obtained results demonstrate the need for an in-depth study of the green mass of *M. lupulina* for the presence of compounds that are indicators of resistance to environmental stress factors for use in breeding to create new resistant and highly nutritious fodder varieties of *M. lupulina*.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made equal contribution to the conception of the study, acquisition, analysis,

interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the work. Contribution of each author: N.Yu. Malysheva — collection and processing of materials, research concept and design, writing text, literature review; A.V. Solovyova, T.V. Shelenga — collection and processing of materials, writing text, literature review; L.L. Malyshev — analysis of the data obtained, writing text, literature review.

Competing interests. The authors declare that they have no competing interests.

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