The version of microevolutionary relationships between the Siberian species *Elymus margaritae*, *E. komarovii*, and *E. transbaicalensis* (Poaceae) according to sequencing of the nuclear gene GBSS1 (waxy)

Alexander Agafonov1*, Maria Emtseva1, Elena Shabanova (Kobozeva)1, and Sergey Asbaganov1

1Central Siberian Botanical Garden SB RAS, Zolotodolinskaya st., 101, Novosibirsk, Russia, 630090

**Abstract.** A comparative study was made of the sequence of the GBSS1 gene fragment in accessions of closely related species *Elymus margaritae*, *E. komarovii*, and *E. transbaicalensis* from Siberia. Nucleotide sequences of the gene were determined for St subgenomes in accessions of *E. margaritae* and *E. komarovii* from classical habitats. The StH-genomic constitution was confirmed, and microevolutionary relationships between species were evaluated by constructing the NJ dendrogram. It was shown that variants of the St subgenomes in accessions *E. margaritae* GUK-1009 and *E. komarovii* AUK-9803 belong to the North American ancestral line St2 of the genus *Pseudoroegneria*, in contrast to accessions *E. margaritae* AUK-0650 and *E. komarovii* GAR-0501. The latter belong to the Asian branch of St1, together with variants of subgenomes in the species *E. gmelinii* and *E. pendulinus*. That is, according to the differentiation levels of the St subgenome, accession *E. margaritae* GUK-1009 (holotype) is significantly distanced from the accession AUK-0650 (paratype), which in turn is close to accessions of *E. komarovii* and *E. transbaicalensis* from East Sayan. According to levels of differentiation of the H subgenome, the studied species did not show noticeable differences.

**1 Introduction**

The species *Elymus margaritae* A.V. Agaf., Kobozeva et B. Salomon was described in collectings from the Altai Republic (holotype: Ust-Koksinsky District, Krasnaya Gora; paratype: Kosh-Agachsky District, Ukok Plateau), but initially these accessions were referred to *E. komarovii* (Nevski) Tzvelev [1]. Before the description of the new species *E. margaritae*, plants were observed in conditions of open ground and climate chamber when generations changing.

* Corresponding author: agalex@mail.ru

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The conclusion was drawn, that specimens of the supposed new species maintained natural morphology, which is significantly different from the typical *E. komarovii* forms. Actual questions when studying a new *E. margaritae* species are its origination, variability, genetic specificity and crossability with other species.

Previously results were presented of the study of GBSS1 gene sequence in *Elymus* species from Siberian and Russian Far East areas to determine their genomic constitution and assess the phylogenetic differentiation [2]. Here the accession numbers of clones in GenBank NCBI are indicated. But no sequences from St genome were revealed in the sets of 8 sequenced clones of both *E. komarovii* GAR-0501 and *E. margaritae* GUK-1009 accessions. Therefore additional procedures of isolation and sequencing of GBSS1 genes from St genomes in these key accessions were performed, because GAR-0501 accession was gathered in the classical habitat of *E. komarovii* [3], and GUK-1009 accession – in the point of collecting of *E. margaritae* holotype [1, 4].

The purpose of this research was to study the relationships of *E. margaritae* with morphologically close *E. komarovii* and *E. transbaicalensis* species, to reveal their specificity and possibility of mutual introgression.

### 2 Materials and Methods

Besides accessions of three mentioned species, the comparative research included clones of previously studied reference species from different regions of Asian Russia, which we assigned to two different groups according to St subgenome classification: *E. kamczadalorum* and *E. jacutensis* (St1), *E. kronokensis* and *E. caninus* (St2) (Table 1). Locations of accessions and authors of collectings were added in the publication [4]. Sequences of GBSS1 gene from species with St, H and Y genomes were drawn from GenBank NCBI (http://www.ncbi.nlm.nih.gov/nuccore) (Table 2). Techniques of preparation of probes and GBSS1 sequences were carried out as described earlier [2]. The construction of dendrograms by Neighbor-Joining method was performed with TREECON ver. 1.3b software [5]. To avoid erroneous results of St clones sequencing in accessions *E. komarovii* GAR-0501 and *E. margaritae* GUK-1009, additional St clones of these accessions were used to construct the dendrogram.

**Table 1.** Clones of *E. margaritae*, *E. komarovii*, *E. transbaicalensis* accessions and 4 reference species of the genus *Elymus*, included in a comparative study of GBSS1 gene sequences.

| Species                          | Accession | Clone (sequence in GenBank NCBI) |
|----------------------------------|-----------|----------------------------------|
| *E. margaritae* A.V. Agaf., Kobozeva et Salomon | GUK-1009  | marGUK09_4-St (MT263972)         |
|                                  |           | marGUK09_1-H (MN164818)          |
| *E. margaritae*                  | AUK-0650  | marAUK50_4-St (MN164819)         |
|                                  |           | marAUK50_2-H (MN164820)          |
| *E. komarovii* (Nevski) Tzvel.   | GAR-0501  | komGAR01_3-St (MT263971)         |
|                                  |           | komGAR01_2-H (MN136107)          |
| *E. komarovii*                   | AUK-9803  | komAUK03_3-St (MN164816)         |
|                                  |           | komAUK03_1-H (MN164817)          |
| *E. transbaicalensis* (Nevski) Tzvel. | GAR-0530 | trnGAR30_4-St (MN164821)         |
|                                  |           | trnGAR30_1-H (MN164822)          |
| *E. kamczadalorum* (Nevski) Tzvel. | KSO-9623 | kamKSO23_1-St (MN126577)         |
|                                  |           | kamKSO23_2-H (MN126576)          |
| *E. jacutensis* (Drob.) Tzvel.   | 12-0135   | jac0443_1-St (MN136106)          |
|                                  |           | jac0443_5-H (MN136105)           |
| *E. kronokensis* (Kom.) Tzvel.   | KES-9603  | kroKES03_2-St (MN126581)         |
|                                  |           | kroKES03_1-H (MN126580)          |
| *E. caninus* (L.) L.             | ABZ-1654  | canABZ54_2-St (MN164839)         |
3 Results and Discussions

The dendrogram built on the basis of nucleotide sequences of GBSSI gene in St and H subgenomes is shown in Fig. 1. It confirms the StH-genomic constitution of studied species. It is shown, that variants of St subgenomes of E. margaritae GUK-1009 and E. komarovii AUK-9803 accessions belong to the North American ancestral line St2 of Pseudoroegneria genus together with reference species E. caninus and E. kronokensis, unlike the accessions of E. margaritae AUK-0650 and E. komarovii GAR-0501. The latter accessions most probably belong to Asian St1 clade with St subgenome variants of E. gmelinii and E. pendulinus species [2]. Studied species showed no noticeable differences on levels of H subgenome differentation, but they confirmed their belonging to the evolutional H1 variant in contrast to the reference E. kamczadalorum and E. jacutensis species, related to H2 variant.

The main differences between subgenome variants St1 and St2 were in 11, 12 and 13 introns of GBSSI gene and are as follows. Clones with St2 variant have insertions of 8 bp (GCCCTCCCTC), 3 bp (CAG), 2 bp (TT), 1 bp (C and A), which are lack in St1, and they don’t have insertions of 3 bp (GTA) and 1 bp (T), which present in St1-subgenomic clones. The two variants of St subgenome also differ from each other by substitutions: GT in St1 was substituted by AG in St1, TAT was substituted by CCG, CT – by TC, GT – by CA, and AT – by CC. In addition, clones with St1 and St2 variants differ by substitutions of single nucleotides: 17 transitions (3 A/G and 14 C/T) and 10 transversions (1 A/T, 5A/C, 2 C/G and 2 G/T). These distinctions allowed clones with St1 and St2 subgenome variants to be separated into different clades.

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

| Species                        | Accession (origin) | Sequence in GenBank NCBI (source*) | Designation on dendrograms |
|-------------------------------|-------------------|----------------------------------|---------------------------|
| *Elymus gmelinii* (Lede.) Tzvel. | PI 499447 China  | St GQ847726^1 Y GQ847727         | gme_7726_S                |
|                               |                   |                                  | gme_7727_Y                |
| *E. pendulinus* (Nevski) Tzvel. | PI 499452 China  | St GQ847731^1 Y GQ847732         | pen_7731_S                |
|                               |                   |                                  | pen_7732_Y                |
| *Pseudoroegneria strigosa* (M.Bieb.) A. Löve | PI 499637 China  | St EU282323^2                  | Ps.str_323                |
|                               |                   |                                  |                           |
| *P. strigosa*                 | PI 531755 China  | St AY360823^3                   | Ps.str 823                |
|                               |                   |                                  |                           |
| *P. spicata* (Pursh) A. Löve  | PI 232117 USA    | St AF079281^4                   | P.spi_281                 |
|                               |                   |                                  |                           |
| *P. spicata*                  | PI 610986 USA    | St AY010999^5                   | P.spi 999                 |
|                               |                   |                                  |                           |
| *Hordeum jubatum* L.          | RJMG 106 USA     | H AY010963^6                   | H.jub 63 H                |
|                               |                   |                                  |                           |
| *H. brevisubulatum* (Trin.) Link | PI 401387 Iran  | H AY010961^3                   | H.bre_61_H                |
|                               |                   |                                  |                           |
| *Bromus tectorum* L.          |                   |                                  |                            |
|                               |                   | AY362757                        | Bromus_AY3                |

^1 [6]; ^2 [7]; ^3 [8]; ^4 [9]; ^5 [10].
The main differences between H and St subgenomes of GBSS1 gene are in the presence of sequences in St-subgenomic clones, which are absent in H-subgenomic carriers: 17 bp (with single distinctions in it between St 1 and St2 subgenomes), 2 bp (TT), 3 bp (AGT) and 4 bp (CATT). H-subgenomic clones also differ from St-subgenomic ones by a number of substitutions of 2, 3, 4, 5 bp, and by 52 single nucleotide substitutions: 32 transitions (16 С/Т and 16 А/G) and 20 transversions (8 А/C, 4 G/T, 5 G/C and 3 А/T).

H-subgenomic clones have several short sequences, identical with St2-subgenomic clones, but differing from St1-subgenomic ones. So, H and St2 subgenomes have sequences 6 bp (GCCTCC), 2 bp (TT) and 1 bp (С and А), which are absent in St1. Also H and St2 subgenomes have substitutions compared to St1 subgenome: GT in Н and St2 substituted by AG in St1, ТАТ – by CCG, CT – by TC, and GT – by СА.

Clones of E. kamczadalorum, E. jacutensis, E. kronokensis and E. caninus with H-subgenome have an insertion of 2 bp in 9 intron, which is absent in all other clones, but differing from St1-subgenomic ones. So, H and St1 subgenomes have sequences 6 bp (GCCTCC), 2 bp (TT) and 1 bp (C and A), which are absent in St1. Also H and St2 subgenomes have substitutions compared to St1 subgenome: GT in H and St2 substituted by AG in St1, TAT – by CCG, CT – by TC, and GT – by CA.

Clones of E. kamczadalorum, E. jacutensis, E. kronokensis and E. caninus with H-subgenome have an insertion of 2 bp in 9 intron, which is absent in all other clones, but presents in Hordeum jubatum clone. H-subgenomic clone of E. caninus have substitutions of 2, 3, 4, 6 bp and two deletions of 4 and 5 bp in 10 intron, which are identical to those of H. jubatum clone and absent in all other clones. This probably influenced the isolation of clone canABZ54_1 with H. jubatum clone into the separate clade within the common clade with H-subgenomic accessions. Clone canABZ54_1 have substitutions of 3, 3 and 4 bp in 12 intron compared to all other clones, among which first two substitutions are identical to those of E. kronokensis clone, what also has been reflected on dendrogram.

Clones of E. gmelinii and E. pendulinus – gme7727_Y and pen7732_Y – have a substitution of 3 bp (GAT) in 12 intron and insertion of 2 bp (CA) in 13 intron, due to what they separated into Y-subgenomic clade.
Thus, according to differentiation levels of the St subgenome, the accession *E. margaritae* GUK-1009 (holotype) is significantly separated from the AUK-0650 accession (paratype), which, in turn, is close to *E. komarovii* and *E. transbaicalensis* accessions from East Sayan.

At the same time our previous research has shown that accession AUK-0650 of *E. margaritae* contains clones of GBSSI gene both with St1 and St2 subgenome variants [2]. This phenomenon demonstrates the appearance of controversial results when comparing data of morphology and molecular genetics. Nevertheless, close clustering of clones of studied species on the dendrogram is consistent with obtained preliminary data about the tendency of species to mutual introgression.

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