

Microevolutionary relationships between biotypes of *Elymus confusus*, *E. peschkovae*, and *E. sibiricus* (Poaceae) according to hybridization and sequencing of the nuclear gene *GBSS1* (*waxy*)

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Abstract. According to descriptions, the Siberian species *E. peschkovae* and *E. confusus* differ in the presence or absence of trichomes on lemma and rachilla surfaces only. Two methods were used for study microevolutionary relationships between these species: hybridization of biotypes from different locations and analysis of nucleotide sequences of the low-copy nuclear gene *GBSS1*. Created and grown hybrids in combinations AMU-8804 × BER-0807 and AMU-8804 × BUK-1109 showed complete seed sterility in two field vegetations. Taking into account wide species areas, 3 hybrids were created between Magadan biotypes from a common habitat in the combination *E. confusus* MOL-1887 × *E. peschkovae* MOL-1882. The plants showed seed fertility (SF) of 0–3 %. This result does not obscure the possibility to assess SF in the F₂ generation and the character of inheritance of diagnostic traits. A comparative study of the *GBSS1* gene sequences in accessions was carried out in comparison with clones of reference species. Differentiation of clones by the St₂ subgenome in *E. confusus*, *E. peschkovae*, *E. sibiricus* and *E. caninus* did not reveal a clear relationships between the species. Meanwhile, a certain species specificity for the H₁ subgenome was noted, confirming the existence of microevolutionary isolation of these species.

1 Introduction

Elymus sibiricus L. and *E. confusus* (Roshev.) Tzvel. are spreaded everywhere in southern regions of Siberia [1]. In the Russian Far East *E. confusus* area is shifted to the North, where it totally replaces *E. sibiricus* [2]. Both species are characterized by glabrous lemmas, but *E. sibiricus* rarely have shortly pilous lemmas. It was shown in the previous experiment that interspecific introgression is possible under the joint growth of these two species in Siberia, as a result of sexual hybridization [3].

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Created and analyzed hybrids of East-Sajan biotypes in combination *E. sibiricus* BUR-0569 × *E. confusus* BUM-0505 in generation F₁ showed mostly opened anthers, values of seed fertility (SF) accounted for 2–7 seeds per spike.

Among 20 plants of the F₂ generation, grown on the opened plot, 17 corresponded to *E. sibiricus* on the trait of doubled spikelets on spike ledge, 3 plants carried single spikelets on all spikes. These results gave reason to refer *E. sibiricus* and *E. confusus* to a unified recombination gene pool with reproductive compatibility at the $\alpha 2$ level at least within the Southern Siberia area.

Elymus peschkovae Tzvelev (— *Agropyron confusum* var. *pubiflorum* Roshev. — *Elymus confusus* var. *pubiflorus* (Roshev.) Tzvelev — *E. pubiflorus* (Roshev.) Peschkova) differs from *E. confusus* by pilose lemmas (Fig. 1). In Siberia the area of *E. peschkovae* is distinctly shifted to the North-East part in comparison with *E. confusus*.



Fig. 1. Seeds (lemmas) of accessions *E. confusus* MOL-1887 (1) and *E. peschkovae* MOL-1882 (2)

The purpose of this research was to study microevolutionary relationships between *E. peschkovae* and *E. confusus* biotypes. Two experimental methods were used: hybridization of biotypes from different points of species area, and analysis of nucleotide sequences of low-copy nuclear gene *waxy* (granule-bound starch synthase 1, *GBSSI*).

2 Materials and Methods

Parental biotypes for hybridization were grown from seeds of wild-growing accessions collected at different points of areas, or from seeds of their offspring grown under conditions of self-pollination. Plants were hybridized according to the previously developed technique [4]. Techniques of probe preparation and sequences study of the *GBSSI* gene in three species were performed as described earlier [5]. The origin of accessions and clone numbers submitted to the GenBank NCBI (<http://www.ncbi.nlm.nih.gov/nucleotide>) are shown in Table 1. In addition to accessions of three species and monogenic markers, clones of *E. caninus* (L.) L. from GenBank NCBI, as a morphologically related species, were included in the study of *GBSSI* sequences (Table 2). The construction of dendrograms by the Neighbor-Joining method was carried out in the TREECON ver. 1.3b soft [6].

Table 1. Accessions and clones of *GBSS1* gene sequences in *E. confusus*, *E. peschkovae*, *E. sibiricus* which have been submitted to the GenBank NCBI. Accessions taken for hybridization are marked by bold

Accession	Clones (sequences) in NCBI	Location of collecting
<i>E. confusus</i>		
TAR-0730	conTAR30_1-St MN164827 conTAR30_4-H MN164828	The Tyva Republic, Tes-Khemsy District, alt. 1545 m N 50° 36.870' E 95° 10.729'
BER-0807	conBER07_2-St MN164825 conBER07_1-H MN164826	The Republic of Buryatia, Eravnsky District, alt. 958 m N 53° 00.776' E 112° 03.673'
BUR-0557	conBUR57_7-St MZ605026 conBUR576a-H MZ605025	The Republic of Buryatia, Okinsky District, alt. 1858 m N 51°57.383' E 100°33.564'
BUM-0505	conBUM05_1-St MZ605028 conBUM05_7-H MZ605027	The Republic of Buryatia, Tunkinsky District, alt. 1738 m N 51°42.610' E 100°59.967'
BUK-1109	conBUK09_8-St MZ605030 conBUK09_6-H MZ605029	The Republic of Buryatia, Eravnsky District, alt. 750 m N 50°32.762' E 107°47.435'
MOL-1887	conMOL87_2-H MZ605031	Magadan Region, Olsky District, alt. 6 m N 59° 34.913' E 151° 16.585'
MOK-1895	conMOK95_3-St MZ605033 conMOK95_4-H MZ605032	Magadan Region, Olsky District, alt. 24 m N 59° 36.706' E 150° 27.629'
<i>E. peschkovae</i>		
AMU-8804	pesAMU04_3-St MN164829 pesAMU04_1-H MN164830	Amur region, Tyndinsky District, alt. 441 m N 54° 56.216' E 125° 21.854'
MJA-1106	pesMJA06_3-St MN164831 pesMJA06_2-H MN164832	Magadan Region, Khasynsky district, alt. 755 m N 60° 19.467' E 151° 10.540'
MOL-1882	pesMOL82_1-St MZ634450 pesMOL82_7-H MZ634449	Magadan Region, Olsky District, alt. 6 m N 59° 34.913' E 151° 16.585'
JTO-1708	pesJTO08_2-St MZ605035 pesJTO08_3-H MZ605034	The Sakha (Yakutia) Republic, Oymyakonsky District, alt. 810 m N 63° 20.705' E 141° 48.050'
<i>E. sibiricus</i>		
JAC-1504	sibJAC04_1-St MN164833 sibJAC04_2-H MN164834	The Sakha (Yakutia) Republic, vicinity of Yakutsk town
MOK-1896	sibMOK96_3-H MZ634451 sibMOK96_4-H MZ634452	Magadan Region, Olsky District, alt. 24 m N 59° 36.706' E 150° 27.629'

Table 2. Species accessions and their clones drawn from the GenBank NCBI.

Species	Accession (origin)	Sequence in NCBI	Designation on dendrograms
<i>Elymus sibiricus</i>	PI 628699, Russia	St HM035280 H HM035281	sib_5280_S sib_5281_H
<i>E. sibiricus</i>	PI 499461, China	St HM035282 H HM035283	sib_5282_S sib_5283_H
<i>E. caninus</i>	ABZ-1654, Russia	St MN164839 H MN164840	canABZ54_2 canABZ54_1

<i>E. caninus</i>	PI 314612 Kazakhstan	St b-HM035270 H a-HM035271	can_5270_S can_5271_H
<i>E. caninus</i>	PI 499413 China	St a-HM035272 H b-HM035273	can_5272_S can_5273_H
<i>E. caninus</i>	PI 531571 Poland	St a-HM035274 H b-HM035275	can_5274_S can_5275_H
<i>E. gmelinii</i> (Ledeb.) Tzvelev	PI 499447 China	St GQ847726 Y GQ847727	gme_7726_S gme_7727_Y
<i>E. pendulinus</i> (Nevski) Tzvelev	PI 499452 China	St GQ847731 Y GQ847732	pen_7731_S pen_7732_Y
<i>Pseudoroegneria</i> <i>a strigosa</i> (M.Bieb.) A. Löve	PI 499637 China	St EU282323	P.str_323
<i>P. strigosa</i>	PI 531755 China	St AY360823	P.str_823
<i>P. spicata</i> (Pursh) A. Löve	PI 232117 USA	St AF079281	P.spi_281
<i>P. spicata</i>	PI 610986 USA	St AY010999	P.spi_999
<i>Hordeum</i> <i>jubatum</i> L.	RJMG 106 USA	H AY010963	H.jub_63_H
<i>H. californicum</i> Covas & Stebbins	MA-138-1-4 USA	AF079273	H.cal_73_H
<i>Bromus</i> <i>tectorum</i> L.	–	AY362757	Bromus_AY3

3 Results and Discussions

Hybrids between *E. peschkovae* (Amur region) and *E. confusus* (The Republic of Buryatia), created and grown by us in combinations **AMU-8804** × **BER-0807** (2 plants) and **AMU-8804** × **BUK-1109** (2 plants), have shown total seed sterility. This phenomenon forced us to return to the problem of phylogenetical relationships between three species, especially considering their wide distribution areas within the Asian part of Russia.

In vegetation period 2020 we have created 3 hybrids between Magadan biotypes from the common ecotope in the valley of river Ola in combination *E. confusus* **MOL-1887** × *E. peschkovae* **MOL-1882**. In the greenbox all three plants had a little part of opened anthers and showed low fertility (SF) 0-3%, which was confirmed on the open experimental plot. This result does not obscure the possibility to estimate inheritance of the distinctive (diagnostic) traits and dynamics of SF in the **F₂** generation.

At the same time we carried out a comparative research of the *GBSSI* gene sequences in collection accessions *E. peschkovae* and *E. confusus* from the different points of area compared to clones of reference species. Dendrogram, built on the data of *GBSSI* gene sequences (exons from 9 to 14 only), is shown on the Fig. 2.

Based on these results, the following conclusions were made:

1. The **StH**-genomic constitution was confirmed for *E. confusus*, *E. peschkovae* and *E. sibiricus*.

2. All gene variants of St subgenome in studied species are phylogenetically close to the North American ancestral line **St₂** of *Pseudoroegneria* genus, unlike the **St** subgenome of reference Asian StY-genomic species *E. pendulinus* and *E. gmelinii*. The group of **St₂** clones of Siberian *E. confusus* accessions separated from the larger group of *E. confusus*, *E. peschkovae* and *E. sibiricus* species with the bootstrap support value 87.

3. As for the H subgenome, all studied species showed relation only within the Asian group **H₁**, which is phylogenetically close to *H. jubatum*. Wherein all *E. caninus* clones separated from other species with the bootstrap support value 58.

4. Three of the four *E. peschkovae* clones belonging to the H subgenome were separated from the *E. confusus* clones into a particular clade, which is probably not an accidental event and supports the microevolutionary isolation of the two species. We can suppose an independent origination of the trait «pilose lemmas» in the mountane biotype *E. peschkovae* JTO-1708, which is located in the other separated branch *E. confusus* – *E. sibiricus*.

5. The largest group of **H₁** subgenome comprised all *E. sibiricus* clones except the one from Yakutia–Sakha, and four *E. confusus* clones. This fact can hypothetically explain the high reproductive compatibility of the *E. confusus* BUM-0505 accession when hybridized with the accession *E. sibiricus* BUR-0569 from Buryatia (Agafonov, Gerus, 2009).

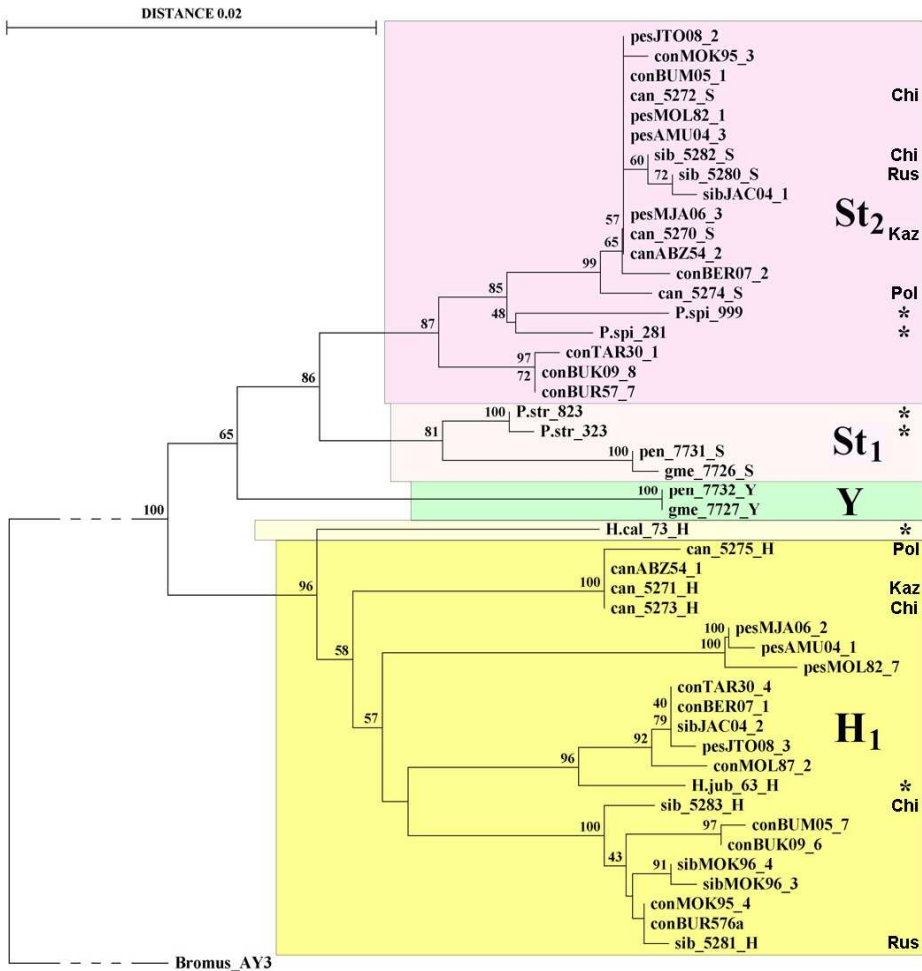


Fig. 2. NJ dendrogram, based on the results of analysis of *GBSSI* gene sequences (only exons 9-14) in accessions *E. confusus*, *E. peschkovae*, *E. sibiricus* compared to reference species from the GenBank NCBI. Monogenomic species that carry subgenomes St and H are marked by asterisks.

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