

Identification of genome compositions in allopolyploid species of the genus *Elymus* (Poaceae: Triticeae) in the Asian part of Russia by CAPS analysis

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Abstract. The genus *Elymus* L., together with wheat, rye, and barley, belongs to the tribe Triticeae. Apart from its economic value, this tribe is characterized by abundance of polyploid taxa formed in the course of remote hybridization. Single-copy nuclear genes are convenient markers for identification of source genomes incorporated into polyploids. In the present work, a CAPS-marker is developed to distinguish basic St, H, and Y genomes comprising polyploid genomes of Asiatic species of the genus *Elymus*. The test is based on electrophoretic analysis of restriction patterns of a PCR-amplified fragment of the gene coding for beta-amylase. There are about 50 *Elymus* species in Russia, and most of them are supposed to possess one of three haplome combinations, StH, StY and StHY. Boreal StH-genomic species endemic for Russia are the least studied. On the basis of nucleotide sequences from public databases, *TaqI* restrictase was selected, as it produced patterns of restriction fragments specific for St, H, and Y haplomes easily recognizable in agarose gel. A sample of 68 accessions belonging to 32 species was analyzed. In 15 species, the earlier known genomic constitutions were confirmed, but in *E. kamoji* this assay failed to reveal the presence of H genome. This unusual H genome was suggested to originate from a different *Hordeum* species. In 16 species, genomic constitutions were identified for the first time. Fifteen accessions from Asian Russia possessed the genomic constitution StStHH, and *E. amurensis*, phylogenetically close to the StY-genomic species *E. ciliaris*, had the genomic constitution StStYY. It is inferred that the center of species diversity of the StH-genomic group is shifted to the north as compared to the center of origin of StY-genomic species, confined to China.

Key words: *Elymus*; taxonomy; allopolyploids; genome constitution; CAPS markers.

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Выявление геномного состава аллополиплоидных видов рода *Elymus* (Poaceae: Triticeae) Азиатской России с помощью CAPS-анализа

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Аннотация. Род *Elymus* L. наряду с пшеницей, рожью и ячменем принадлежит к трибе Triticeae. Помимо своего хозяйственного значения, эта триба характеризуется широким распространением аллополиплоидных таксонов, которые формируются в ходе межвидовой и межродовой гибридизации и последующих преобразований вовлеченных в гибридизацию диплоидных геномов. Для идентификации исходных геномов в составе полиплоидов удобны малокопийные ядерные гены, менее подверженные процессам реорганизации, чем повторенные некодирующие элементы. В настоящей работе разработан удобный CAPS-маркер для различения базисных геномов St, H, Y, входящих в состав азиатских видов рода *Elymus*, с помощью электрофоретического анализа фрагментов рестрикции ПЦР-амплифицированного участка гена, кодирующего β-амилазу. В России распространено около 50 видов *Elymus* предположительно трех гапломных комбинаций: StH, StY и StHY. Наименее изученными остаются бореальные StH-геномные виды – эндемики Российской Федерации. По результатам анализа ранее изученных разными авторами нуклеотидных последовательностей гена β-амилазы была отобрана эндонуклеаза рестрикции *TaqI*, которая имела различающиеся по положению сайты узнавания в составе вышеуказанного фрагмента из геномов St, H и Y. В результате расщепления ПЦР-продукта эндонуклеазой *TaqI* у каждого из исходных гапломов формировался специфичный паттерн фраг-

ментов рестрикции, легко визуализируемый в агарозном геле. Проанализирована выборка из 68 образцов, принадлежащих 32 видам. У 15 видов была подтверждена ранее известная геномная конституция, у *E. kamoji* этот метод не позволил выявить присутствие генома H. Предполагается возможное происхождение данного варианта генома H от другого вида *Hordeum*. У 16 видов геномная конституция определена впервые. Показано, что большинство изученных видов бореальной группы видов из Сибири и Российского Дальнего Востока имеют геномную конституцию StStHH. Исключение составил *E. amurensis*, филогенетически близкий к StY-геномному виду *E. ciliaris* и также имеющий геномный состав StStYY. Сделан вывод, что основное видовое разнообразие StH-геномной группы находится севернее центра происхождения большинства StY-геномных видов рода.

Ключевые слова: *Elymus*; таксономия; аллополиплоиды; геномная конституция; CAPS-маркеры.

Introduction

The genus *Elymus* L. is the largest in the tribe Triticeae Dum. and, according to different estimates, counts from 150 to 200 species (Dewey, 1984; Barkworth, 2000). It is represented only by allopolyploid taxa with genome compositions including several basic genomes (haplomes) in different combinations. The genetic basis of the genus *Elymus* is formed by five haplomes descending from different genera of the tribe Triticeae: (St) *Pseudoroegneria*, (H) *Hordeum*, (P) *Agropyron*, (W) *Astralopyrum*, (Y) donor unknown. Genome constitution was proposed as a stable genetic criterion for taxonomic classification of *Elymus* species (Löve, 1984). Within a relatively short span of time, substantial changes occurred in the taxonomy of the tribe Triticeae on the basis of the genomic system of classification suggested by D.R. Dewey (1984). During the next 20 years, six genera were identified according to variants of genome constitution: *Douglasdeweya* C. Yen, J.L. Yang & B.R. Baum (PPStSt), *Roegneria* C. Koch (StStYY), *Anthosachne* Steudel (StStWWYY), *Kengyia* C. Yen & J.L. Yang (PPStStYY), *Campeiostrachys* Drobov (HHStStYY), and *Elymus* L. (StStHH, StStStHH, StStHHHH).

However, departing from A. Löve's principles, many botanists still attribute several genome combinations to the single genus *Elymus* s. l. With all this, genome constitutions are not yet determined in about 40 % of species (Okito et al., 2009). According to current evidence, 53 species of the genus *Elymus* subdivided into four sections occur in Russia (Tsvelyov, 2008; Tsvelyov, Probatova, 2010). Two of the sections, *Elymus* and *Gouldardia* (Husn.) Tzvelev, contain species with different genomic constitutions, which obviously contradicts the phylogenetic principle of their formulation. We suppose that Russia is home to species with only three haplome combinations: StH, StY, and StHY (Agafonov et al., 2015). Boreal StH-genomic endemics of Russia are less studied. According to the taxonomic system based on the genome constitution, the *Elymus* species should be attributed to three genera: *Elymus*, *Roegneria*, and *Campeiostrachys*. However, in our view, the division of the species inhabiting Russia into three genera is impractical due to the difficulties of morphologic identification of these genera. With all this, taxonomic classification within the genus based on genome constitutions is indispensable for the construction of a phylogenetically oriented taxonomy of the genus.

Earlier, Cleaved Amplified Polymorphic Sequences (CAPS) markers were used to distinguish individual genomes in representatives of the tribe Triticeae (Gostimsky et al., 2005; Li et al., 2007; Hu et al., 2014; Shavrukov, 2016). Some advantages of CAPS markers are their codominance, moderate sensitivity to the amount of genomic DNA, and relatively low cost.

We were first to use CAPS-markers to identify the genomic constitutions of species of the genus *Elymus* (Kobozeva et al., 2017). For this purpose, primers were designed based on the known sequences of the gene coding for β amylase (Mason-Gamer, 2013), which included 38 sequences of haplome St, 23 of haplome H, and 15 of haplome Y, belonging to 24 *Elymus* species. Of them, 14 species had the genomic composition StStHH; 9, StStYY; and 1, StStHHUkUk (*Elytrigia repens*). Variable positions were sought that would discriminate representatives of an individual genome from the other two. Special attention was paid to those genome-specific sequence variants that resulted in appearance/disappearance of recognition sites for restriction endonucleases. It was found that digestion of the PCR products with *TaqI* endonuclease resulted in the formation of genome-specific restriction patterns. In the present work, we apply CAPS analysis to a large sample of *Elymus* species from Asian Russia to reveal their genome constitutions unknown hitherto.

Materials and methods

Plant material included 68 accessions of the species with known (Table 1) and unknown (Table 2) genome constitutions found in Russia. The species nomenclature is given according to N.N. Tsvelyov and N.S. Probatova (2010). The accessions analyzed were received from the scientific collection of biological resources of the Central Siberian Botanic Garden SB RAS "Collections of living plants indoors and outdoors"; their identification numbers are given in Tables 1 and 2. Prefixes correspond to the geographic origin of the accessions.

Total DNA was extracted from 20 mg of dried green matter with the use of NucleoSpin Plant II Kit (Macherey-Nagel, Germany) according to manufacturer's recommendations. Amplification of the β amylase gene fragment was made in a C-1000 thermocycler (Bio-Rad, USA) with the following primers: El_balq_F4 (5'-GGTACCATCGTGGACATTGAA-3') and El_balq_R4 (5'-CTGTACCACCAGTGAATGCC-3') (Kobozeva et al., 2017). The PCR reaction mixture of 15 μ L in volume contained 1 \times buffer for Taq polymerase, 0.2 mM each dNTP, 1.5 mM MgCl₂, 1 μ M each of primers, 20 ng of genomic DNA, and 1 U of HS Taq DNA polymerase (Eurogene, RF). The following settings were used: predenaturation at 94 °C for 4 min; 40 cycles: denaturation at 94 °C for 20 s, primer annealing at 60 °C for 25 s, elongation at 72 °C for 90 s; postextension at 72 °C for 5 minutes. CAPS-analysis (Konieczny, Ausubel, 1993) was made as follows: 8 μ L of the PCR reaction mixture was mixed with MQ-H₂O and *TaqI* buffer up to 1 \times concentration in a volume of 15 μ L, and 1 unit of *TaqI* restrictase (Thermo Scientific, USA) was added. The mixture was incubated at 65 °C for 1 hour and resolved in

Table 1. Accessions of *Elymus* species with known genomic constitutions determined by the classical cytogenetic method

No	<i>Elymus</i> species; accession	Genomic constitutions	Location and collectors
1	<i>E. kamoji</i> ; CCH-1395	StHY (Zhou et al., 1999)	People's Republic of China (PRC), prov. Sichuan, Chengdu outskirts, a forest patch in Panda park, alt. 527 m, N 30°44.253' E 104°8.453' (A. Agafonov, E. Kobozeva)
2	<i>E. kamoji</i> ; SLA-1276	»	Russia, Primorskiy Krai, Khasan raion, Slavyanka Town, coastal meadow, alt. 1 m, N 42°52.101' E 131°22.987' (A. Agafonov)
3	<i>E. dahuricus</i> ; BUD-0781	StHY (Dewey, 1984)	Russia, Republic of Buryatia, Dzhirga raion, Nizhniy Torey village outskirts, the Toreyka River valley, meadow slope, alt. 863 m, N 50°34.567' E 104°52.571' (N. Badmaeva)
4	<i>E. dahuricus</i> ; CHJ-1516	»	People's Republic of China (PRC), Jilin Province, nearby Lake Tsagan-Nur, forest plantation, alt. 138 m, N 45°12.106' E 124°25.074' (S. Asbaganov)
5	<i>E. schrenkianus</i> ; AKA-0702	StHY (Lu, Bothmer, 1992)	Russia, Altai Republic, Kosh-Agach raion, stony meadow, Kalanegir River valley, alt. 2283 m, N 49°37.896' E 88°29.441' (S. Lukjanchikov)
48	<i>E. schrenkianus</i> ; AUK-0652	»	Russia, Altai Republic, Kosh-Agach raion, stony meadow in a brook valley, alt. 2445 m, N 49°30.418' E 88°5.012' (A. Agafonov, B. Salomon)
6	<i>E. pendulinus</i> ; VOK-0738	StY (Jensen, 1990)	Russia, Vladivostok City outskirts, Okeanskaya Station, shrubbery; alt. 7 m, N 43°14.10' E 132°0.19' (A. Agafonov)
7	<i>E. pendulinus</i> ; CHE-1044	»	Russia, Altai Republic, Chermal raion, confluence of the Chermal and Katun Rivers, alt. 434 m, N 51°23.533' E 86°00.197' (E. Kobozeva)
8	<i>E. gmelinii</i> ; AUS-1013	StY (Jensen, Hatch, 1989)	Russia, Altai Republic, Shebalino raion, Ust-Sema settlement outskirts, shingle bank in the Katun River left floodplain, alt. 341 m, N 51°37.620' E 85°45.923' (A. Agafonov, E. Kobozeva)
9	<i>E. sibiricus</i> ; ACH-1601	StH (Dewey, 1974)	Russia, Altai Republic, Chike-Taman Pass, right side of the road, 500 m N of the viewpoint, alt. 1250 m, N 50°38.911' E 86°18.789' (E. Kobozeva)
10	<i>E. caninus</i> ; OSE-1423	StH (Dewey, 1968)	Russia, North Osetia-Alania Republic, Alagir raion, ecological path in the Tseydon River valley, alt. 1951 m, N 42°47.139' E 43°53.605' (A. Agafonov, M. Agafonova)
11	<i>E. caninus</i> ; OSE-1427	»	Russia, North Osetia-Alania Republic, Iraf raion, Stur-Digora village outskirts, Great Caucasus Mt. Range northern slope, the valley of a right Uruk River tributary, alt. 1996 m, N 42°52.898' E 43°35.959' (S. Asbaganov)
12	<i>E. caninus</i> ; UKU-1617	»	Russia, Republic of Bashkortostan, Beloretskiy district, Novoabzakovo village outskirts, dry meadow in a mixed forest, alt. 618 m, N 53°48.718' E 58°40.377' (A. Agafonov, S. Asbaganov)
13	<i>E. gmelinii</i> ; BKA-0962	StY (Jensen, Hatch, 1989)	Russia, Primorskiy Krai, Bolshoy Kamen Town outskirts, alt. 41 m, N 43°7.513' E 132°25.133' (A. Agafonov)
14	<i>E. fedtschenkoi</i> ; KSA-0935	StY (Liu, Dewey, 1983)	Kazakhstan, South Altai Mt. Range, the W principal slope, alt. 1791 m, N 49°05.077' E 86°04.483' (D. Gerus)
49	<i>E. fedtschenkoi</i> ; KME-1729	»	Kazakhstan, Trans-Ili Alatau Mts., Medeu village outskirts, stony highland meadow, alt. 3223 m, N 43°07.101' E 77°06.828' (S. Asbaganov)
50	<i>E. fedtschenkoi</i> ; AUR-1714	»	Russia, Altai Republic, Ulagan raion, the Yarly-Amry River, stony highland meadow, alt. 2180 m, N 50°19.044' E 087°43.049' (E. Kobozeva)
51	<i>E. nevskii</i> ; KME-1728	StY (Dewey, 1980)	Kazakhstan, Trans-Ili Alatau Mts., Medeu village outskirts, stony highland meadow, alt. 3223 m, N 43°07.101' E 77°06.828' (S. Asbaganov)
15	<i>E. fibrosus</i> ; ABZ-1602	StH (Dewey, 1984)	Russia, Republic of Bashkortostan, Beloretsk raion, Novoabzakovo village outskirts, a ground road side, alt. 546 m, N 53°47.845' E 58°37.291' (A. Agafonov, S. Asbaganov)
16	<i>E. mutabilis</i> ; KHA-1210	»	Russia, Krasnoyarskiy Krai, Shushenskoe raion, Shushenskiy Bor pine forest, alt. 495 m, N 52°49.622' E 91°26.609' (S. Asbaganov)
17	<i>E. mutabilis</i> ; ABZ-1607	»	Russia, Republic of Bashkortostan, Beloretsk raion, Novoabzakovo village outskirts, a ground road side, alt. 546 m, N 53°47.845' E 58°37.291' (A. Agafonov, S. Asbaganov)
26	<i>E. abolinii</i> ; BUD-0780	StY (Jensen, 1989)	Russia, Republic of Buryatia, Dzhida raion, Nizhniy Torey village outskirts, the Toreyka River valley, meadowy slope, alt. 863 m, N 50°34.634' E 104°52.781' (N. Badmaeva)
27	<i>E. ciliaris</i> ; VOK-0711	StY (Dewey, 1984)	Russia, Vladivostok City env., between Sanatornaya and Okeanskaya Stations, shrubbery, alt. 4 m, N 43°13.94' E 131°59.95' (D. Gerus, A. Agafonov)
38	<i>E. panormitanus</i> ; H4152*	StY (Lu, Salomon, 1992)	USSR: Ukraine, Crimea
39	<i>E. caucasicus</i> ; H3207*	StY (Jensen, Wang, 1991)	USSR: Armenia, Dilidjan city outskirts
66	<i>E. confusus</i> ; BUM-0505	StH (Lu et al., 1995)	Russia, Republic of Buryatia, Tunka raion, Mondy village outskirts, the Tunka Range southern slope, a stony brook bank, alt. 1738 m, N 51°42.610' E 100°59.967' (D. Gerus, A. Agafonov)
67	<i>E. confusus</i> ; TAR-0730	»	Russia, Republic of Tyva, Tes-Khem raion, Khorumnug-Taiga Mt. Range, Shuurnak-Samagaltay Pass, spruce-larch forest, alt. 1545 m, N 50°36.870' E 95°10.729' (I. Artemov)

Note. The numbering of accessions corresponds to the lane numbering in Fig. 2.

* Accessions kindly provided by Dr. B. Salomon (Swedish University of Agricultural Sciences, Department of Plant Breeding, Alnarp, Sweden).

Table 2. Accessions of *Elymus* species with unknown genomic constitutions collected in Russia

No.	<i>Elymus</i> species; accession	Location and collectors
18	<i>E. uralensis</i> ; UKU-1617	Republic of Bashkortostan, Beloretsk raion, Novoabzakovo village outskirts, dry meadow in a mixed forest, alt. 618 m, N 53°48.718' E 58°40.377' (A. Agafonov, S. Asbaganov)
19	<i>E. viridiglumis</i> ; UKU-1618	Republic of Bashkortostan, Beloretsk raion, Novoabzakovo village outskirts, tall herbage meadow in a birch open stand, alt. 619 m, N 53°48.718' E 58°40.377' (A. Agafonov, S. Asbaganov)
20	<i>E. transbaicalensis</i> ; AKU-0422	Altai Republic, Kosh-Agach raion, 10 km N of Chagan-Uzun village along the Chuya Highway, Kuyaktanar valley, alt. 1815 m, N 50°9.783' E 88°19.054' (A. Agafonov, D. Gerus)
21	<i>E. transbaicalensis</i> ; GAR-0530	Republic of Buryatia, Oka raion, the road to Orlik town, shingle bank of the Gargan River, alt. 1610 m, N 52°05.947' E 100°23.005' (A. Agafonov, D. Gerus)
22	<i>E. margaritae</i> ; GUK-1009	Altai Republic, Ust-Koksa raion, Krasnaya Mt., a complex of screes and highland meadows, alt. 2028 m, N 50°4.495' E 85°13.073' (D. Nikonova, E. Kobozeva)
23	<i>E. margaritae</i> ; AUK-0650	Altai Republic, Kosh-Agach raion, Ukok Plateau, stony meadow in a brook valley, alt. 2438 m, N 49°30.418' E 88°05.012' (A. Agafonov, B. Salomon)
24	<i>E. komarovii</i> ; AKU-0458	Altai Republic, Kosh-Agach raion, 10 km N of Chagan-Uzun village along the Chuya Highway, Kuyaktanar valley, alt. 1815 m, N 50°9.783' E 88°19.054' (A. Agafonov, D. Gerus)
25	<i>E. transbaicalensis</i> ; TUV-9697	Republic of Tyva, Todzha raion, Azas State Nature Reserve, Ilgi-Chul ranger post (D. Shaulo)
28	<i>E. komarovii</i> ; AKT-0417	Altai Republic, Kosh-Agach raion, North-Chuya Range, Aktry Gorge, ground road edge at forest margin, alt. 2061 m, N 50°6.518' E 87°48.193' (A. Agafonov, D. Gerus)
29	<i>E. komarovii</i> ; GAR-0501	Republic of Buryatia, Oka raion, the Oka River valley, forest glade 50 m from the Gargan River mouth, alt. 1607 m, N 52°05.947' E 100°23.005' (A. Agafonov, D. Gerus)
30	<i>E. komarovii</i> ; JPO-1505	Republic of Sakha-Yakutia, Khangalas raion, Pokrovsk Town outskirts, a meadow at the gas station, alt. 131 m, N 61°29.367' E 129°08.225' (E. Kobozeva, S. Asbaganov)
31	<i>E. subfibrosus</i> ; ANA-1118	Chukotskiy Autonomous district, Anadyr' Town outskirts (D. Lysenko)
32	<i>E. subfibrosus</i> ; LEN-1524	Republic of Sakha-Yakutia, Khangalas raion, the nature reserve "Lenskies Stolby", alt. 156 m, N 61°6.370' E 127°21.593' (E. Kobozeva, S. Asbaganov)
33	<i>E. macrourus</i> ; 12-0135	Taymyr Peninsula, the shingle floodplain of the Bolshaya Lesnaya Rassokha River at its mouth, alt. 2 m, N 72°37.363' E 101°17.793' (E. Pospelova)
34	<i>E. jacutensis</i> ; 13-0443	Taymyr Peninsula, the Anabar Plateau margin, Eriehka and Nyamakit-Daldyn Rivers, a small meadow below rocks, alt. 218 m, N 71°15.250' E 105°37.452' (I. Pospelov)
35	<i>E. sajanensis</i> ; ZUN-0502	Republic of Buryatia, Oka raion, Zun-Kholbo village outskirts, alt. 1682 m, N 52°10.092' E 100°57.581' (A. Agafonov, D. Gerus)
36	<i>E. sajanensis</i> ; ART-0202	Altai Republic, Kosh-Agach raion, Chikhacheva Range, shingle bank of a Buguzun River left tributary, alt. 2254 m, N 50°1.914' E 89°23.620' (I. Artemov)
37	<i>E. amurensis</i> ; MES-1111	Primorskiy Krai, Khasan raion, Andreevka village outskirts, meadow patch at a ground road edge, alt. 93 m, N 42°37.045' E 131°8.650' (E. Kobozeva, A. Agafonov)
40	<i>E. transbaicalensis</i> ; AKT-0628	Altai Republic, Kosh-Agach raion, North-Chuya Range, Aktry Gorge, willow thickets at the mountaineering camp, alt. 2118 m, N 50°5.038' E 87°46.820' (A. Agafonov, D. Gerus)
41	<i>E. kronokensis</i> ; BER-0804	Republic of Buryatia, Eravnoe raion, SE of the temporary settlement Ozernyy, larch forest, alt. 1154 m, N 52°58.625' E 111°38.166' (O. Anenkhonov)
42	<i>E. kronokensis</i> ; MMA-1103	Magadan oblast, Madaun village outskirts, a burnt area in the Arman' Rover floodplain, alt. 627 m, N 60°35.861' E 150°40.862' (D. Lysenko)
43	<i>E. kronokensis</i> ; KES-9603	Kamchatka Krai, Bystraya raion, southern slope of a mountain N of Esso village, alt. 627 m, N 55°55.945' E 158°41.275' (A. Agafonov, B. Salomon)
44	<i>E. lenensis</i> ; 12-0125	Taymyr Peninsula, Bolshaya Rassokha and Novaya Rivers, alt. 39 m, N 72°39.613' E 101°17.079' (I. Pospelov)
45	<i>E. kamczadalarum</i> ; KSO-9623	Kamchatka Krai, Elizovo raion, Sosnovka village outskirts, alt. 247 m, N 53°5.046' E 158°17.918' (A. Agafonov, B. Salomon)
46	<i>E. charkeviczii</i> ; KES-9670	Kamchatka Krai, Bystraya raion, Esso village outskirts, ground road margin, alt. 484 m, N 55°55.014' E 158°42.116' (A. Agafonov, B. Salomon)
47	<i>E. charkeviczii</i> ; MSN-1202	Magadan City, Snezhnyy settlement, path at a forest margin, alt. 145 m, N 59°43.466' E 150°52.677' (N. Badmaeva)
52	<i>E. lenensis</i> ; LEN-1520	Republic of Sakha (Yakutia), Khangalas raion, meadow slope at the Lena River right bank, alt. 114 m, N 61°6.369' E 127°21.593' (E. Kobozeva, S. Asbaganov)

Table 2 (end)

No.	<i>Elymus</i> species; accession	Location and collectors
53	<i>E. lenensis</i> ; ALD-1539-3	Republic of Sakha (Yakutia), Aldan raion, the Aldan River bank, shrubbery at a sandy bank, alt. 228 m, N 58°40.878' E 128°33.081' (E. Kobozeva, S. Asbaganov)
54	<i>E. kronokensis</i> ; KRT-1611	Krasnoyarsk Kray, Evenk raion, Tura village outskirts, the Nizhnyaya Tunguska River, path side in a larch forest, alt. 169 m, N 64°16.478' E 100°16.445' (L. Krivobokov)
55	<i>E. kronokensis</i> ; 12-0137	Taymyr Peninsula, the Bolshaya Rassokha River bank bluff, alt. 2 m, N 72°35.808' E 101°15.900' (E. Pospelova)
56	<i>E. kronokensis</i> ; TAL-0602	Altai Republic, Kosh-Agach raion, the Taldura River valley, larch forest on a mountain slope, alt. 2095 m, N 49°57.472' E 87°57.552' (D. Gerus, A. Agafonov)
57	<i>E. subfibrosus</i> ; KRT-1612	Krasnoyarsk Kray, Evenk raion, Tura village, a ground road edge, alt. 309 m, N 64°16.920' E 100°14.880' (L. Krivobokov)
58	<i>E. subfibrosus</i> ; JRO-1733	Republic of Sakha (Yakutia), Tompo raion, the Kolyma Riad, Verkhoianskiy Mt. Range southern spurs, the Rosomakha River valley, alt. 460 m, N 63°2.879' E 137°52.610' (N. Badmaeva)
59	<i>E. jacutensis</i> ; ALU-1711	Altai Republic, Ulagan raion, herbaceous meadow at the Chulyshman River left bank under Katu-Yaryk Pass, alt. 733 m, N 50°55.497' E 088°12.226' (E. Kobozeva)
60	<i>E. jacutensis</i> ; GAN-1516	Altai Republic, Chemal raion, Anos village outskirts, slope above the Anos River left bank at the bridge, alt. 380 m, N 51°30.014' E 85°57.160' (E. Kobozeva)
61	<i>E. jacutensis</i> ; ALD-1541	Republic of Sakha (Yakutia), Aldan raion, the Aldan River bank, shrubbery at a sandy bank, alt. 228 m, N 58°40.878' E 128°33.081' (E. Kobozeva, S. Asbaganov)
62	<i>E. macrourus</i> ; MTE-1210	Magadan oblast, Tenka raion, roadside at a mixed forest margin, alt. 970 m, N 60°26.034' E 150°58.558' (N. Badmaeva)
63	<i>E. macrourus</i> ; LEN-1524_1	Republic of Sakha (Yakutia), Khangalas raion, meadow slope at the Lena River right bank, alt. 114 m, N 61°6.369' E 127°21.593' (E. Kobozeva, S. Asbaganov)
64	<i>E. turuchanensis</i> ; KRE-1440	Krasnoyarsk Kray, Turukhansk raion, Bor village, Yenisey River sandy bank, alt. 30 m, N 61°36.265' E 90°0.143' (M. Lomonosova)
65	<i>E. peschkovae</i> ; MJA-1106	Magadan oblast, Khasyn raion, Yablonovyy Pass, floodplain meadow at a road, alt. 755 m, N 60°19.467' E 151°10.540' (D. Lysenko)
68	<i>E. peschkovae</i> ; AMU-8804	Amur oblast, 50 km downstream the Gilyuy River from Tynda City, sandy bank, alt. 445 m, N 54°56.216' E 125°21.854' (O. Potemkin)

Note. The numbering of accessions corresponds to the lane numbering in Fig. 2.

1.7 % agarose gel in TAE buffer. Molecular weight marker: 100+ bp DNA Ladder (Evrogen, RF).

Results and discussion

The comparative analysis of sequences of the β amylase gene published in R. Mason-Gamer (2013) showed that the studied fragment of Y genome of about 1100 bp in length did not contain recognition sites for *TaqI* endonuclease, while St genome contained one recognition site in the fragment of interest at a distance of about 170 bp from the primer El_balq_R4. The same site was present in some H genomes; besides, all H genomes contained a recognition site at a distance of about 280 bp from the primer El_balq_F4. Visualized on gels, restriction patterns of the studied genomes were differentiated according to the lengths of the longest fragments: H genome was distinguished by the presence of a band at about 650 bp; St genome, 930 bp; and Y genome, 1100 bp (Fig. 1).

Restriction patterns of the CAPS marker employed were studied in 68 accessions (see Tables 1, 2). Electrophoretic patterns formed after *TaqI* digestion are shown in Fig. 2. Based on the results of CAPS analysis, genomic constitutions of the accessions studied were determined. Previously known genomic constitutions were confirmed in 15 species of 16, *E. kamoji* being the only exception. In 16 species, genomic compositions were determined for the first time: 15 of them had the

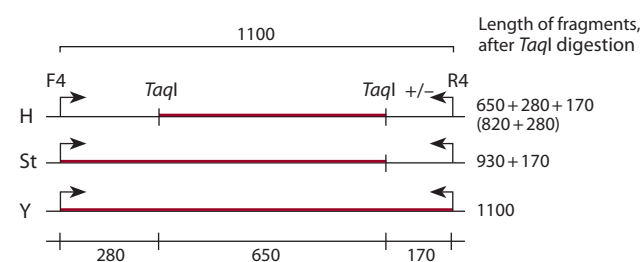


Fig. 1. Map of recognition sites for *TaqI* endonuclease in the β amylase gene fragment amplified from the basic haplomes constituting the polyploid *Elymus* genome.

genomic constitution StStHH, and one species, *E. amurensis*, had StStYY (Table 3). However, some limitations of the approach were met. For example, in two accessions of *E. kamoji* CAPS-analysis revealed only two haplomes, St and Y (Fig. 2, lanes 1 and 2), whereas it is known to be hexaploid according to the number of chromosomes, thus, it should contain three basic genomes (haplomes). It is improbable that the absence of restriction fragments corresponding to haplome H was due to incomplete digestion. Since all representatives of the genus contain St haplome, possessing a recognition site for *TaqI* endonuclease, the presence of St-specific fragments serves as an

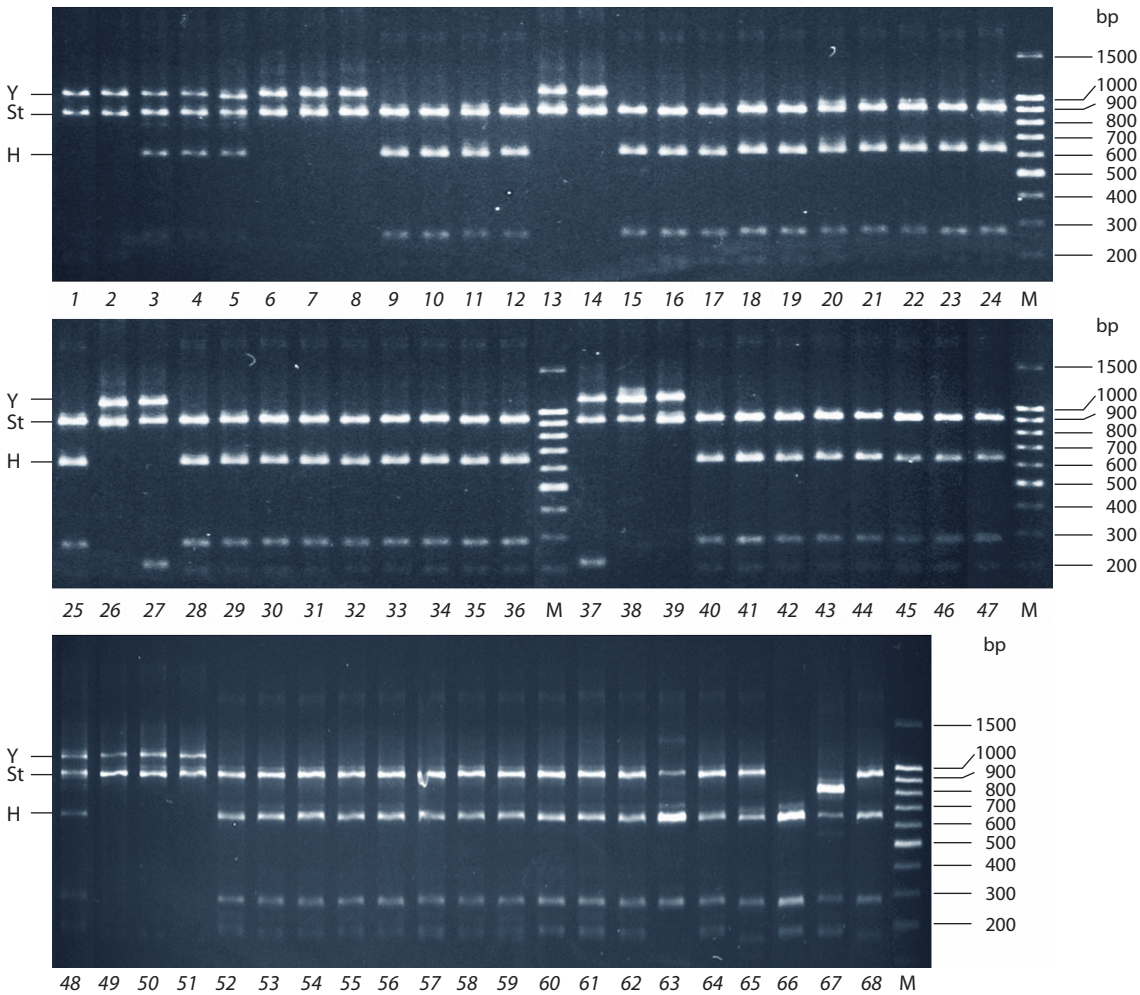


Fig. 2. Polymorphism of restriction fragment lengths (CAPS) after *TaqI* digestion of the PCR-amplified fragment of the β amylase gene in species of the genus *Elymus*.

Lane numbers correspond to the accession numbering in Tables 1 and 2. M – molecular weight ladder: 100+bp DNA Ladder (Evrogen).

Table 3. The list of boreal *Elymus* species in Asian Russia in which genome constitutions (GC) were determined by the CAPS method

No.	<i>Elymus</i> species	Number of accessions studied	GC	No.	<i>Elymus</i> species	Number of accessions studied	GC
1	<i>E. amurensis</i>	1	StY	9	<i>E. margaritae</i>	2	StH
2	<i>E. charkeviczii</i>	2	StH	10	<i>E. sajanensis</i>	2	StH
3	<i>E. jacutensis</i>	4	StH	11	<i>E. subfibrosus</i>	4	StH
4	<i>E. kamczadalarum</i>	1	StH	12	<i>E. transbaicalensis</i>	4	StH
5	<i>E. komarovii</i>	4	StH	13	<i>E. uralensis</i>	1	StH
6	<i>E. kronokensis</i>	6	StH	14	<i>E. viridiglumis</i>	1	StH
7	<i>E. lenensis</i>	3	StH	15	<i>E. turuchanensis</i>	1	StH
8	<i>E. macrourus</i>	3	StH	16	<i>E. peschkovae</i>	2	StH

internal control for the completeness of hydrolysis. According to the classification system based on genomic compositions, *E. kamoji* belongs to the genus *Campeiosiachys* (Baum et al., 2011) which embraces species with the genomic composition StHY. In fact, we performed a cytological analysis, which

showed that both accessions of *E. kamoji* possessed the chromosome number $2n = 42$, corresponding to hexaploid. The presence of the H genome lacking two recognition sites for *TaqI* endonuclease in *E. kamoji* brings its origin into a question. It is not inconceivable that different representatives of

the genus received their H genomes from different ancestor species, which agrees with the assumption of polyphyly of the donors of basic haplomes (Mason-Gamer, 2013).

An interesting pattern of restriction fragments was observed in two accessions of *E. confusus* (see Fig. 2, lanes 66 and 67), with the genome constitution formerly determined as StStHH (Lu et al., 1995). In accession TAR-0730 (see Fig. 2, lane 67), the longer fragment corresponding to the allele from St genome is truncated, possibly, as the result of a deletion or acquisition of an additional restriction site. The spectrum of restriction fragments in accession BUM-0505 (see Fig. 2, lane 66) lacks the fragment of about 930 bp characteristic of St genome, while the smaller fragment of about 170 bp corresponding to this haplome is clearly seen. This phenomenon might be attributed to a mutation in the St genome of the accession, for example, appearance of a recognition site for *TaqI*. Another possibility is a recombination and/or introgression between genomes St and H in the course of intense microevolutionary processes indirectly confirmed by the high morphologic variability within this species.

According to the CAPS analysis undertaken in the present work, almost all newly studied accessions of the boreal group of species from Siberia and Russian Far East have the StH genomic composition. One exception was *E. amurensis*, phylogenetically close to the StY-genomic species *E. ciliaris* and possessing the genome composition StY. This implies that the center of species diversity of the Asiatic StH-genome group is shifted to the north as compared to that of the StY-genome group, which is considered to be situated in China (Lu, Salomon, 1992). In this context, it is worth noting that in North America, the genus *Elymus* is also represented mainly by StH-genome species (except for *Elymus californicus* with unclear origin) (Mason-Gamer, 2001). Besides, in that territory a number of adventive Asiatic StHY- and StY-genome species were found (Barkworth et al., 2007).

In general, the applied method showed a high accuracy: in the present work earlier known genome constitutions were confirmed by CAPS analysis in 15 *Elymus* species of 16. For 10 species, the genomic composition newly determined by CAPS analysis as StH, was independently corroborated by sequencing of a cloned fragment of the *GBSSI* (*waxy*) gene (Kobozeva et al., 2018; Agafonov et al., 2019). It should be noted that the sequencing of DNA from polyploid species has a disadvantage, as it is rather laborious, requiring additional gene cloning manipulations.

Conclusion

The main advantage of CAPS markers is the ease of their methodic implementation, which permits one to analyze many specimens with extensive morphologic and genetic variability from broad ranges. The present work involves CAPS analysis with the use of a fragment of the gene for β amylase and demonstrates rather good predictive power of the method. However, it should be kept in mind that no molecular marker taken by itself can unambiguously identify a genome or species; it serves as a marker, not diagnostic. Therefore, the development of additional simple and accessible approaches for genome identification in new and poorly studied biotypes from local habitats remains vital.

References

- Agafonov A.V., Asbaganov S.V., Shabanova (Kobozeva) E.V., Morozov I.V., Bondar A.A. Genome constitution and differentiation of subgenomes in Siberian and Far Eastern endemic species of the genus *Elymus* (Poaceae) according to the sequencing of the nuclear gene *waxy*. *Vavilovskii Zhurnal Genetiki i Selekcii* = Vavilov Journal of Genetics and Breeding. 2019;23(7):817-826. DOI 10.18699/VJ19.555. (in Russian)
- Agafonov A.V., Kobozeva E.V., Asbaganov S.V., Shmakov N.A. Current achievements and prospects of construction of a phylogenetically oriented taxonomy of the genus *Elymus* (Poaceae: Triticeae). Proceedings of the 14th International Scientific and Practical Conference "Problems of Botany of Southern Siberia and Mongolia". Barnaul. 2015;314-322. (in Russian)
- Barkworth M.E. Changing perceptions of the Triticeae. In: Jacobs S.W.L., Everett J. (Eds.). *Grasses: Systematics and Evolution*. Melbourne: CSIRO, 2000;110-120.
- Barkworth M.E., Cambell J.J.N., Salomon B. *Elymus* L. In: Barkworth M.E., Capels K.M., Long S., Anderton L.K., Piep M.B. (Eds.). *Flora of North America*. New York; Oxford: Oxford University Press, 2007;24:288-343.
- Baum B.R., Yang J.-L., Yen C., Agafonov A.V. A taxonomic revision of the genus *Campeiostrachys* Drob. J. Syst. Evol. 2011;49(2):146-159.
- Dewey D.R. Synthetic *Agropyron-Elymus* hybrids. III. *Elymus canadensis* × *Agropyron caninum*, *A. trachycaulum* and *A. striatum*. *Amer. J. Bot.* 1968;55:1133-1139.
- Dewey D.R. Cytogenetics of *Elymus sibiricus* and its hybrids with *Agropyron tauri*, *Elymus canadensis* and *Agropyron caninum*. *Bot. Gaz.* 1974;135:80-87.
- Dewey D.R. Cytogenetics of *Agropyron ugamicum* and six of its interspecific hybrids. *Bot. Gaz.* 1980;141:305-312.
- Dewey D.R. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson J.P. (Ed.). *Gene Manipulation in Plant Improvement*. New York: Plenum Publ. Corp., 1984;209-279.
- Gostimsky S.A., Kokaeva Z.G., Kononov F.A. Studying plant genome variation using molecular markers. *Russ. J. Genet.* 2005;41(4):378-388. DOI 10.1007/s11177-005-0101-1.
- Hu C.Y., Tsai Y.Z., Lin S.F. Development of STS and CAPS markers for variety identification and genetic diversity analysis of tea germplasm in Taiwan. *Bot. Stud.* 2014;55:12. DOI 10.1186/1999-3110-55-12.
- Jensen K.B. Cytology, fertility, and origin of *Elymus abolinii* (Drob.) Tzvelev and its F₁ hybrids with *Pseudoroegneria spicata*, *E. lancaolatus*, *E. dentatus* ssp. *ugamicus*, and *E. drobovii* (Poaceae: Triticeae). *Genome*. 1989;32:468-474. DOI 10.1139/g89-470.
- Jensen K.B. Cytology and morphology of *Elymus pendulinus*, *E. pendulinus* ssp. *multiculmis*, and *E. parviglume* (Poaceae: Triticeae). *Bot. Gaz.* 1990;151(2):245-251.
- Jensen K.B., Hatch S. Genome analysis, morphology, and taxonomy of *Elymus gmelinii* and *E. strictus* (Poaceae: Triticeae). *Bot. Gaz.* 1989;150(1):84-92.
- Jensen K.B., Wang R.R.C. Cytogenetics of *Elymus caucasicus* and *Elymus longearistatus* (Poaceae: Triticeae). *Genome*. 1991;34:860-867.
- Kobozeva E.V., Asbaganov S.V., Agafonov A.V. Genome composition and assessment of the divergence between Russian boreal species in the genus *Elymus* (Poaceae) detected on the basis of sequencing of the nuclear gene *GBSSI*. In: *Prospects of Development and Challenges of Modern Botany*. BIO Web Conf. 2018;11.00023. DOI 10.1051/bioconf/20181100023.
- Kobozeva E.V., Mglinets A.V., Agafonov A.V. Identification of the genomic composition in allopolyploid species of the genus *Elymus* (Poaceae: Triticeae) with CAPS-analysis. In: Proceedings of the 6th International Conference "Issues in the Study of the Vegetation Cover in Siberia". Tomsk. 2017;155-157. DOI 10.17223/9785946216371/51. (in Russian)

- Konieczny A., Ausubel F.M. A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. *Plant J.* 1993;4(2):403-410.
- Li X.-M., Lee B.S., Mammadov A.C., Koo B.-C., Mott I.W., Wang R.R.C. CAPS markers specific to Eb, Ee, and R genomes in the tribe Triticeae. *Genome.* 2007;50:400-411.
- Liu C.W., Dewey D.R. The genome constitution of *Elymus fedtschenkoi*. *Acta Genet. Sinica.* 1983;10:20-27.
- Löve A. Conspectus of the Triticeae. *Feddes Repert.* 1984;95:425-521.
- Lu B.-R., Bothmer R. von. Interspecific hybridization with *Elymus himalayanus* and *E. schrenkianus*, and other *Elymus* species (Triticeae: Poaceae). *Genome.* 1992;35:230-237.
- Lu B.-R., Salomon B. Differentiation of the SY genomes in Asiatic *Elymus*. *Hereditas.* 1992;116:121-126.
- Lu B.-R., Salomon B., Bothmer R. von. Interspecific hybridization with *Elymus confusus* and *E. dolichaterus*, and their genomic relationships (Poaceae: Triticeae). *Plant Syst. Evol.* 1995;197:1-17.
- Mason-Gamer R.J. Origin of North American *Elymus* (Poaceae: Triticeae) allotetraploids based on granule-bound starch synthase gene sequences. *Syst. Bot.* 2001;26:757-768.
- Mason-Gamer R.J. Phylogeny of a genomically diverse group of *Elymus* (Poaceae) allopolyploids reveals multiple levels of reticulation. *PLoS One.* 2013;8(11):e78449. DOI 10.1371/journal.pone.0078449.
- Okito P., Mott I.W., Wu Y., Wang R.R. A Y genome specific STS marker in *Pseudoroegneria* and *Elymus* species (Triticeae: Gramineae). *Genome.* 2009;52(4):391-400.
- Shavrukov Y.N. CAPS markers in plant biology. *Russ. J. Genet.: Appl. Res.* 2016;6(3):279-287. DOI 10.1134/S2079059716030114.
- Tsvelyov N.N. On the genus *Elymus* (Poaceae) in Russia. *Botanicheskiy Zhurnal = Botanical Journal.* 2008;93(10):1587-1596. (in Russian)
- 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. In: V.L. Komarov Memorial Lectures. Vladivostok: Dalnauka Publ., 2010;57:5-102. (in Russian)
- Zhou Y.-H., Wu B.-H., Fu T.-H., Zheng Y.-L. Morphology, fertility and cytogenetics of intergeneric hybrid between *Roegneria kamoji* Ohwi and *Dasyphyrum villosum* (L.) Candargy (Poaceae: Triticeae). *J. Syst. Evol.* 1999;37(2):125-130.

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