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

A.V. Agafonov¹, E.V. Shabanova (Kobozeva)¹, S.V. Asbaganov¹, A.V. Mglinets², V.S. Bogdanova²

¹ Central Siberian Botanical Garden of Siberian Branch of the Russian Academy of Sciences, Russia, Novosibirsk, Russia
² Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

**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, *Taq*I 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 StSTHY. 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

А.В. Агафонов¹, Е.В. Шабанова (Кобозева)¹, С.В. Асбаганов¹, А.В. Мглинец², В.С. Богданова²

¹ Центральный сибирский ботанический сад Сибирского отделения Российской академии наук, Новосибирск, Россия
² Федеральный исследовательский центр Институт цитологии и генетики Сибирского отделения Российской академии наук, Новосибирск, Россия

**Аннотация.** Род *Elymus* L. наряду с пшеницей, рожью и ячмением принадлежит к трибе Triticeae. Помимо своего хозяйственного значения, эта триба характеризуется широким распространением аллополиплоидных таксонов, которые формируются в результате между видовой и межродовой гибридизации и последующих преобразований включенных 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 гапломерных 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 *Taq*I, которая имела различающиеся по положению сайты узнавания в составе вышеуказанного фрагмента из геномов St, H и Y. В результате расщепления ПЦР-продукта эндонуклеазой *Taq*I у каждого из исходных гапломеров формировался специфический паттерн фраг-
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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 allopoloid 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 (PPSStl), *Roegneria* C. Koch (StStYY), *Anthosachne* Steudel (StStWWYY), *Kengylia* C. Yen & J.L. Yang (PPSStlYY), *Campeiostachys* Drobow (HHlStlYY), and *Elymus* L. (StStHl, StStlStHl, StStlHHH).

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 *Goulardia* (Hun.). 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 *Campeiostachys*. 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 (Gostinsky 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 StSSHH; 9, StStYY; and 1, StStHlUk (*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_balg_F4 (5’-GGTACCACGCTGGGACATTGGA-3’) and El_balg_R4 (5’-CTGTACCCACACTGGAATGCC-3’) (Kobozeva et al., 2017). The PCR reaction mixture of 15 μL in volume contained 1× buffer for *Taq* polymerase, 0.2 mM each dNTP, 1.5 mM MgCl₂, 1 μM 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 μL of the PCR reaction mixture was mixed with MQ-H₂O and *Taq* buffer up to 1× concentration in a volume of 15 μL, and 1 unit of *Taq* restricctase (Thermo Scientific, USA) was added. The mixture was incubated at 65 °C for 1 hour and resolved in

Ключевые слова: *Elymus*; таксономия; аллополиплоиды; геномная конституция; CAPS-маркеры.
Table 1. Accessions of Elymus species with known genomic constitutions determined by the classical cytogenetic method

| No  | Elymus species; accession | Genomic constitutions | Location and collectors                                                                                                                                 |
|-----|--------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1   | *E. kamoji*; CCH-1395    | StHY                  | 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   | *E. kamoji*; 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   | *E. dahuricus*; BUD-0781  | StHY                  | Russia, Republic of Buryatia, Dzhibira 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   | *E. dahuricus*; CHU-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   | *E. schrenkianus*; AKA-0702 | StHY                 | Russia, Altai Republic, Kosh-Agach raion, stony meadow, Kalganir River valley, alt. 2283 m, N 49°37.896'E 88°29.441' (S. Lukjanchikov)                      |
| 6   | *E. schrenkianus*; AUK-0652 |                       | Russia, Altai Republic, Kosh-Agach raion, stony meadow in a brook valley, alt. 2445 m, N 49°30.418'E 88°50.012' (A. Agafonov, B. Salomon)                        |
| 7   | *E. pendulinus*; VOK-0738 | STY                   | Russia, Vladivostok City outskirts, Okeanskaia Station, shrubbery; alt. 7 m, N 43°14.10'E 132°0.19' (A. Agafonov)                                        |
| 8   | *E. pendulinus*; CHE-1044 |                       | Russia, Altai Republic, Chemal raion, confluence of the Chemal and Katun Rivers, alt. 434 m, N 51°23.533'E 86°00.197' (E. Kobozeva)                             |
| 9   | *E. gmelinii*; AUS-1013   | STY; 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) |
| 10  | *E. sibiricus*; OCE-1423  | STH                   | 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  | *E. sibiricus*; OCE-1427  |                       | Russia, North Osetia-Alania Republic, Iraf raion, Styr-Digora village outskirts, Great Caucasus Mt. Range, a road in the valley of a right Uruk River tributary, alt. 1996 m, N 42°52.898'E 43°35.959' (S. Asbaganov) |
| 12  | *E. caucasicus*; UKU-1617 |                       | Russia, Bashkorstotan, Beloretsky district, Novoabzakovo village outskirts, dry meadow in a mixed forest, alt. 618 m, N 53°48.718'E 58°40.377' (A. Agafonov, E. Kobozeva) |
| 13  | *E. gmelinii*; BKA-0962   | STY; Hatch, 1989      | Russia, Primorskiy Krai, Bolshoy Kamen Town outskirts, alt. 41 m, N 43°7.513'E 132°25.133' (A. Agafonov)                                               |
| 14  | *E. fedtschenkoi*; KSA-0935 | STY                 | Kazakhstan, South Altai Mt. Range, the W principal slope, alt. 1791 m, N 49°05.077'E 86°04.483' (D. Gerus)                                             |
| 15  | *E. fedtschenkoi*; KM-1729 |                       | Kazakhstan, Trans-III Alatay Mts., Medeu village outskirts, stony highland meadow, alt. 3223 m, N 43°07.101'E 77°06.828' (S. Asbaganov)                  |
| 16  | *E. fedtschenkoi*; AUR-1714 |                       | Russia, Altai Republic, Ulagan raion, the Yarly-amy River, stony highland meadow, alt. 2180 m, N 50°19.044'E 087°43.049' (E. Kobozeva)                       |
| 17  | *E. nevski*; KME-1728     | STY                   | Kazakhstan, Trans-III Alatay Mts., Medeu village outskirts, stony highland meadow, alt. 3223 m, N 43°07.101'E 77°06.828' (S. Asbaganov)                  |
| 18  | *E. fitraeus*; ABZ-1602   | STH                   | Russia, Republic of Bashkorstotan, Beloretsk region, Novoabzako village outskirts, a road in the valley, alt. 456 m, N 53°47.845'E 58°37.291' (A. Agafonov, S. Asbaganov) |
| 19  | *E. mutabilis*; KHA-1210  |                       | Russia, Krasnoyarskiy Krai, Shushenskoe raion, Shushenskiy Bor pine forest, alt. 495 m, N 52°49.622'E 91°25.133' (A. Agafonov)                        |
| 20  | *E. mutabilis*; ABZ-1607  |                       | Russia, Bashkorstotan, Beloretsky district, Novoabzako village outskirts, a road in the valley, alt. 456 m, N 53°47.845'E 58°37.291' (A. Agafonov, S. Asbaganov) |
| 21  | *E. abolinii*; BUD-0780   | STY                   | Russia, Republic of Buryatia, Dzhiba raion, Nizhiy Torey village outskirts, the Toreyka River valley, meadow slope, alt. 863 m, N 50°34.634'E 104°52.781' (N. Badmaeva) |
| 22  | *E. ciliaris*; VOK-0711   | STY                   | Russia, Alagir raion, ecological path in the Tseydon River valley, meadow slope, alt. 863 m, N 50°34.634'E 104°52.781' (N. Badmaeva)                  |
| 23  | *E. panormitana*; H4152*  | STY                   | USSR: Crimea, Severomorsk, Altai Republic, Ust-Sima settlement outskirts, a stony brook bank, alt. 1980 m, N 53°48.718'E 58°40.377' (A. Agafonov, S. Asbaganov) |
| 24  | *E. caucasicus*; H3207*   | STY; Wang, 1991       | USSR: Armenia, Dilijan city outskirts                                                                                                                                 |
| 25  | *E. confusus*; BUM-0505   | STY                   | Russia, Republic of Buryatia, Tunka raion, Mondy village outskirts, Tunka Range southern slope, a stony brook bank, alt. 1738 m, N 51°42.610'E 100°59.967' (D. Gerus, A. Agafonov) |
| 26  | *E. confusus*; TAR-0730   |                       | Russia, Republic of Tyva, Tes-Khem raion, Khorumnuig-Taiga Mt. Range, Shuurmak-Samagaltay Pass, spruce-larch forest, alt. 1545 m, N 50°36.870'E 95°10.729' (I. Artemov) |
Table 2. Accessions of *Elymus* species with unknown genomic constitutions collected in Russia

| No. | *Elymus* species; accession | Location and collectors |
|-----|-----------------------------|------------------------|
| 18  | *E. uralensis*; UKU-1617     | Republic of Bashkortostan, Beloretsk raion, Novoobazakovo village outskirts, dry meadow in a mixed forest, alt. 618 m, N 53°48.718' E 58°40.377' (A. Agafonov, S. Asbaganov) |
| 19  | *E. virigilumis*; UKU-1618   | Republic of Bashkortostan, Beloretsk raion, Novoobazakovo 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  | *E. transbaicalensis*; AKU-0422 | Altai Republic, Kosh-Agach raion, 10 km N of Chagan-Uzun village along the Chuya Highway, Kuyuktanar valley, alt. 1815 m, N 50°9.783' E 88°19.054' (A. Agafonov, D. Gerus) |
| 21  | *E. transbaicalensis*; 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  | *E. marginata*; GUK-1009     | Altai Republic, Ust-Koksra raion, Krasnaya Mt., a complex of screes and highland meadows, alt. 2028 m, N 50°4.945' E 85°13.073' (D. Nikonova, E. Kobozeva) |
| 23  | *E. marginata*; AUK-0650     | Altai Republic, Kosh-Agach raion, Ulok Plateau, stony meadow in a brook valley, alt. 2438 m, N 49°30.418' E 88°05.012' (A. Agafonov, B. Salomon) |
| 24  | *E. komarovi*; AKU-0458      | Altai Republic, Kosh-Agach raion, 10 km N of Chagan-Uzun village along the Chuya Highway, Kuyuktanar valley, alt. 1815 m, N 50°9.783' E 88°19.054' (A. Agafonov, D. Gerus) |
| 25  | *E. transbaicalensis*; TUV-9697 | Republic of Tyva, Todzha raion, Azas State Nature Reserve, Ilgi-Chul ranger post (D. Shaulo) |
| 26  | *E. komarovi*; 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°48.192' (A. Agafonov, D. Gerus) |
| 27  | *E. komarovi*; 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) |
| 28  | *E. komarovi*; 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) |
| 29  | *E. subfibrosus*; ANA-1118   | Chukotskiy Autonomous district, Anadyr' Town outskirts (D. Lysenko) |
| 30  | *E. subfibrosus*; LEN-1524   | Republic of Sakha-Yakutia, Khangalas raion, the nature reserve "Lenskie Stolby", alt. 156 m, N 61°3.670' E 127°21.593' (E. Kobozeva, S. Asbaganov) |
| 31  | *E. macrorus*; 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) |
| 32  | *E. jacutensis*; 13-0443      | Taymyr Peninsula, the Anabar Plateau margin, Eriechka and Nyamakit-Daldyn Rivers, a small meadow below rocks, alt. 218 m, N 71°15.250' E 105°37.452' (E. Pospelova) |
| 33  | *E. sajanensis*; 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) |
| 34  | *E. sajanensis*; 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) |
| 35  | *E. amurensis*; MES-1111     | Primorsky Krai, Khasan raion, Andreevka village outskirts, meadow patch at a ground road edge, alt. 93 m, N 42°37.015' E 131°8.650' (E. Kobozeva, A. Agafonov) |
| 36  | *E. transbaicalensis*; AKT-0628 | Altai Republic, Kosh-Agach raion, North-Chuya Range, Aktry Gorge, willow thickets at the mountaineering camp, alt. 2118 m, N 50°3.038' E 87°46.820' (A. Agafonov, D. Gerus) |
| 37  | *E. kronokensis*; BER-0804   | Republic of Buryatia, Eravnoe raion, SE of the temporary settlement Ozerny, larch forest, alt. 1154 m, N 52°58.625' E 111°38.166' (O. Anenkhnov) |
| 38  | *E. kronokensis*; 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) |
| 39  | *E. kronokensis*; 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) |
| 40  | *E. lenensis*; 12-0125       | Taymyr Peninsula, Bolshaya Rassokha and Novaya Rivers, alt. 39 m, N 72°39.613' E 101°17.079' (I. Pospelov) |
| 41  | *E. kamczadalonorum*; KSO-9623 | Kamchatka Krai, Elizovo raion, Zosnovskaya village outskirts, alt. 247 m, N 53°3.046' E 158°17.918' (A. Agafonov, B. Salomon) |
| 42  | *E. charkevicii*; 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) |
| 43  | *E. charkevicii*; MSN-1202   | Magadan City, Snezhny settlement, path at a forest margin, alt. 145 m, N 59°43.466' E 150°52.677' (N. Badmaeva) |
| 44  | *E. lenensis*; 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) |

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Table 2 (end)

| No. | Elymus species; accession | Location and collectors |
|-----|--------------------------|-------------------------|
| 53  | *E. lenensis*; 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  | *E. kronskenoski*; KRT-1611 | Krasnoyarsk Krai, 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  | *E. kronskenoski*; 12-0137 | Taymyr Peninsula, the Bolsheya Rassokha River bank bluff, alt. 2 m, N 72°35.808' E 101°15.900' (E. Pospelova) |
| 56  | *E. kronskenoski*; TAL-0602 | Altai Republic, Kosh-Agach raion, the Taldura River valley, larch forest on a mountain slope, alt. 2095 m, N 50°49.572' E 87°57.552' (D. Gerus, A. Agafonov) |
| 57  | *E. subfibrosus*; KRT-1612 | Krasnoyarsk Krai, Evenk raion, Tura village, a ground road edge, alt. 309 m, N 64°16.920' E 100°14.880' (L. Krivobokov) |
| 58  | *E. subfibrosus*; JRO-1733 | Republic of Sakha (Yakutia), Tompo raion, the Kolyma Riad, Verkhoyanskii Mt. Range southern spurs, the Rosomakha River valley, alt. 460 m, N 63°2.879' E 137°52.610' (N. Badmaeva) |
| 59  | *E. jacutensis*; 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  | *E. jacutensis*; 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, S. Asbaganov) |
| 61  | *E. jacutensis*; 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  | *E. macrourus*; 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  | *E. macrourus*; LEN-1524_1 | Republic of Sakha (Yakutia), Khangalas raion, meadow slope at the Lena River right bank, alt. 114 m, N 61°16.369' E 127°21.593' (E. Kobozeva, S. Asbaganov) |
| 64  | *E. turuchanensis*; KRE-1440 | Krasnoyarsk Krai, Turukhansk raion, Bor village, Yenisey River sandy bank, alt. 30 m, N 61°36.265' E 090°10.143' (M. Lomonosova) |
| 65  | *E. peschkovae*; MJN-1106 | Magadan oblast, Khasyn raion, Yablonyovyy Pass, floodplain meadow at a road, alt. 755 m, N 60°19.467' E 151°10.540' (O. Potemkin) |
| 66  | *E. peschkovae*; 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.

Results and discussion

The comparative analysis of sequences of the $\beta$ 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_balg_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_balg_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 genomic constitution *StStHH*, and one species, *E. amurenensis*, 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
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**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. | *Elymus* species         | Number of accessions studied | GC  | No. | *Elymus* species         | Number of accessions studied | GC  |
|-----|--------------------------|------------------------------|-----|-----|--------------------------|------------------------------|-----|
| 1   | *E. amurensis*           | 1                            | StY | 9   | *E. margaritae*          | 2                            | StH |
| 2   | *E. charkeviczii*        | 2                            | StH | 10  | *E. sajanensis*          | 2                            | StH |
| 3   | *E. jacutensis*          | 4                            | StH | 11  | *E. subfibrosus*         | 4                            | StH |
| 4   | *E. kamczadalorum*       | 1                            | StH | 12  | *E. transbaicalensis*    | 4                            | StH |
| 5   | *E. komarovii*           | 4                            | StH | 13  | *E. uralensis*           | 1                            | StH |
| 6   | *E. kronokensis*         | 6                            | StH | 14  | *E. viridiglumis*        | 1                            | StH |
| 7   | *E. lenensis*            | 3                            | StH | 15  | *E. turuchanensis*       | 1                            | StH |
| 8   | *E. macrourus*           | 3                            | StH | 16  | *E. peschkovae*          | 2                            | StH |

internal control for the completeness of hydrolysis. According to the classification system based on genomic compositions, *E. kamoji* belongs to the genus *Campeiostachys* (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 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).

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**ORCID ID**

A.V. Agafonov orcid.org/0000-0002-1403-5867

S.V. Asbaganov orcid.org/0000-0002-7482-7495

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