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ABSTRACT

Of the dissertation for the degree in Biological Sciences

BIOECOLOGICAL FEATURES AND EFFICIENT UTILIZATION OF *MEDICAGO* L. SPECIES IN THE FLORA OF AZERBAIJAN

Speciality: 2426.01 – Ecology

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GENERAL CHARACTERIZATION

The actuality of the topic. In the past 10 years, the rapid growth of the population of world, the violation of the environmental balance and the decline in biodiversity have led to an increase in demand for food supply and agricultural plants. Therefore, the study and protection of biodiversity and biological resources have become a very urgent problem. In this regard, special importance is attached to the effective use of currently existing biological resources, the determination of the areas of wild-growing ancestors of cultivated plants and natural resources of promising species, and the involvement of hybridization and breeding works.

The family Fabaceae and its various genera are considered one of the higher plant groups in studying the morphobiological features of plants with modern methods and creating their phylogenetic detailed information on natural resources, habitats, ecology, population status and promising species of Alfalfa-*Medicago* L. was not found.

Medicago species, belonging to the family *Fabaceae* Juss. are valuable fodder, medicinal and phytoremedial plants and play an important role in the development of the vegetation of Azerbaijan. In recent years, the addition of a large number of new species from other genera to the genus *Medicago* intraspecific diversity and natural hybridization in the genus, and the complexity of determining their status made the study relevant. Since the intraspecific taxonomy of the genus has not been studied monographically, the taxonomy of the genus always generates interest.

In the World Flora, 83-87 species of Alfalfa are distributed throughout Eurasia, Africa, and especially the Mediterranean countries[1,2]. Distribution of 17 species of *Medicago* are known in different regions of Azerbaijan.

[1]Small E. Alfalfa and relatives: Evolution and classification of *Medicago*. 2011. NRC Research Press Ottawa, Canada.

[2] Azra Gholami, Nathan De Geyter, Jacob Pollier, Sofie Goormachtig and Alain Goossens., (2014) Natural product biosynthesis in *Medicago* species., This journal is The Royal Society of Chemistry., USD, Chicago. 68-(23). pp. 168-193.

The purpose and objectives of the study:

The main purpose of the research is to study the distribution, ecology, biological resources, bioecological and biomorphological features of medicinal plants of the flora of Azerbaijan, to study the possibilities of efficient utilization of populations with large resources and the development of a new abstract of the genus.

The following tasks have been set for achieving these goals:

❖ To identify the range of species of *Medicago* and collect seed herbarium;

❖ To study the bioecological and biomorphological features of *Medicago* L. species;

❖ Study of bioresources of Alfalfa, conduction of biochemical analyzes of promising species and to explore the possibility of utilization

❖ Study of rare and threatened species of *Medicago*;

❖ To investigate the genetic diversity of the species of Alfalfa distributed in Azerbaijan;

❖ To clarify and prepare an abstract of the taxonomic composition of *Medicago* distributed in the flora of Azerbaijan;

Scientific innovations. The ranges of species of Alfalfa, distributed in the flora of Azerbaijan have been determined, and two new ecotypes of species of the genus have been identified. The ecological groups and the role of species in the formation of vegetation are determined. The absence of the species of *M. daghestanica* in Azerbaijan was identified. For the first time, the constant diagnostic micromorphological traits of alfalfa seeds belonging to the flora of Azerbaijan have been studied on an electron microscope. Spectral chemical analysis of seeds of *Medicago* species was conducted and chemical elements collected in the seeds were identified. For the first time in Azerbaijan, the generic monograph of *Medicago* has been studied, the status and specific epithet of the species were determined and a new taxonomic concept of the genus has been developed. 4 subspecies of *Medicago* distributed in the flora of Azerbaijan have been reduced to 1 species status and 3 species were found as synonyms.

The scientific-practical significance of work. The herbarium and seed materials of the species of *Medicago* collected during the study enriched the herbarium fund and the collection stored in the National Genbank of the Genetic Resources Institute. The species of alfalfa are easily hybridized, so *in situ* taxa with high morphological parameters may be used in obtaining new high-yielding and stress factors resistant breeding varieties.

Existing of biologically active substances in the species and subspecies of the genus (*M. littoralis*, *M. truncatula*, *M. sativa*, *M. sativa ssp. caerulea*, *M. rigidula*) were specified, so it is advisable to use these plants in obtaining new medicines.

The developed taxonomic classification can be used in the preparation for a new edition of the Flora of Azerbaijan; also, they can be useful in the preparation of monographs and training tools on *Medicago* (*M. sativa ssp. x varia*, *M. sativa ssp. glomerata*, *M. sativa ssp. caerulea*, *M. papillosa*). The ISSR markers (UBC 827, 834, and 857) can be utilized to access the species of *Medicago* by future researchers.

The main regulations of the dissertation defense:

- The taxonomy of *Medicago* was studied by classical and modern methods, and the existence of 17 species, 4 subspecies and 5 species diversity of *Medicago* in the flora of Azerbaijan was determined.
- An analysis of phytocenoses of bioresources of *Medicago* with specified areas lays the foundation for their utilization as a fodder base.
- A correlation analysis was conducted to determine the relationship between the parameters of biochemical analysis and environmental factors affecting the areas of species collection, and especially, a highly significant positive relationship between the total amount of nitrogen and the zones was detected.
- Utilization of the identified ecotypes belonging to the subspecies *M. sativa* ssp.*caeruleae* and *M. sativa* ssp. *glomerata*, *M. sativa* ssp. *caerulea*, *M. papillosa* taxa that have high biomorphological characteristics and tolerance to stress factors under different environmental conditions in culture may be useful.

- Genotypes of alfalfa were evaluated using molecular markers. *M. monspeliaca* collected from the Lerik district and *M. lupulina* collected from the Shamkir district were determined as the most distant genotypes, while *M. littoralis* and *M. truncatula* collected from Absheron were determined as the closest genotypes according to their systematic position.

Approval of the work: The main results of the dissertation reported in the II International Scientific Conference "Ecology: Nature and Society Problems", dedicated to the 105th anniversary of the notable scientist Hasan Aliyev (Baku, 2012), dedicated to the 110th anniversary of academician Hasan Aliyev:"Ecology: Nature and Society Problems", at the 3rd International Scientific Conference (Baku, 2017), in the International scientific conference "Actual Problems of Contemporary Nature and Economic Sciences" (Ganja, 2018), At the LX International Scientific and Practical Conference dedicated to the memory of Academician N.N. Luzina (Moscow, 2019), at the Scientific Conference on "Innovations and Traditions in Modern Botany" dedicated to 130th anniversary of Academician A.A. Grossheim (Baku, 2019), at the meetings and scientific workshops of the Scientific Council of the Genetic Resources Institute Ministry of Science and Education (2013-2018).

Published proceedings: 13 proceedings related to dissertation work have been published.

Structure and volume of the dissertation: The dissertation consists of an introduction, six chapters, a conclusion, results, recommendations, reference material and an appendix, total volume of the dissertation is 187 pages. Here are 17 tables and 45 figures. The study used 156 citing sources, of which 142 were from foreign publications.

BASIC WORK CONTENT
CHAPTER I. LITERATURE REVIEW

This chapter provides a comprehensive overview of the sources of research on the origin of *Medicago*, origin centres of the genus, evolution history, taxonomy, distribution, macro-micromorphological traits, chemical composition and research by molecular markers.

From the date, that K. Linney published the scientific description of *Medicago* L. in 1753 up to today, the taxonomic composition of the genus is being changed. I. Urban (1873), I.T.Vasilchenko (1949, perennial species), C.C. Hein (1963, annual species), K.A. Lesins (1979), I. Lachashvili (1967, Caucasus) conducted their research on *Medicago*. More detailed information on this genus is provided by E. Small (2011), a monograph researcher of the world flora on *Medicago*.

CHAPTER II. RESEARCH MATERIALS AND METHODS

The research material was the biological resources-herbarium and seed material collected in floristic expeditions held in all botanical and geographical areas of Azerbaijan from 2012 to 2017 years. The collected herbarium materials are conserved in the Herbarium Fund of the Genetic Resources Institute of Ministry of Science and Education.

The collected data have been analysed based on the results of monitoring, data, literature, internet resources, and data obtained from the inspection of materials conserved in the Herbariums of the Tbilisi Botanical Institute (TB), the GöttingenHerbari Foundation (DGT), the Institute of Botany of Ministry of Science and Education (AGI) and the Genetic Resources Institute of Ministry of Science and Education (AGRI).

The names of species with disputable nomenclature-systematic positioning were regulated based on the code adopted by the International Botanical Congress (Austria, Vienna, 2005), and

published in 2009 and have been used a number of monographical studies on genera. The specification has been carried out by biomorphological, microbiomorphological, and comparative-taxonomic, molecular, etc. methods.

Distribution of species is given on the botanical and geographical regions of Azerbaijan, adopted in the "Flora of Azerbaijan".

Hypometric altitude and range coordinates were measured using a Garmin eTex 20 GPS. The habitat-biotope, coordinates of each collected species were recorded in descriptor forms and the photos were taken. The maps, reflecting the distribution range of each species based on the coordinates of the collection point given by the Diva-GIS program have been prepared. In the course of biochemical and spectral analysis, the amount of nitrogen, protein, tryptophan, lysine and microelements collected in the seed materials species were studied.

Seed material of different plant parts (stem, leaf, stalk, etc.) has been used to carry out micromorphological studies. Seed size and color were determined on a Leica EZ4D stereomicroscope. The microstructure of the surface of the seed material and samples taken from various organs of the plant was studied using a JEOL JSM 6060 electron microscope (SEM)[3].

Evaluation of genetic diversity in genotypes of *Medicago* L. was performed using ISSR markers and based on CTAB protocol[4].

CHAPTER III. TAXONOMY OF ALFALFA AND ITS BIOECOLOGICAL PARAMETERS

3.1. Phytocenological analysis of bioecological features and *in-situ* conservation of bioresources of *Medicago* species

To investigate *Medicago*, the biological resources (herbarium and seed materials) of different species of alfalfa were collected

[3] Sima, K. Phenetic analysis of the genera medicagoid *Trigonella* L., *Melilotus* Mill. and *Medicago* (*Fabaceae*) on seed coat in Iran. / Sima K., Assadi M., Nejadsatari, T. [et al.] // Biodiversitas. – 2016, 17(1), – p. 162-171.

[4] Doyle, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue / J.L Doyle, J.J. Doyle // Phytochem., Bull., - 1987, v. 19, - p. 11–15.

during the floristic expeditions, conducted in most regions of Azerbaijan. Expeditions and monitoring were carried out at different morphophysiological stages of plant development to detect taxonomic diagnostic signs. An analysis of the geographical and genetic links of alfalfa of the flora of Azerbaijan shows that the origin and development of these species are closely related to some floristic regions - Central Asia, Western Europe, the Mediterranean basin, Central Asia, Central Asia and the Tien Shan mountain systems.

Most resources of species of alfalfa are found in all botanical and geographical regions of Azerbaijan, from arid to moderate mountainous terrain in a variety of ecological conditions and biotopes, especially in arid lands, meadow slopes, rocky and clay soils, gardens, woods, meadows. *Medicago* species - bioresources are involved in the development of various meadows. However, they do not form large clumpy formations and independent cenosises. The species of alfalfa are found in a variety of plant formations, mainly desert, semi-desert, subalpine, different grain pastures, mountain xerophytes (*M. rigidula*, *M. truncatula*, *M. minima*) and dry grasslands (*M. littoralis*, *M. rigidula*, *M. truncatula*, *M. minima*, *M. majeri*, *M. medicaginoides*, *M. monantha*, *M. monspeliaca*, *M. orthocerac*, *M. radiata*), wormwood-cereal-ephemeral, subalpine meadows (*M. sativa ssp. caerulea*, *M. lupulina*, *M. orbicularis*, *M. sativa* ssp. glomerata, *M. popillosa*, *M. brachycarpa*).

The *Medicago* species belong to the three main ecobiomorphic groups: mesophyte, xerophyte, and mesoxerophyte. 15 species of these are annual and 2 species are perennials. The analyses on Azerbaijan and the Caucasus species shows a high degree of polymorphism of these traits.

3.2. Ecotypes of *Medicago* species

During the expeditions, study of species and species diversity with a more polymorphic structure was carried out. Two ecotypes were found on the territory of Azerbaijan, belonging to the subspecies *M.sativa* ssp.caeruleae (diversity of 5-6 species).

Newly discovered ecotypes were found in populations distributed in the Gabala and Absheron regions in the west of the Greater Caucasus. The ecotypes of blue-flowered *M.sativa* ssp. coerulea subspecies are distinguished by the fact that the stem is erect and dense, develops more intensively, and the leaves are wide and large. Plant seeds were planted on the experimental field and biomorphological evaluation was carried out. Based on the data obtained, a comparative biomorphological analysis was carried out with the blue-flowered *M. sativa* ssp. coerulea.

The obtained results showed that the morphological traits of ecotypes differ from the traits of forms belonging to other *in situ* cultivated subspecies of blue alfalfa (*M.sativa* ssp.coeruleae).

3.3. Rare and threatened species of *Medicago* and their protection

Regardless of the fact that, the herbarium material of some alfalfa species has been collected, most of the taxa (*M. daghestanica* Rupr.), which population area was indicated, have not been found in those territories during the numerous expeditions. Only one herbarium specimen, collected in 1900 and determined as *M. daghestanica* in 1925 is conserved in the herbarium fund of the Institute of Botany of ANAS. Determination of any morphological traits of the plant is impossible. As well as the herbarium specimen of *M. daghestanica* collected from Azerbaijan has not been found in the Herbarium Foundations (Dagestan, Kyiv, Leningrad, Tbilisi), where we collected data about this plant. The biotope of indicated in the herbarium specimen is not identically to the distribution biotope of it. It is well known that the biotope of *M. daghestanica* was found only in chalky-stony slopes. But the collection area of the unique herbarium considered as a specimen of *M. daghestanica* was indicated meadow. Evidently, these data conflict with each other. During the expeditions, conducted to that area and regions around, *M. daghestanica* was not found. Probably the purpose of this species was conducted incorrectly. As the result of the study and analysis, we consider that *M. daghestanica* was not distributed in Azerbaijan territory.

Georgian botanic I. Lachashvili described M. *talyschensis* Latsh. from Talysh. He considered that *M. talyschensis* Latsh. has a limited geographical range, it was found only in the Talysh region of Azerbaijan and is supposed endemic to this zone. Numerous different types of research have been carried out to study the ranges, systematical position, and diagnostical morphological traits of disputable species. Herbarium and seed material of *M. talyschensis* Latsch. have been collected from Lerik village of Lerik region and studied comparatively. Micromorphological features of the stem, leave, pedicle, seeds etc. of the species have been studied in the scanning electron microscope (SEM). The micromorphological features of *M. talyschensis* and *M. arabica* species were nearly the same. As the result of analysis of *Medicago* genotypes with genetic diversity of molecular markers, nucleic acid sequence of *M. arabica* var. heptacycla Urb. (*M. talyschensis* Latsch.) and *M. arabica* (L.) Huds genotypes showed a 0,98% identity coefficient and both of genotypes grouped in the same clusters by their analysis indices. As the result of all obtained research and analysis types, we don't consider *M. talyschensis* as independent species and it is considered to be under *M. arabica* species status (*M. arabica* var. heptacycla Urb.).

CHAPTER IV. ANALYSIS AND SYSTEMATICS OF BIOMORPHOLOGICAL TRAITS OF ALFALFA SPECIES

4.1. General morphological structure of *Medicago* species.

As in the case of all other groups of higher plants, when assessing the biological reserve and the systematic status of species of the alfalfa, morphological traits were initially analyzed.

The genus has a number of morphological traits: the calyx is 5-toothed, the slipcover is blunt, and the pod is 1-7 (9) beans, straight, spear-shaped, kidney-shaped or spiral-shaped, covered with prickles or densely hairy. The leaves are obovate or diamond-shaped, sliced, lanceolate and surrounded by little teeth

on the top. The leaves consist of three parts, stipule and with accrete cutting. The stem is mainly many-branched, erect, lying, thin, scleroticnaked, hairy or glandular-hairy. The shape of the bean is used as the main morphological trait in determining the species.

4.2. Micromorphological characteristics of seeds of *Medicago*.

Seed material and cutting samples of the different parts (stem, leave, pedicle etc.) of the *Medicago, Orbiculares, Lupulariya, Buceras, Lunatae, Hymenocarpos* and *Spirocarpos* species and subspecies of various populations have been taken to conduct the micromorphological study. Collected seed material was placed into specific sterile paper bags and humidity was dried with silica gel in special laboratory conditions. Description of the general shape of the seed is characterized by shape, width and length.

The seeds of most species of *Medicago* are kidney-shaped, elliptical, some of them are cylindrical and oval, and the epidermal layer is granular, wrinkled, creased or smooth. Although the a slight difference in morphological traits in some of the studied taxa, the electron microscope allows the species to be easily distinguished for the different structures of the seed epidermis on the specific microstructural features from others (Figure 1).

The seeds size ranged between 4.2×2.5 1.8×1.4 mm. The short morphobiological characteristics and microstructural structure of the seeds of the studied species are described and given below in the example of *M. minima* species.

M. minima L. is annual (rarely biennial). Stems are thin and hairy. Stems usually numerous 10-40 from one root, slender, sparingly branched, often prostrated, pubescent. The seeds are kidney-shaped, boatshaped. The radicle forms a beak at the tip of the nucleus and is equal to half of the cotyledon. It is 1.8-2.8 x 1.1-1.4 mm in size. The surface is smooth, matte or slightly shiny, yellow, sandy yellow. 2n = 16.

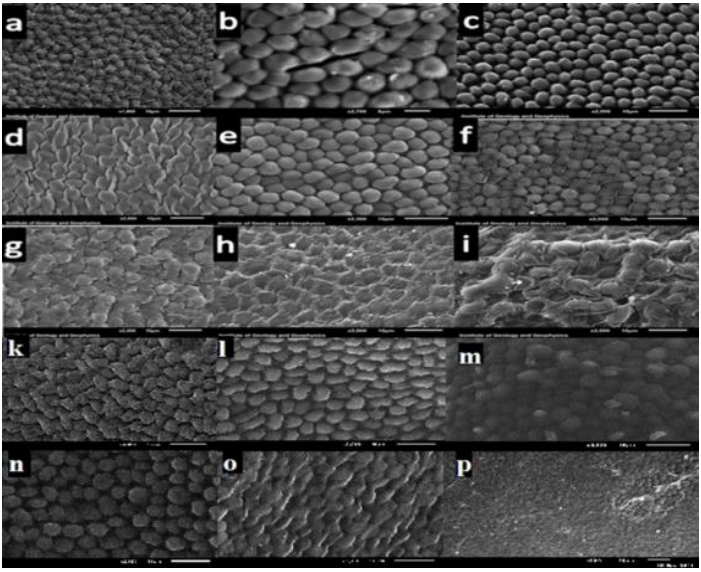

Figure 1. Seed surface traits on electron microscopy (SEM)

The micromorphological traits of the seeds of each taxon studied were indexed according to shape (5 shape index), color (3 color index), surface structure (4character index), size (2 size index) and cluster analysis based on biomorphological indicators was conducted by SPSS method in UPGMA program (Figure 2.

The studied 16 taxa were grouped into 5 clusters. According to macromorphological traits, species belonging to one section are grouped into different clusters. (Figure 2.).

Cluster I includes species of the genus *Trigonella* with disputable taxonomic positions (*M. monantha*, *M. orthocerac*, *M. medicaginoides*, *M. monspeliaca*). In the indexation based on the micromorphological traits of the seed, only the indices belonging to these species were recorded. Separately, all species belonging to this cluster belong to the ecological group of xerophytes.

In the second Cluster, as in the first Cluster, also grouped only one species *M. orbicularis*. Because of the unique macro and

micromorphological features like *M. lupulina*, *M. orbicularis* grouped in a separate cluster.

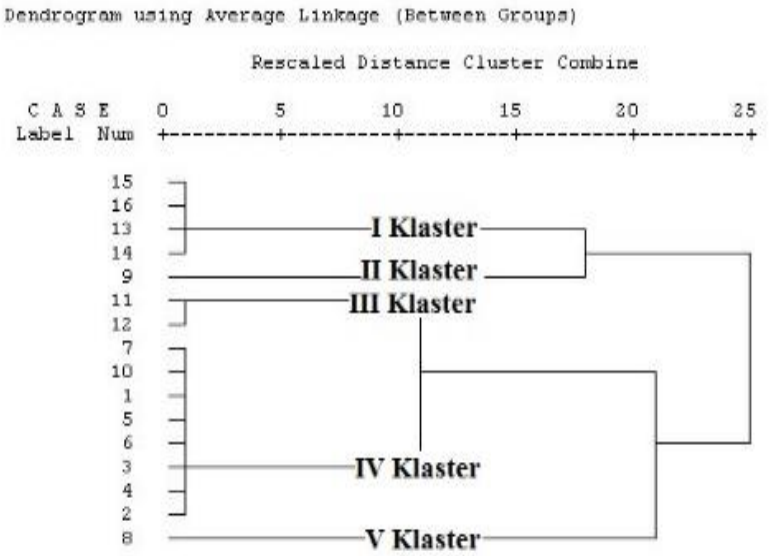

Figure 2. Cluster analysis according to the micromorphological trait indices of the seed

Two taxa (*M. sativa*, *M. sativa ssp. coerulea*) grouped in the third cluster. The traits of the seeds both taxa were almost the same.

Eight species (*M. minima*, *M. meyeri*, *M. littoralis*, *M. rigidula*, *M. truncatula*, *M. arabica*, *M. polymorpha*, *M. talyshensis = M. arabica var. heptacyla*) grouped into the forth cluster. All of these species are from the same section. When looking at the micromorphological traits of the seeds of *M. talyshensis= M. arabica* var. *heptacyla*, taken as separate taxa, are almost identical. Therefore, it proves that *M. talyshensis* species cannot be accepted as independent species.

Only one species (*M. lupulina*) is grouped into the fifth cluster. *M. lupulina* species has specific traits and are grouped into a separate cluster. Fifty-one quantitative and qualitative traits were

selected based on micro- and macromorphological data on the vegetative and reproductive organs of the taxa of alfalfa.

4.3. A complex and synonymous species of Alfalfa.

The *Medicago sativa-falcata* complex, or taxa of the *Medicago sativa* species complex, is a taxonomic group that includes alfalfa. To determine the systematic status of subspecies of the *M. sativa* species complex and species considered synonymous with *M. sativa* species, this issue was clarified by assessing the genetic polymorphism of macromorphological characters of the vegetative and genital organs using comparative analysis and molecular studies. During the analysis, two main critical morphological traits were identified - the colour of the flower and the shape of the bean.

Based on the results of all types of studies conducted, all systematic units of the *Medicago sativa* species complex are accepted as subspecies and the currently accepted and widely used systematic status of these taxa (*M. coerulea* Less. in Ledeb.= *M. sativa ssp. coerulea* Less. in Ledeb., *M. caucasica* Vassilcz.= *M. sativa* L., *M. glomerata* Balb.(*M. glutinosa* Bieb.) = *M. sativa ssp.glomerata* Balb., *M. falcata* L.= *M. sativa ssp. falcata* L., *M. polychroa* Grossh.= *M. sativa* L., *M. viresgens* Grossh.= *M. sativa* L., *M. talyshensis* Latsch.= *M. arabica var. heptacycla* Urb., *M. hemicycla* Grossh.= *M. sativa ssp. varia* (Martyn) Arcang.) was supported.

4.4. Systematic review and phylogeny of *Medicago* species

Six species of *Trigonella* (*Trigonella brachycarpa* M. Bieb., *Trigonella arcuata* C.A Mey., *Trigonella monantha* C.A Mey., *Trigonella monspeliaca* L., *Trigonella orthocerac* (Car. &Kir.) Trautv., *Trigonella radiata* L.*)* Traut. transferred to Alfalfa (*M. coerulea* Less. in Ledeb.= *M. sativa ssp. coerulea* Less. in Ledeb., *M. caucasica* Vassilcz.= *M. sativa* L., *M. glomerata* Balb.(*M. glutinosa* Bieb.) = *M. sativa ssp.glomerata* Balb., *M. falcata* L.= *M. sativa ssp. falcata* L., *M. polychroa* Grossh.= *M. sativa* L., *M.viresgens* Grossh.= *M. sativa* L., *M.talyshensis* Latsch.= *M. arabica var. heptacycla* Urb., *M. hemicycla* Grossh.= *M. sativa ssp.*

varia (Martyn) Arcang. and a new systematic concept of Alfalfa has been developed.

Fifty-one quantitative and qualitative traits were selected based on micro- and macromorphological data on the vegetative and reproductive organs of the taxa of alfalfa. Each of the qualitative traits for statistical analysis was analyzed and coded and linked to the standardized quantitative traits. MVSP Ver. 3.2 (Kovach, 1985-2002) and the UPGMA method have been used in the phenetic analysis, and changes in morphological traits of phenograms on species. The analysed taxa were grouped in seven clusters of phenogram based on constant morphological traits[5]. Species within each cluster were grouped according to specific conventional micro- and macromorphological traits. These common characteristic traits have played an important role in the identification of taxa and in their taxonomic status. a taxonomic structure consisting of 7 sections, 4 subsections, 17 species, 4 subspecies, and 5 species diversities was compiled into based on all obtained research data.

4.5. Botanical characteristics of *Medicago* species (specific and intraspecific taxa).

The botanical characteristics of each 17 alfalfa species distributed in Azerbaijan are given as an example of the *M. arabica* species:

M. arabica (L.) All. Fl. Pedem. 1 (1785): 315; Grossg. 1945, Fl. USSR, 11: 166; Grossg. 1952, Fl. Caucasus.2, 5: 190; İsayev. 1954, Fl. Azerb., 5: 261; Small 2011, Evol. and classif. of *Medicago*. : 727; Askerov 2016, plant world of Az.: 268. – Basionim - *M. polymorpha* var. *arabica* L. 1753, Sp. Pl. : 780.– Arabic m.

Description: Annual; root slender; stems rather slender, often decumbent, 4-angled, to 50-60 cm long, branched from base cind in

[5] В.М. Гувандиев, А.М. Аскеров, Таксономия видов рода Medicago L. (Fabaceae Lindl.) Флоры Азербайджана и микроморфологические признаки семян некоторых видов., 2019, РФ, Красноярский, Вестнк КрасГАУ. № 1 (142):192-200 с.

upper part, covered, as are more profusely stipules and calyces, with soft articulate hairs; stipulesrather large, green, broadly ovate-lanceolate, deeply pectinate-toothed or incised; leaflets commonly glabrous, to 2.5 cm long and to 2 cm broad, broadly triangular-obcuneate, emarginate and obtusely toothed at apex, often with a broad dark spot at base; petiole slender, weak, to 10 cm long or longer; corolla 4.5 mm long, yellow or orange-yellow, twice as long as calyx; standard rather broad, about twice as long as keel and wings, these equal in length; calyx broadly campanulate, glabrous or nearly so, the triangular lanceolate teeth as long as or longer than tube; pedicel slender, usually shorter than calyx; bracteoles triangular- subulate, membranous, as long as or shorter than pedicel; inflorescence loosely 1-5 flowered; peduncle slender, often pendent, shorter than the subtending leaf; pod globular cylindric, glabrous, 5-8 mm in diameter, with 3-7 turns; veins 4-6 from ventral suture, reticulately confluent with concentric loops, the outer margin of coils somewhat thickened, with 4 ridges separated by 3 grooves, and a double row of spines, these long, deeply sulcate, usually arched-recurved; seeds cinnamon-brown, reniform. Flowering occurs in April-May, and the fructification period begins in June. 2n=16, 2200 m. d.s.h.

Type: Described from Italy. Lectotype: Morison, PI. Hist. Oxon. 2: tab. 13. fig. 12 (1680) (Heyn, 1959).

Biotope: It was found in the meadow slopes, river banks, canals, gardens, sowing areas, shrubberies from lowlands up to mountain zones.

Distribution in Azerbaijan: GC (Greater Caucasus) (Guba mountain massive), Samur-Davachi lowland, around Caspiansea, Apsheron, Gobustan, Kur-Araz lowland., LC- (Lesser Caucasus) centre.), Lank. lowland, Lank. mountains.

General distribution: North Africa, Balkan Peninsula, Asia Minor.

Areal type: Mediterrian Sea.

Species diversity: var. *heptacycla* Urb. Fruit is a coiled pod with seven spirals. Spines are short. It was found in the Lankaran mountains.

CHAPTER V. BIOCHEMICAL COMPOSITION SPECIES OF OF ALFALFA AND USE POSSIBILITIES

5.1. Importance of plant resources

The Alfalfa dry herb has many biological reserve substances - protein, phosphorus, calcium and essential amino acids (valine, leucine, isoleucine, lysine, methionine, threonine, tryptophan, phenylalanine, arginine, histidine, peronein, etc.) and is distinguished by its high nutritional value. The leaves and stems of annual species common in situ are also rich in protein, vitamins and minerals. Species of the genus contain compounds that include the three main groups of isoflavones, lignans, and phytoestrogens[6].

5.2. Biochemical and spectral analysis

Plants absorb macro and microelements, inorganic substances from the upper layers of the atmosphere, hydrosphere and lithosphere and use them in their ontogenetic development, and some of them accumulate in their body as biological resources. Biochemical and spectral analyzes were carried out on the seeds of species of alfalfa collected from different geographical regions and ecological conditions of Azerbaijan, as a result of which some biological organic substances and micromacroelements were found (Figure 3.), (Table 1.). Despite the fact that the species were collected from different geographical and ecological conditions, as a result of the analysis, the same set of micro- and macroelements was found in them, which underlies the above substances.

According to the results of the analysis, the element with the largest mass fraction was 66.62 mg (O2) of oxygen in the seeds of *M. littoralis* species, and the smallest was magnesium

[6] Dong, X.N. Study on extraction technology and antibacterial of flavonoids from space breeding alfalfa / X.N. Dong, H.F. Zhao, Q. Zhao [et al.] // Pratacult Sci., – 2013, 31. – p. 771-776.

(*Mg*) with a value of 0.02 mg in M., the minimum species. Minor elements include sulfur (*S*) and calcium (*Ca*)[7].

The biochemical analysis determined the amount of total nitrogen, protein, lysine, and tryptophan from amino acids that make up the majority of valuable bioresources in seeds. Based on the results, it was found that the largest amount of total protein was in *M. rigidula* species (28.31 mg), and the smallest in *M. popillosa* species (24.38 mg). The highest amount of the amino acid tryptophan was in *M. rigidula* species (455 mg) and the lowest in *M. orbicularis* species (345 mg). For the existence of all living organisms, the main elements of their vital activity, the analysis of the lysin, nitrogen and other amino acids are given in the table (Table 1.).

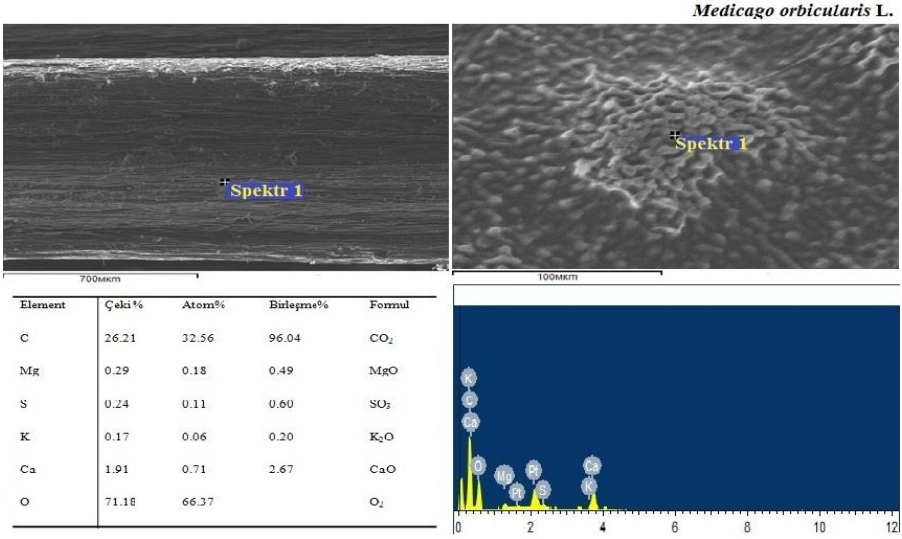

Figure 3. *M. orbicularis* (L.) Bartalini. spectral analysis indicator

[7] Güvəndiyev, V.M., Əsgərov, A.M., Azərbaycan florasının Qarayonca (Medicago L.) cinsi növlərinin kimyəvi tərkibi və əhəmiyyəti // AMEA Genetik Ehtiyatlar İnstitunun Elmi əsərləri, – 2017. – c. 6. № 1, – s. 182-186.

Table 1.

Biochemical analysis indicators

Taxa	Acc.to air dry matter		In 100 gr, mq		1000 seeds weight (gr)	1000 beans weight (gr)
	Total nitrogen	Protein	Tryptophan	Lysine		
M. sativa	4.06	25.31	250	719	2.7	54
M. sativa ssp. Coerulea	4.32	27.06	267	761	2	46
M. sativa ssp. glomerata	4.37	27.31	222	846	1.8	39
M. popillosa	3.90	24.38	240	973	1.6	37
M. truncatula	4.02	25.13	375	634	5	89
M. littoralis	4.25	26.56	360	676	4	83
M. rigidula	4.53	28.31	455	550	5	140
M. lupulina	4.23	26.44	320	634	2	4
M.orbicularis	3.86	24.13	345	423	3	43

To determine the relationship between the indicators of biochemical analysis and environmental factors affecting the collection areas of the analyzed species, a correlation analysis was carried out, and a significant positive and negative relationship between some features was found. In particular, a high correlation between zones with total nitrogen, DSH (height above sea level) and precipitation was observed.

In some countries, the leaves of some annual species are used in salads, soups and sandwiches, as well as herbal tea due to the high protein content[8].

Species of *Medicago* are used for medicinal purposes (inducing milk during lactation and the ability to lower cholesterol levels in the body, in certain cancers, to reduce the effects of many diseases, such as coronary heart disease, osteoporosis, anemia,

[8] Çakmak, Y.S. Medicago rigidula (L.) ALL. növünün Antioksidan ve Enzim İnhibisyon Aktiviteleri ve Fenolik Bileşiminin İncelenmesi / Y.S.Çakmak, G.Zengin, B.Eskin [ve b.] // Marmara Pharmaceutical Journal, – Stambul: – 2017, 21(3), – s. 522-529

diabetes and ulcers), in the pharmacological field[9], important biologically active substances are obtained from Alfalfa, such as antioxidant, antibacterial, antiallergic and sedative (pain reliever)[10]. Species of *Medicago* are used as green manure, a source of industrial enzymes in biotechnology.

CHAPTER VI. EVALUATION OF THE GENETIC DIVERSITY OF SPECIES OF *MEDICAGO* USING ISSR MARKERS

6.1. Genetic diversity of alfalfa genotypes (*M. sativa*)

Genetic structure of *Medicago* L. species are diploid (2n = 2x = 14 or 2n = 2x = 16), tetraploid (2n = 4x = 32) or hexaploid (2n = 6x = 48).

High polymorphic ISSR primers were used to assess genetic diversity among the studied 46 alfalfa genotypes. Eight out of 10 primers were selected. All primers amplified fragments with the expected length.

In the UBC841 ISSR primer, nine alleles were synthesized and the length of these alleles ranged between 100-1000 nc (Figure 4.). In genotypes of *M. orbicularis* species collected from Khizi, Goychay and Shamkir, the length of the 7th fragment amplified by this primer was synthesized, this fragment was between 125-150 nc. Totally, 9 fragments synthesized by this primer were distributed among 3-44th genotypes. Fragment 8, synthesized by the UBC841 primer, was found in 5 genotypes, 4 of which were genotypes of *M. lupulina*. fragment 5 occurs in most genotypes (44).

[9] Hanif, M.A. Chemical characterisation of bioactive compounds in *Medicago sativa* growing in the deser of Oman // Nat. Prod. Res. – 2015, 29, – p. 2332-2339.

[10] Azra Gholami, Nathan De Geyter, Jacob Pollier, Sofie Goormachtig and Alain Goossens., (2014) Natural product biosynthesis in *Medicago* species., This journal is The Royal Society of Chemistry., USD, Chicago. 68-(23). pp. 168-193.

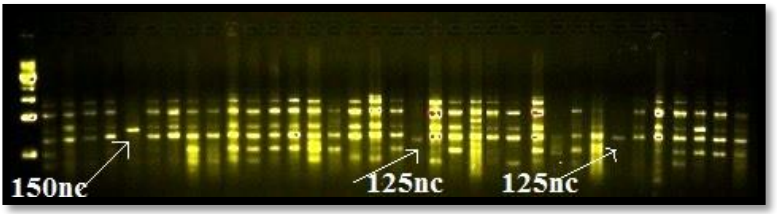

Figure 4. Alleles length synthesized by primer UBC841

The length of the fragments synthesized by the UBC834 primer ranged between 100-1000 nc. 6 fragments were synthesized by this primer, which were distributed between genotypes differently.

The second fragment synthesized here was found in 8 genotypes, 3 of them were *M. orbicularis* and 3 were genotypes of *M. lupulina*. In genotypes of *M. orbicularis* species collected from Khizi, only the 4th fragment was synthesized, the length of its fragment was 150 nc (Figure 5.).

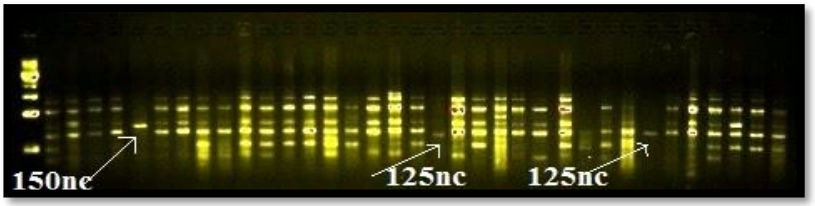

Figure 5. Length of fragments synthesized by primer UBC841

6.2. Assessment of genetic diversity of alfalfa genotypes and determination of genetic similarity indices

In the studied 46 alfalfa genotypes, the frequency of distribution of 58 fragments ranged between 0.01 - 0.78. In fragments, the average score of frequency was 0.28. The frequency of occurrence of 20 fragments ranged between 0.01-0.15, only 7 fragments were found in more than 40 samples and their frequency ranged between 0.70 and higher.

3 of 58 fragments with a frequency of less than 1% were found to be rare fragments, 25 fragments with a frequency of 1-20% were found to be common fragments, and the frequency 30

fragments was higher more than 20% and these fragments were included in the group of high-frequency fragments.

High polymorphism was observed in the fragments obtained from the conducted analysis results. Polymorphism ranged between 67-100%, with a mean value of 82.0%. The mean polymorphism for each ISSR primer was 5.8.

In the research in alfalfa, the Genetic Diversity (GDI) index was calculated per ISSR locus.

The level of the genetic diversity index ranged from 0.87 to 0.98, with a mean score was 0.93. The high rate of genetic diversity can be explained by the fact that the evaluated genotypes of the alfalfa have been collected from different regions of Azerbaijan and belong to different species. The UBC 834 primer had a high genetic diversity index (0.98), and this primer also showed a high polymorphism index (100%)[11].

UBC827 and UBC857 were the most reliable primers with 0.44PIC unit.

EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands and Mi is the product of Pic and eMR, therefore the high level of polymorphism provides high eMR. In our experiment, the eMR and MI scores in primer ranged between 3.2-7.11 and 0.88-3.13. The highest score of EMR and MI polymorphism on two indicators is a sign of their informativeness with UBC827 (7,11-3,13) and UBC864 (6-1,62) primers.

Among the evaluated genotypes of alfalfa, *M. monspeliaca* collected from Gusar and *M. lupulina* (GS = 0.10) collected from Shamkir were considered to be the most distant genotypes, and *M. littoralis* and *M. truncatula* (GO = 1) collected from Absheron were determined as the closest genotypes, respectively.

[11] Гувандиев В.М., Аскеров А.М., Гювендиева Х.М., Калантарова Н.С., Гаджиев Э.С. Исследование генетического разнообразия рода Люцерна (Medıcago L.) с применением ISSR маркеров // – Махачкала: Проблемы Развития АПК Региона., Дагестанский ГАУ., – 2020. № 1 (41), – с. 27-34.

RESULTS

1. For the first time in Azerbaijan, monographical study of Alfalfa genus has been conducted, a systematic synopsis of the genus was prepared. It was determined that the species of the genus consists of 7 sections, 17 species on 4 subsections, 4 subspecies and 9 species diversity. The nomenclature of some species has been clarified. 6 species of the genus *Trigonella* (*Trigonella brachycarpa* M. Bieb., *Trigonella arcuata* C.A Mey., *Trigonella monantha* C.A Mey., *Trigonella monspeliaca* L., *Trigonella orthocerac* (Car. &Kir.) Trautv., *Trigonella radiata* L.) have been transferred to the *Medicago* L. Based on the obtained results, previously given as independent species *M. coerulea, M. glomerata, M. falcata, M. hemicycle* species were reduced to *M. sativa* L. subspecies, *M. viresgens*, *M. caucasica*, *M. polychroa* species were reduced to synonym of *M. sativa* L. *M. talyshensis* was considered to present in status of species diversity of *M. arabica (M. arabica var. heptacyla* Urb.).

2. Distribution of the species of *Medicago* in the flora of Azerbaijan was specified and their digital mapping has been drawn up. The absence of the area of the species of *M. daghestanica* in Azerbaijan was identified. 2. *M. talyschensis* Lasch.= *M. arabica* var. *heptacyla* Urb.the species was found after a long time, and herbarium and seed materials were collected.

3. Ecological groups of the species of *Medicago* (3 mesophyte species, 11 xerophyte species and 3 mesoxerophyte species) and biomorphs (3 hemicryptophyte species and 14 therophyte species) and phytocenoses inhabited by the species were determined. *M. littoralis, M. minima, M. radiata* Ph- steppe and cereal phytocenosis, *M. monspeliaca, M. polymorpha, M. meyeri, M. truncatula, M. rigidula* - arid and semi-arid, - *M. monantha, M. minima, M. orthoceras, M. rigidula, M. medicaginoides* in Mountainous xerophytic vegetation, as well as *M. lupulina, M. orbicularis, M.arabica, M. sativa, M. polymorpha, M. papillosa*. in subalpine and alpine meadows.

4. Ecotypes of alfalfa were studied and two new ecotypes were identified (Gabala stony ecotype, Absheron sandy ecotype) belonging to the subspecies *M. sativa* ssp. *caerulea*.

5. Spectral and biochemical analyzes of seeds of species of the alfalfa were carried out. Various chemical elements (potassium, magnesium, calcium, oxygen, carbon, chlorine, sulfur) were found in the seeds of the species. The amount of nitrogen, total protein, lysine and tryptophan in seeds, which are valuable biological resources were also determined. Although the seed material was collected from different geographical and ecological conditions, chemical elements and biologically active substances found in these samples were similar.

6. Forty-six genotypes of 16 species of *Medicago* distributed in Azerbaijan have been evaluated by molecular markers, close and distant genotypes were identified according to their systematic position, phylogenetic relationships were determined according to the genetic characteristics of the species and their ecological conditions. *M. monspeliaca* collected from Lerik region and *M. lupulina* (GO = 0.10) collected from Shamkir were identified as the most distant genotypes, and *M. littoralis* and *M. truncatula* (GO = 1) collected from Absheron were identified as the closest genotypes.

7. Electron microscopic (SEM) diagnostic micromorphological features (seed shape, color, size, surface structure of the exodermal layer, etc.) of the seeds and stems of the genus were detected. Constant macro and micromorphological features were analyzed comparatively and used to determine the status of taxa with disputed systematic position.

RECOMMENDATIONS

1. Since species of the genus (*M. littoralis, M. truncatula, M. sativa, M. sativa* ssp. caerulea, *M. rigidula*) synthesize biologically active substances and are rich in valuable reserves, it is advisable to use those species in the field of pharmacology.

2. Species of alfalfa (*M. sativa* ssp. *glomerata, M. sativa* ssp. caerulea, *M. papillosa*) grown *in situ* can be used in breeding

because they are stress factors tolerant and have some high biomorphological characteristics.

3. Micromorphological characteristics of species of Alfalfa can be used as a marker in the selection of promising species.

4. The ISSR markers UBC 827, 834, and 857 used in our assessment can be used by future researchers to evaluate the species of alfalfa.

5. Due to nitrogen-fixing properties of the species, the green mass of annual species can be used as a fertilizer to enrich marginal soils with nitrogen.

6. During the monographic study of the genus, the newly developed systematic classification may be used for the writing of flora, the preparation of monographs and tutorials on genus.

LIST OF PUBLISHED WORKS ON THE THEME OF THE DISSERTATION

1. Güvəndiyev, V.M., Böyük Qafqazin mərkəzi hissəsinin *Medicago* (*Fabaceae*) cinsi növlərinin ekoloji-botaniki tədqiqi // "Ekologiya: Təbiət və Cəmiət Problemləri" mövzusunda Həsən Əliyevin 105 illik yubileyinə həsr olunmuş II Beynəlxalq Elmi Konfrans, – Bakı: – 2012, – s. 385-386.
2. Güvəndiyev, V.M., Vəliyeva, L.İ., Azərbaycan florasının *Medicago* L. (*Fabaceae* Lindl) cinsinin taksanomik tədqiqi // – Bakı: AMEA Mərkəzi Nəbatat Bağının əsərləri, – 2017. XV c., – s. 196-201.
3. Güvəndiyev, V.M. Azərbaycanda yayılan *Medicago* L. (*Fabaceae* Lindl) cinsinin ekobotaniki təhlili və bioekoloji xüsusiyyətləri // "Ekologiya: Təbiət və Cəmiət Problemləri" mövzusunda Həsən Əliyevin 110 illik yubileyinə həsr olunmuş III Beynəlxalq Elmi Konfrans, – Bakı: – 2017, – s. 288-290.
4. Güvəndiyev, V.M. Abşeron flora biomüxtəlifliyində *Medicago* L. (*Fabaceae* Lindl.) cinsi növlərinin tədqiqi // – Bakı: Əkinçilik Elmi Tədqiqat İstitutunun elmi əsərləri məcmuəsi, – 2018. XXIX c., – s. 173-178.
5. Güvəndiyev, V.M. Azərbaycan florasının Qarayonca cinsinin nadir növləri // Müasir Təbiət və Iqtisad Elmlərinin Aktual Problemləri. Beynəlxalq Elmi Konfrans, – Gəncə: – 4 may – 5 may, – 2018, II c., – s. 68-72.
6. Güvəndiyev, V.M. Əsgərov A. M., Azərbaycan Florasının *Medicago* L. (*Fabaceae* Lindl) cinsinin bəzi növlərinin Elektron Mikroskopda (SEM) tədqiqi // – Bakı: Azəraycan Milli Elmlər Akademiyasının Xəbərləri, Biologiya və tibb elmlər seriyası, – 2018. 73 c., №1, – s. 41-47.
7. Güvəndiyev, V.M., Əsgərov, A.M., Azərbaycan florasının Qarayonca (*Medicago* L.) cinsi növlərinin kimyəvi tərkibi və əhəmiyyəti // – Bakı: Azəraycan Milli Elmlər Akademiyası

Genetik Ehtiyatlar İnstitunun Elmi əsərləri, – 2017. – VI c. № 1, – s. 182-186.

8. В.М. Гувандиев, А.М. Аскеров, Таксономия видов рода *Medicago* L. (*Fabaceae* Lindl.) Флоры Азербайджана и микроморфологические признаки семян некоторых видов // – Красноярск: Вестнк КрасГАУ., – 2019. № 1 (142), – с. 192-200.
9. Güvəndiyev, V.M. Azərbaycan florasının Əkin qarayoncası (*M. sativa* L.) Növ kompleksinə daxil olan taksonların tədqiqi // – Bakı: Əkinçilik Elmi-Tədqiqat İstitutunun elmi əsərləri məcmuəsi, – 2019. C. 1(30)., –28-36. s.
10. Гувандиев, В.М. Новые сведения о роде Люцерна (*Medicago* L.) Флоры Азербайджана // LX Международной научно-практической конференции (памяти Н.Н. Лузина). – Москва, 2019, – с. 17-18.
11. Güvəndiyev, V.M. Azərbaycan Qarayonca (*Medicago* L.) cinsi növlərinin ISSR markerlərlə genetik profilinin qiymətləndirilməsi // "Müasir Botanikada İnnovasiya və Ənənələr" mövzusunda A.A. Qrossheymin 130 illik yubileyinə həsr olunmuş Elmi Konfrans, – Bakı: – 2019, 52 s.
12. Guvendiyev, V.M., Asgarov, A.M. Micromorphology of seeds of *Bucerates*, *Lunatae*, *Hymenocarpos* sections of *Medicago* L. species in Azerbaijan // – Delhi: International Journal of Botany Studies, – 2020.Vol. 5(1), – p. 109-113.
13. Гювендиев, В.М., Аскеров, А.М., Калантарова, Н.С., Гювендиева, Х.М., Гаджиев, Э.С. Исследование генетического разнообразия рода Люцерна (*Medicago* L.) с применением ISSR маркеров // – Махачкала: Дагестанский ГАУ., – 2020. № 1 (41), – с. 27-34.

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