Electrophoretic Variation of Endosperm Proteins and its Use for Taxonomy and Biosystematics in the Genus *Leymus* (*Poaceae*)

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Abstract: Eighteen species of the genus *Leymus* Hochst. are present in Siberia. They grow in a large number of local, geographically isolated populations. Herbarium and living material of the species collected in Southern Siberia were 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 was carried out. All populations (except for *L. racemosus* ssp. *crassinervius*) were characterized by wide polymorphism in polypeptide patterns. Certain seeds from heterogeneous populations were been identified as interspecific hybrids.

Keywords: taxonomy; systematics; Leymus; SDS-electrophoresis; storage proteins

The genus *Leymus* includes perennial, rhizomatous, cross-pollinating grasses with genomic constitution of NsXm haplomes and $2n = 28, 42, 56, 84$ (Dewey 1984; Löve 1984; Wang *et al*. 1994). Löve (1984) followed Tzvelev (1976) in respect of taxa of the genus from the former Soviet Union. Peshkova (1990, 2001) has presented a revised treatment of the genus for Siberia. Eighteen species and 2 subspecies were recognized, 6 of which were new (Table 1).

In southern Siberia, *Leymus* is represented by a large number of local populations, some of which are geographically isolated. Analysis of populations in different regions showed that most of them were heterogeneous morphologically, differing each other by a range of polymorphism.

In the last years many new species have been described from the territory of Southern Siberia (Peshkova 1985, 1987) and from China (Cai 1995, 2000, 2001; Cui 1998). Therefore, the current number of species could be increased more by using the monotypic concept or geographical criteria. No biosystematic data concerning Asiatic taxa were found.

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

MATERIALS AND METHODS

Herbarium, seed and living material of *Leymus* species was collected in Irkutskaya Oblast and Buryatia (Southern Siberia). Taxonomic identification of accessions was carried out following Peshkova (1990). Preparation of endosperm protein extracts and SDS-electrophoresis were carried out according to Laemmli (1970) with modifications (Kostina *et al*. 1998). The geographic origin of *Leymus* accessions studied by electrophoresis is shown in Figure 1.

Table 1. Taxa of the genus *Leymus* distributed in Siberia according to Peshkova (1990, 2001)

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

RESULTS AND DISCUSION

A detailed study of species type specimens in St. Petersburg (LE) and Novosibirsk (NS) did not answer many questions of intra-generic differentiation. Reproduction in the species is both vegetative, via rhizomes, and sexual, via cross-pollination. As a result, even rather distant (intersectional) hybrids that are completely sterile can produce viable clones that can expand over an area. The descriptions of some species and diagnostic keys are overly complicatedly and contradictory in a series of positions. For example, some morphologically heterogeneous populations in Altai and Buryatia

Figure 1. The map of origin of *Leymus* accessions from Buryatia and Irkutskaya oblast

included from 3 to 7 morphotypes; plants of each of them produced different amounts of seeds. Because some of the variation is in the basic diagnostic characteristics (pubescence of leaves and lemmas, glume length and width, number of spikelets per node), boundaries between some species seem to be extremely problematic. Nevertheless, until new biosystematic data are available, it seems best to use Peshkova's (1990) system in Siberia.

The analysis of populations of *Leymus secalinus* s. str. in several regions of Buryatia (including the type location) showed that anthers were closed and seeds were completely absent. We hypothesized that this taxon arises permanently and polyphyleticly as an inter-specific hybrid *L. littoralis* × *L. chinensis*, or exists as an aneuploid race. A number of mixed subpopulations of these species and semi-fertile individuals were collected and studied electrophoretically (see below). Moreover, plants in the type locality of *L. buriaticus* appear to consist of hybrid (introgressive) combinations of *L. littoralis* and *L. chinensis*. Furthermore, there is some doubt about the close relationships of these genotypes with those from Yakutia-Sakha Republic.

Leymus ordensis which, according to Peshkova (1985), is comparatively widespread in Southern Siberia, is another problematic Siberian taxon. It was described as a new cespitose species but a study of the type specimens (LE) showed that they were long rhizomatous.

These problems and hypotheses can be addressed using biosystematic methods. We did so, by examining the electrophoretic variation in endosperm proteins.
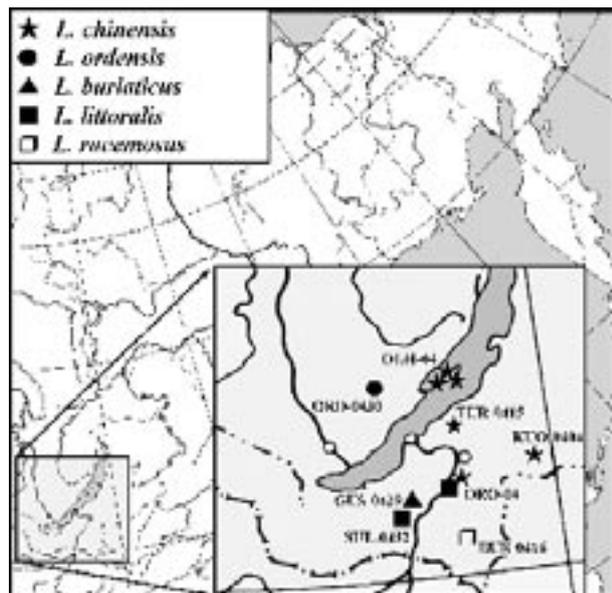
St – *Elymus sibiricus,*
    ALT-8401
A – *L. ordensis* ORD-0430;
    L. chinensis:
B – OLH-0409;
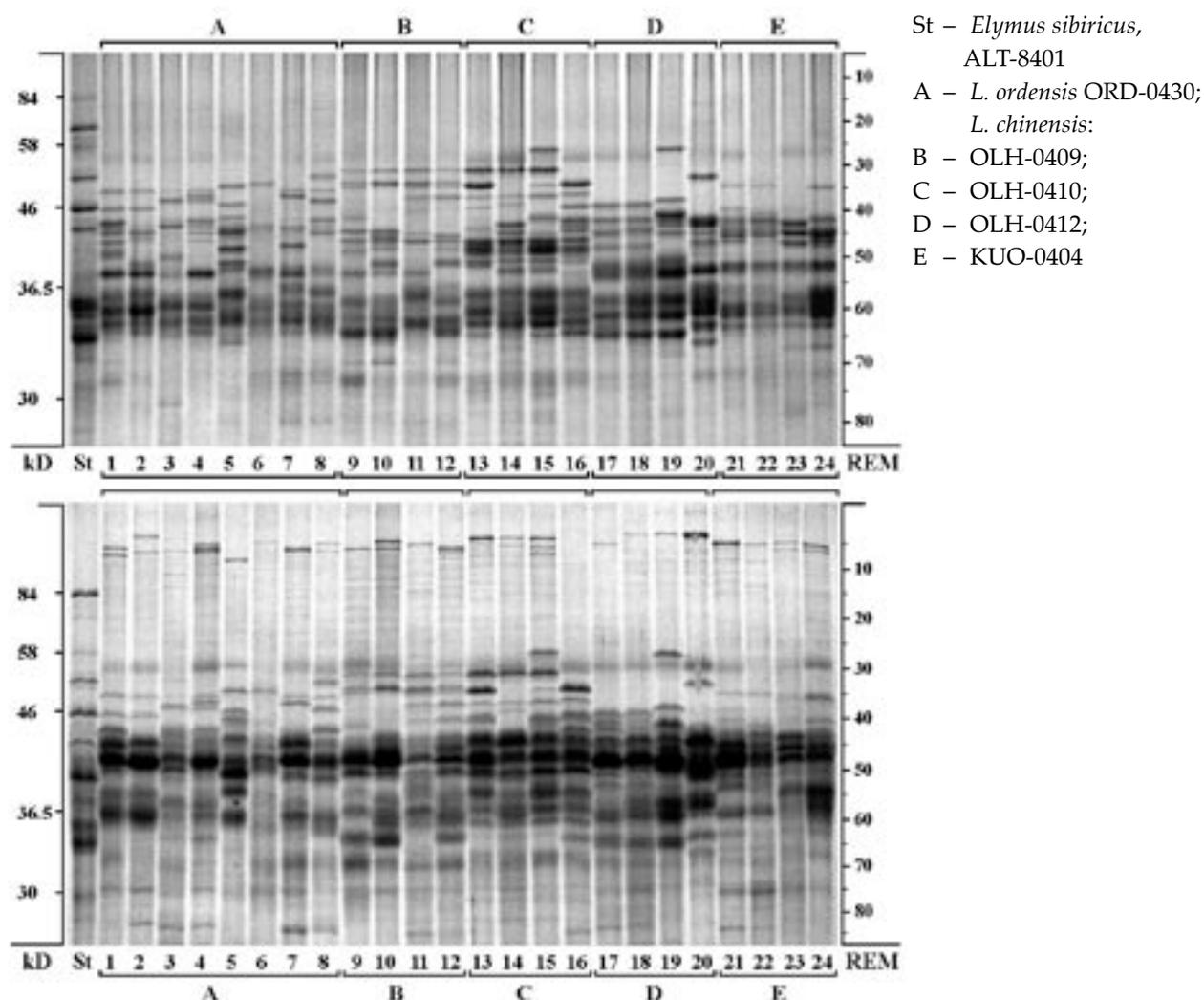C – OLH-0410;
D – OLH-0412;
E – KUO-0404

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, same seeds in electrophoretic variants –Me (above) and + Me (below)

Electrophoretic analysis

SDS-PAGE of storage endosperm proteins (prolamine-gluteline complex) can be used for electrophoretic characterization of genotypes and as indicators of a population status in the genus *Elymus* (AGAFONOV 1994; KOSTINA *et al.* 1998, 2002). 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.

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 the variation of polypeptide patterns in populations of *Leymus ordensis* in comparison with 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 *Leymus ordensis* and *L. chinensis*. Invariant prolamine polypeptides were revealed with relative electrophoretic mobility (REM) in the range of 25–37. These kinds of polypeptides kept their REM after 2-mercaptoethanol treatment, as was the case in *Elymus* (KOSTINA *et al.* 1998). Polypeptides with a molecular weight of 28 to 35 kD changed their REM, which indicated a presence of internal S-S bonds (cystine) in this type of prolamine. In the variant +Me several electrophoretic bands were revealed on gels, which
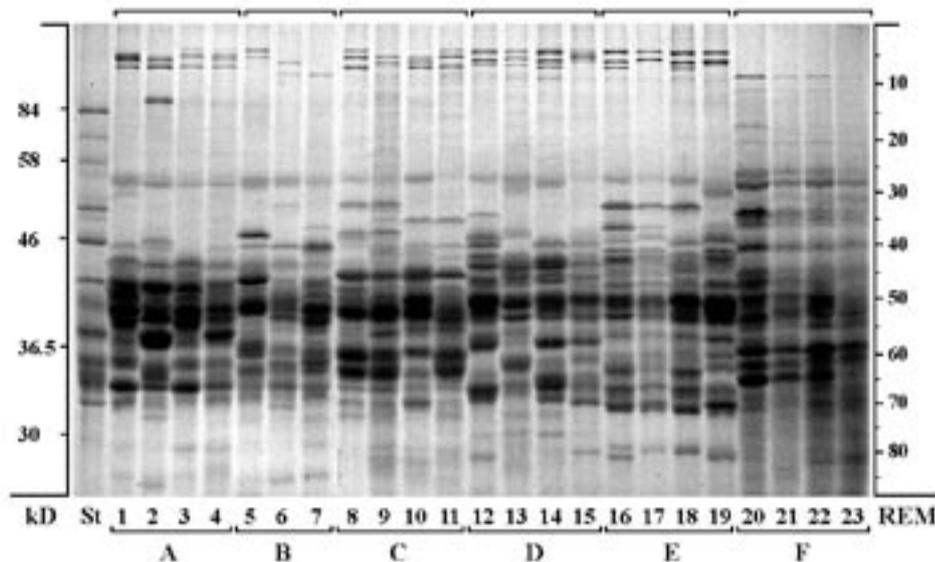
Figure 3. SDS-PAGE polypeptide patterns of endosperm proteins of *Leymus* species. Random seed samples from different plants of populations, electrophoretic variant +Me

A – *L. chinensis* TUR-0415;
B – *L. ordensis* ORD-0430;
C – *L. buriaticus* GUS-0429; D – *L. littoralis* ORO-0417;
E – *L. littoralis* SUL-0432;
F – *L. racemosus* ssp *crassinervius* BUR-0416

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 status of *L. ordensis* and makes numerical analysis difficult. Nevertheless, presence of common components of REM 3–8 and REM 43–50 in patterns of two species suggests that they have a common gene pool and may be crossable.

Electrophoretic patterns from random seed samples from different *Leymus* species were compared (Figure 3). The greatest similarity in individual polypeptides for species *L. chinensis*, *L. ordensis* and *L. buriaticus* had 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, indicating that its gene pool is narrow.

To confirm permanent interspecific hybridization in mixed populations, an electrophoretic test was made. Besides typical plants of *Leymus littoralis* and *L. chinensis*, seed samples from intermediate plants (conditionally "I-seeds") were studied (Figure 4). Seed set in all plants in the population was low, with no more than 5–10 grains per spike. Some plants that were morphologically close to
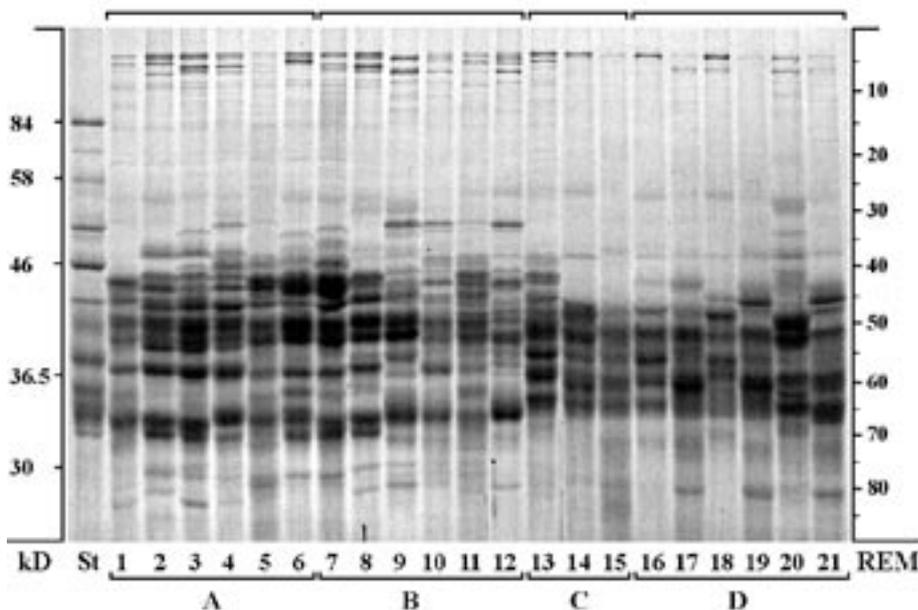
A – seeds from an identical spike;
B, C, D – seeds from different plants

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). Single seeds in the variant +Me

L. chinensis had long pubescent lemmas and (or) elongated glumes. Seeds from these plants were weaker than seeds from typical plants.

The results of the test showed that, for *Leymus littoralis*, variation within the seed samples from the same spike (A) was just slightly less than variation within the seed samples from different plants of population (B), particularly in the range of REM 30–85. These data support the conclusion that sexual reproduction in *L. littoralis* is predominantly via cross-pollination, as was shown for other species of *Leymus* by JENSEN *et al.* (1990). In the samples of I-seeds the pattern C-13 was similar to those of *L. littoralis* B-11 in a range of REM 37-52, whereas patterns C-14 and C-15 were similar to those of *L. chinensis* D-16 and D-18 in a total range of REM. The data also supported a hybrid origin of intermediate plants which had low but not zero seed fertility and possibility for stable vegetative reproduction.

The electrophoretic analysis of storage endosperm proteins of individual seeds in selected taxa of the genus *Leymus* showed that all populations, except those of *L. racemosus* ssp *crassinervius*, were characterized by wide polymorphism in polypeptide patterns. Some 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 is likely that that some narrowly circumscribed species will be relegated to infraspecific rank within large-scale species on the basis of phylogenetic relationships.

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