IRSTI: 34.31.29
DOI: 10.52301/1684-940X-2024-1-8-14

ELECTROPHORETIC STUDY OF POLYMORPHISM OF GRAIN STORAGE PROTEINS IN THE SIBERIAN SPECIES OF THE *GENUS LEYMUS HOCHST. (POACEAE)*

***N.K. Badmaeva[1], A.V. Agafonov[2]**

[1]Institute of General and Experimental Biology' Siberian Branch of the Russian Academy of Sciences, Ulan-Ude, Russia
[2]Central Siberian Botanical Garden Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
**badmayevan@mail.ru*

Summary

Eighteen species of the genus Leymus *Hochst. in Siberia are represented by a large number of local populations geographically isolated. Herbarium and living material of the species collected in Southern Siberia have been studied. Most of populations were heterogeneous morphologically and more or less differed from each other in the range of variation. An electrophoretic analysis of storage endosperm proteins of individual seeds in selected populations has been carried out. All populations (except for* L. racemosus ssp crassinervius*) have been characterized by wide polymorphism in polypeptide patterns. Certain seeds from heterogeneous populations have been identified as interspecific hybrids.*

Keywords: *SDS-electrophoresis, storage proteins, taxonomy, systematics, Lemus.*

Introduction. The genus *Leymus* contains perennial rhizomatous cross-pollinating grasses with genomic constitution of NsXm haplomes and 2n=28, 42, 56, 84 [1-3]. A. Löve [2] followed N.N. Tzvelev [4] in respect of taxa of the genus from the territory of the former Soviet Union. For the Siberian area a taxonomic system was proposed by G.A. Peschkova [5].The list of taxa of the genus Leymus in the flora of the Asian region of Russia has been expanded to 24 taxa by Baikov and Lipin [6]. At the last revision of the genus N.N. Tsvelyov and N.S. Probatova [7] included 32 taxa in the genus *Leymus* for the flora of Russia.

Eighteen species and 2 subspecies have been recognized, 6 from them have been described as new ones (Table 1) [5].

Table 1. Taxa of the genus *Leymus* distributed in Siberia according to Peschkova [5].

Sect. ***Leymus*** **Hochst.:**	Sect. ***Aphanoneuron*** **(Nevski) Tzvel.:**
L. interior (Hulten) Tzvel.	*L. akmolinensis* (Drob.) Tzvel.
L. racemosus (Lam.) Tzvel. subsp. *crassinervius*	*L. angustus* (Trin.) Pilger
(Kar. et Kir.) Tzvel.	*L. chakassicus* Peschkova
L. racemosus (Lam.) Tzvel. subsp. *klokovii* Tzvel.	*L. dasystachys* (Trin.) Pilger
L. villosissimus (Scribn.) Tzvel.	*L. jenisseiemis* (Turcz.) Tzvel.
Sect. *Anisopyrum* (Griseb.) Tzvel.	*L. littoralis* (Griseb.) Peschkova
L. buriaticus Peschkova	*L. ordensis* Peschkova
L. chinensis (Trin.) Tzvel.	*L. ovatus* (Trin.) Tzvel.
L. multicaulis (Kar. et Kir.) Tzvel.	*L. paboanus* (Claus) Pilger
L. ramosus (Trin.) Tzvel.	*L. secalinus* (Georgi) Tzvel.
L. tuvinicus Peschkova	*L. sphacelatus* Peschkova

In Southern Siberia the genus is represented by a large number of local populations that sometimes are geographically isolated. The analysis of populations in different regions showed that most of them were heterogeneous morphologically and more or less differed from each other by a range of polymorphism.

In the last years many new species have been described from the territory of Southern Siberia [8, 9] and from China [10-13]. Therefore, the current number of species could be increased more by using the monotypic concept or geographical criteria. The biosystematic data concerning Asian taxa were not found.

An attempt to identify biotypes with the use of SDS-electrophoresis of storage endosperm proteins was undertaken.

Materials and methods. Herbarium, seed and living material of Leymus species were collected in the Irkutsk region and Buryatia (Southern Siberia). Taxonomic identification of accessions was carried out following Peschkova [5]. Preparation of endosperm protein extracts and SDS-electrophoresis were carried out according to Laemmli [14] with modifications [15]. A geographic origin of Leymus accessions studied electrophoreticly is shown in Figure 1.

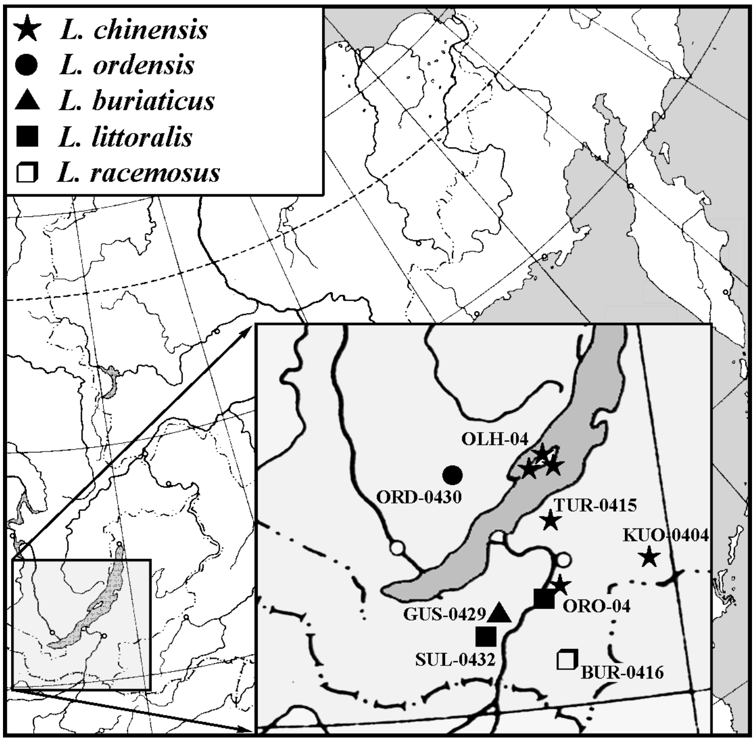

Figure 1. The map of the origin of Leymus *accessions from Buryatia and The Irkutsk region*

Results and discussion. A detailed study of species type specimens in St. Petersburg (LE) and Novosibirsk (NS) did not give answers to many issues of intra-generic differentiation. The main mode of species reproduction is the combination of vegetative (rhizomes) and spermatic (cross-pollination) ones. As a result even rather distant (conditionally intersectional) hybrids possessing absolute sterility can produce vital clones and take up a certain territory. The descriptions of some species and diagnostic keys are constructed overly complicatedly and contradictorily in a series of positions. Thus, some morphologically heterogeneous populations in Altai and Buryatia included from 3 to 7 morphotypes, the plants of each of them produced more or less amount of seeds. Since variation of basic diagnostic characteristics (pubescence of leaves and lemmas, glume length and width, number of spikelets per node) was revealed, boundaries between some minute species seemed to be extremely problematic. Therefore, concerning Siberian taxa of Leymus, we will share Peschkova‘s [5] system until new biosystematic data are obtained.

Thus, the analysis of populations of *L. secalinus s.str.* in several regions of Buryatia (including classic location) showed that anthers were closed and seeds were absolutely absent. By hypothesis this taxon arises permanently and polyphyleticly as an interspecies hybrid *L. littoralis* x *L. chinensis* or exists as one of polyploid (aneuploid) race. On the other hand, a number of mixed subpopulations of these species and semi-fertile individuals were collected and studied electrophoreticly (see below). Probably, one of hybrid (introgressive) combinations of *L. littoralis* and *L. chinensis* has been recognized as L. buriaticus in the classic location [8]. Furthermore, some doubt should arise about close relationships of these genotypes with those from Yakutia-Sakha Republic.

L. ordensis which according to G.A. Peschkova [8] comparatively widely spreads in Southern Siberia could be mentioned as the most problematic taxon in Siberia. In our opinion this taxon was described as a new cespitose species quite erroneously. The study of the type specimens (LE, St. Petersburg) showed that they were long rhizomatous.

It is obvious that all assumptions above should be verified and confirmed by biosystematic methods.

Conclusion. Previously it has been shown that SDS-PAGE of storage endosperm proteins (prolamine-gluteline complex) can be used for electrophoretic characterization of genotypes and as the indicators of a population status in the genus Elymus [15-17]. The main advantage of grain proteins as genetic markers is that living plants are not required because the endosperm proteins keep their electrophoretic properties for many years.

The weight of dry grains isolated from lemmas and paleas of different *Leymus* species averages between 1.0–1.8 mg in *L. chinensis*, 1.2–2.0 mg in *L. littoralis* and 10–24 mg in *L. racemosus*.

Figure 2 shows a variation of polypeptide patterns in populations of *L. ordensis* in comparison with the populations of *L. chinensis* in electrophoretic variants –Me and +Me.

Monomeric proteins (variant –Me) mostly have to be considered among prolamines and are characterized by a range of molecular weight from 30 to 60 kD. A high level of prolamine polymorphism was observed in all populations of *L. ordensis* and *L. chinensis*. Invariant prolamine polypeptides were revealed in the range of REM 25–37. This kind of polypeptides kept their relative electrophoretic mobility (REM) after 2-mercaptoethanol treatment analogously to those in some *Elymus* species [15].

Polypeptides with a molecular weight of 28 to 35 kD changed their REM, which indicated the presence of internal S-S bonds (cystine) in this type of prolamines. In the variant +Me several electrophoretic bands were revealed on gels, which probably were gluteline subunits. The HMW subunits in *L. ordensis* and *L. chinensis* occurred in the range of REM 3–8.

Significant variation in electrophoretic patterns has neither confirmed nor disproved the taxonomic rank of *L. ordensis* and makes numerical analysis difficult. Nevertheless, the presence of common components of REM 3–8 and REM 43–50 in patterns of two species could testify to their common gene pool and mutual crossability.

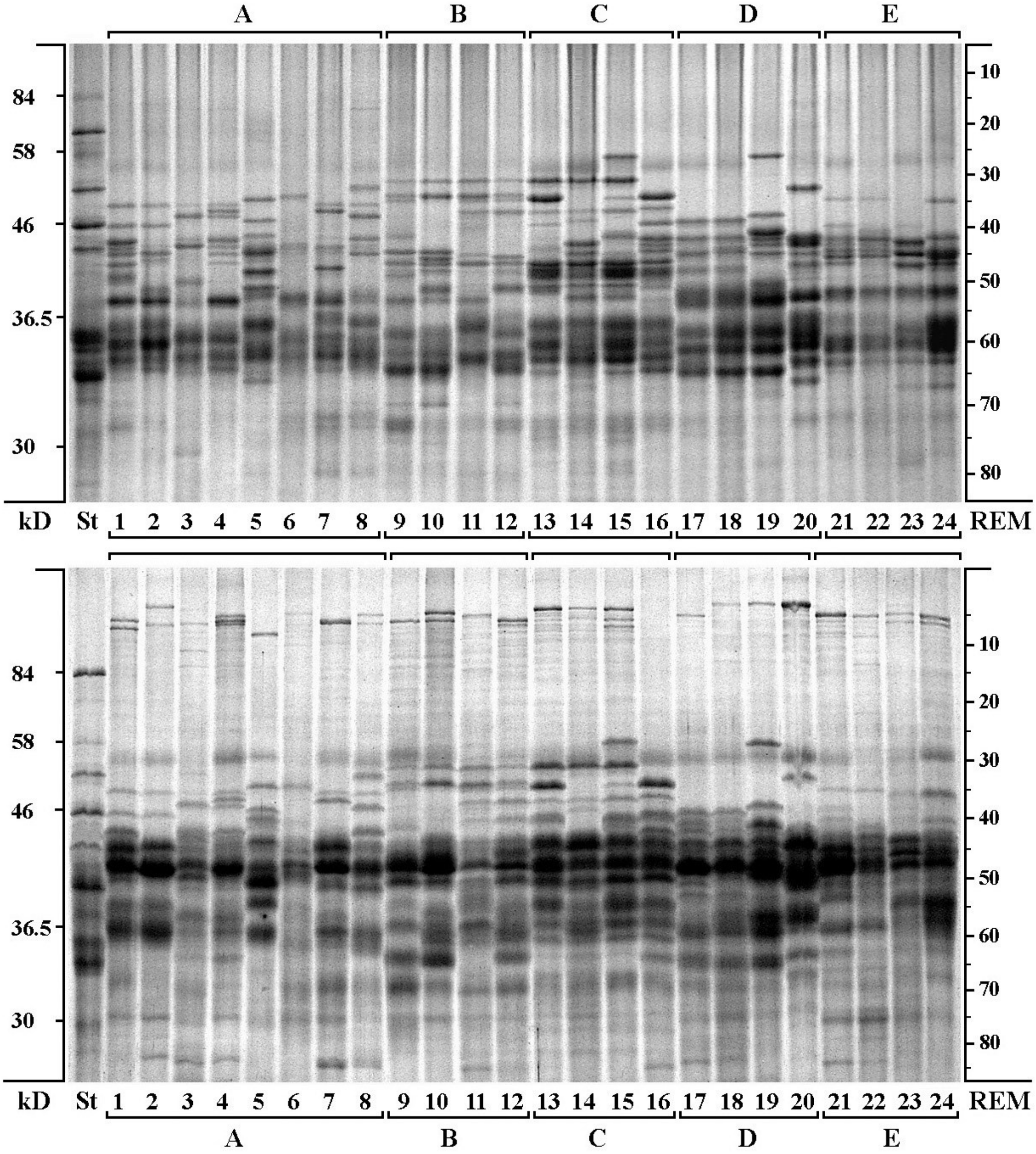

Figure 2. SDS-PAGE polypeptide patterns of endosperm proteins of **Leymus ordensis** *(A) in comparison with geographically close* ***(B–D)*** *and relatively distant* ***(E)*** *populations of* **L. chinensis.** Random *seed samples from different plants of population, the same seeds in electrophoretic variants –Me (above) and +Me (below). St –* Elymus sibiricus*, ALT-8401*

The most overlapping of individual polypeptides for the speies *L. chinesis*, *L. ordensis* and *L. buriaticus* was found out in the range of REM 3–8, that corresponded to HMW subunits. These polypeptides were found to be similar in REM with those in *L. littoralis*, but distinguished from subunits of *L. racemosus* in REM 9 and 11. The electrophoretic patterns of separate seeds of the latter species differed from each other in very few components that evidently reflected a narrow gene pool of its population.

To confirm a permanent interspecific hybridization in mixed populations, an electrophoretic test was made. Besides the typical plants of *L. littoralis* and *L. chinensis*, seed samples from intermediate plants (conditionally “I-seeds”) were studied (Fig 4). A seed set in all plants in the population was low, as not more than 5–10 grains per spike. Some plants being morphologically close to *L. chinensis* had long pubescent lemmas and (or) elongated glumes. The seeds from these plants were weaker than those from typical ones.

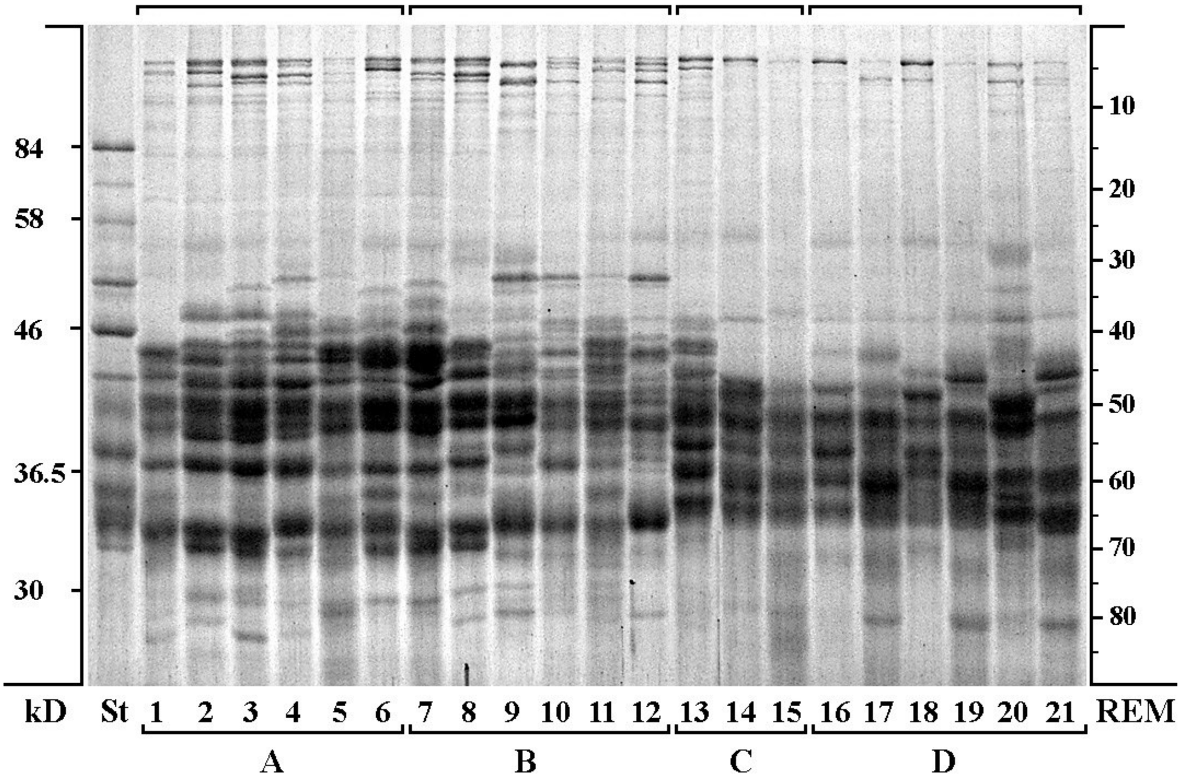

Figure 4. SDS-PAGE polypeptide patterns of endosperm proteins in the mixed population of Leymus *consisting of* **L. littoralis** *ORO-0417 (A, B),* **L. chinensis** *ORO-0416, **(D)** and morphologically intermediate plants **(C)***

The results of the test showed that for *L. littoralis* variation within the seed samples from the same spike **(A)** was just slightly less than the variation within the seed samples from different plants of population **(B),** particularly in the range of REM 30–85. These data support predominantly cross-pollination in *Leymus* species as it was shown by K. Jensen et al. [18]. In the samples of I-seeds the pattern C-13 was similar with *L. littoralis* B-11 in a range of REM 37 -52, whereas the patterns C-14 and C-15 were similar to *L. chinensis* D-16 and D-18 in a total range of REM. It also supported a hybrid origin of the intermediate plants which had low but not zero seed fertility and possibility for stable vegetative reproduction.

Thus, the electrophoretic analysis of the storage endosperm proteins of the individual seeds in the selected taxa of the genus *Leymus* showed that all populations (except for *L. racemosus ssp crassinervius*) were characterized by wide polymorphism in polypeptide patterns. The certain seeds from heterogeneous populations were identified as interspecific hybrids.

A living collection of natural biotypes of *Leymus*, which is being created in the Central Siberia Botanical Garden for Siberian taxa, is required for biosystematic study. It could be presupposed that some minute species should be relegated to infraspecific rank within large-scale species on the basis of phylogenetic relationships.

References

1.Dewey D.R. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae // Gene Manipulation in Plant Improvement / Ed. J.P. Gustafson. – New York: Plenum Publ. Corp., 1984. – P. 209–279.

2.Löve A. Conspectus of the Triticeae // Feddes Repertorium. – 1984. – Vol. 95. – P. 425–521.

3.Wang R. R.-C., von Bothmer R., Dvorak J., Fedak G., Linde-Laursen I., Muramatsu M. Genome symbols in the Triticeae (Poaceae) // Proc. 2nd Int. Triticeae Symp., Logan, Utah, USA / Eds. R.R.-C. Wang, K.B. Jensen, C. Jaussi. – 1994. – P. 29–34.

4.Tzvelev N.N. Zlaki SSSR. – Leningrad: Nauka, 1976. – 788 p.

5.Peschkova G.A. Leymus // Flora Sibiriae. Poaceae (Gramineae) / Eds. L.I. Malyschev, G.A. Peschkova. – Novosibirsk: Nauka, Siberian division, 1990. – Vol. 2. – P. 41–52.

6.Baikov K.S., Lipin S.A. Check-list of genus Leymus (Poaseae) in flora of Asian Russia // Bulletin of Moscow Society of Naturalists. Biological series. – 2008. – Vol. 113, № 5. – P. 83–88.

7.Tsvelyov N.N., Probatova N.S. The genera Elymus L., Elytrigia Desv., Agropyron Gaertn., Psathyrostachys Nevski and Leymus Hochst. (Poaceae: Triticeae) in the flora of Russia // V.L. Komarov Memorial Lectures. – Vladivostok: Dalnauka, 2010. – Issue 57. – P. 5–102.

8.Peschkova G.A. New species of the genus Leymus (Poaceae) from Siberia // Botanicheskii Zhurnal (Leningrad). – 1985. – Vol. 11. – P. 1554–1557.

9.Peschkova G.A. About Leymus secalinus s.l. (Georgi) Tzvel. (Poaceae) // Novosti Sistematiki Vysshikh Rastenii (Leningrad). – 1987. – Vol. 24. – P. 21–26.

10.Cai L.-B. New taxa of Leymus from China // Acta Phytotaxonomica Sinica. – 1995. – Vol. 5. – P. 491–496.

11.Cai L.-B. Two new species of Leymus (Poaceae: Triticeae) from Qingai, China // Novon. – 2000. – Vol. 10. – P. 7–11.

12.Cai L.-B. A new species and a new variety of Leymus Hochst. (Poaceae) from Qingai, China // Acta Phytotaxonomica Sinica. – 2001. – Vol. 39, № 1. – P. 75–77.

13.Cui D.-F. New taxa of Leymus Hochst. from Xinjiang // Bulletin of Botanical Research. – 1998. – Vol. 2. – P. 144–148.

14.Laemmli U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4 // Nature. – 1970. – Vol. 227. – P. 680–685.

15.Kostina E.V., Agafonov A.V., Salomon B. Electrophoretic properties and variability of endosperm proteins of Elymus caninus (L.) L. // Triticeae III / Ed. A.A. Jaradat. – Enfield, New Hampshire, USA: Science Publishers, 1998. – P. 265–272.

16.Agafonov A.V. The Principle of Recombination Gene Pools (RGP) and Introgression Gene Pools (IGP) in the biosystematic treatment of Elymus species // Proc. 2nd Int. Triticeae Symp., Logan, Utah, USA / Eds. R.R.-C. Wang, K.B. Jensen, C. Jaussi. – 1994. – P. 254–260.

17.Kostina E.V., Salomon B., Agafonov A.V. Biosystematic relationships between Elymus mutabilis and E. transbaicalensis (Poaceae) as indicated by morphology, grain proteins, and crossability // Triticeae IV / Eds. P. Hernández et al. – Sevilla, Spain: Consejeria de Agricultura y Pesca, 2002. – P. 63–68.

18. Jensen K.B., Zhang Y.F., Dewey D.R. Mode of pollination of perennial species of the Triticeae in relation to genomically defined genera // Canadian Journal of Plant Science. – 1990. – Vol. 70. – P. 215–225.

Material received on 24.01.24

Эндоспермнің қор ақуыздарының полиморфизмін электрофорез әдістерімен зерттеу Leymus Hochst . (Poaceae) туысының сібірлік түрлерінде

Аңдатпа

Leymus (Hochst.) туысының он сегіз түрі Сібірде географиялық тұрғыдан оқшауланған жергілікті популяциялармен кеңінен ұсынылған. Оңтүстік Сібірде жиналған гербарий үлгілері мен тірі материал зерттелді. Көптеген популяциялар морфологиялық тұрғыдан әртекті болып, өзгергіштік диапазонында бір-бірінен азды-көпті ерекшеленді. Әртүрлі популяциялардағы жеке тұқымдардың эндосперм қор ақуыздары электрофоретикалық талдаудан өтті. Барлық популяциялар (L. racemosus subsp. crassinervius популяцияларын қоспағанда) полипептидтік спектрлерде кең полиморфизммен сипатталды. Әртекті популяциялардағы кейбір тұқымдар аралық түрлердің гибридтері ретінде анықталды.

***Түйінді сөздер**: SDS-электрофорез, қор ақуыздары, таксономия, систематика,* Leymus.

Материал баспаға 24.01.24 түсті

Исследование полиморфизма запасных белков эндосперма методами электрофореза у сибирских видов рода Leymus Hochst. (Poaceae)

Аннотация

Восемнадцать видов рода колосняк Leymus Hochst. в Сибири представлены большим количеством географически изолированных локальных популяций. Изучены гербарные образцы и живой материал видов, собранных в Южной Сибири. Большинство популяций были неоднородными морфологически и более или менее отличались друг от друга в диапазоне изменчивости.

Проведен электрофоретический анализ запасных белков эндосперма отдельных семян в разных популяциях. Все популяции (за исключением популяций L. racemosus subsp. crassinervius) характеризовались широким полиморфизмом в полипептидных спектрах. Некоторые семена из гетерогенных популяций были идентифицированы в качестве межвидовых гибридов.

***Ключевые слова**: SDS-электрофорез, запасные белки, таксономия, систематика,* Leymus.

Материал поступил в редакцию 24.01.2024

Acknowledgements. The authors are very grateful to D. Gerus for the essential help in electrophoretic analysis. The work was supported by the Assignment № VI.52.1.9 and from the Russian Foundation for Basic Research: 04-04-48720, 11-04-00861.

Disclosure statement. The authors declare that there are no conflicts of interest to disclose in this article.