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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)

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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 St_2 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 St_1, 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 distanted 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.

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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* (St_1), *E. kronokensis* and *E. caninus* (St_2) (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)

		canABZ54_1-H (MN164840)

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 (Ledeb.) 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[5]	H.jub_63_H
H. brevisubulatum (Trin.) Link	PI 401387 Iran	H AY010961[5]	H.bre_61_H
Bromus tectorum L.		AY362757	Bromus_AY3

[1] [6]; [2] [7]; [3] [8]; [4] [9]; [5] [10].

3 Results and Discussions

The dendrogram built on the basis of nucleotide sequences of *GBSS1* 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 St$_2$ 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 St$_1$ clade with St subgenome variants of *E. gmelinii* and *E. pendulinus* species [2]. Studied species showed no noticeable differences on levels of H subgenome differentiation, but they confirmed their belonging to the evolutional H$_1$ variant in contrast to the reference *E. kamczadalorum* and *E. jacutensis* species, related to H$_2$ variant.

The main differences between subgenome variants St$_1$ and St$_2$ were in 11, 12 and 13 introns of *GBSS1* gene and are as follows. Clones with St$_2$ variant have insertions of 8 bp (GCCTCCTC), 3 bp (CAG), 2 bp (TT), 1 bp (C and A), which are lack in St$_1$, and they doesn’t have insertions of 3 bp (GTA) and 1 bp (T), which present in St$_1$-subgenomic clones. The two variants of St subgenome also differ from each other by substitutions: GT in St$_2$ was substituted by AG in St$_1$, TAT was substituted by CCG, CT – by TC, GT – by CA, and AT – by CC. In addition, clones with St$_1$ and St$_2$ 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 St$_1$ and St$_2$ subgenome variants to be separated into different clades.

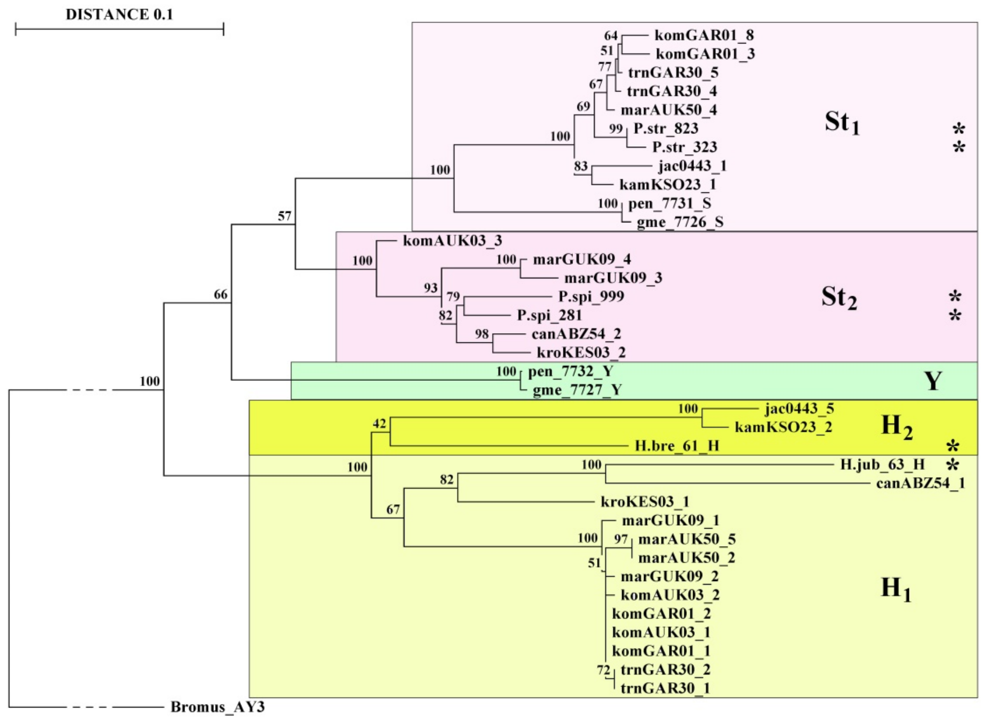

Fig.1. The NJ dendrogram based on the analysis of *GBSS1* gene sequences (exons and introns from 9 through 14) of the Siberian species *E. komarovii, E. transbaicalensis* and *E. margaritae* in comparison with the reference species from the NCBI GenBank. Asterisks indicate species — monogenomic carriers of the St and H subgenomes.

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 St_2 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 C/T and 16 A/G) and 20 tansversions (8 A/C, 4 G/T, 5 G/C and 3 A/T).

H-subgenomic clones have several short sequences, identical with St_2-subgenomic clones, but differing from St_1-subgenomic ones. So, H and St_2 subgenomes have sequences 6 bp (GCCTCC), 2 bp (TT) and 1 bp (C and A), which are absent in St_1. Also H and St_2 subgenomes have substitutions compared to St_1 subgenome: GT in H and St_2 substituted by AG in St_1, 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 *GBSS1* gene both with St_1 and St_2 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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