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Genetic patterns of *Hordeum jubatum* L. (Poaceae) and × *Elyhordeum arcuatum* W. W. Mitch. et H. J. Hodgs. in Siberia and Far East of Russia

Генетические паттерны *Hordeum jubatum* L. (Poaceae) и × *Elyhordeum arcuatum* W. W. Mitch. et H. J. Hodgs. (Poaceae) в Сибири и на Дальнем Востоке России

Kulakova N. V.[1, 2], Verkhozina A. V.[1]

Кулакова Н. В.[1, 2], Верхозина А. В.[1]

[1] Сибирский институт физиологии и биохимии растений СО РАН, г. Иркутск, Россия
E-mails: kulakova@sifibr.irk.ru; allaverh@list.ru
[1] Siberian Institute of Plant Physiology and Biochemistry SB RAS, Irkutsk, Russia

[2] Иркутский государственный университет, г. Иркутск, Россия. E-mail: ninkul@mail.ru
[2] Irkutsk State University, Irkutsk, Russia

Summary. *Hordeum jubatum* (foxtail barley) is a weed and ornamental plant with a natural range in Eastern Siberia, the Russian Far East and North America. Over the past decades, its range significantly expanded in Eurasia, rapidly spreading westward, while its genetic characteristics remain poorly understood. PCR, molecular cloning, and sequencing of nuclear (ITS) and chloroplast (*mat*K) DNA markers were performed for comparative characteristics of *H. jubatum* from different parts of its modern range (Western and Eastern Siberia) and for one of the hybridogenic species × *Elyhordeum arcuatum*. Two variants of the *Hordeum* H-genome were found in all studied samples. No significant differences in the nucleotide structure of ITS and *mat*K were found in *H. jubatum* from Western and Eastern Siberia and North America. ITS sequences specific to *Elymus sibiricus* and *H. jubatum* prove the participation of these species in the formation of × *E. arcuatum*, as well as the maternal origin of *mat*K (chloroplast DNA) from *H. jubatum*. The obtained results complement the genetic characteristics of the studied species and determine further directions of their study.

Key words. *Elymus sibiricus*, genetic markers, H-genome, hybrid, ITS, *mat*K.

Реферат. *Hordeum jubatum* (ячмень гривастый) – сорное и декоративное растение, с естественным ареалом в Восточной Сибири, на Дальнем Востоке России и в Северной Америке. За последние десятилетия вид значительно расширил свой ареал в Евразии, быстро распространяясь на запад, при этом его генетические характеристики остаются малоисследованными. Для сравнительной характеристики *H. jubatum* из разных частей его современного ареала (Западная и Восточная Сибирь) и анализа одного из гибридогенных видов × *Elyhordeum arcuatum* мы проводили ПЦР, молекулярное клонирование и секвенирование ядерных (ITS) и хлоропластного (*mat*K) ДНК маркеров. Два варианта H-генома *Hordeum* обнаружены во всех исследованных образцах. Существенных отличий в нуклеотидной структуре ITS и *mat*K *H. jubatum* из Западной и Восточной Сибири и Северной Америки не выявлено. Последовательности ITS, специфичные для *Elymus sibiricus* и *H. jubatum*, доказывают участие этих видов в формировании × *E. arcuatum*, а также материнское происхождение хлоропластной ДНК из *H. jubatum*. Полученные результаты дополняют генетические характеристики исследованных видов и определяют дальнейшие направления их изучения.

Ключевые слова. Генетические маркеры, гибрид, H-геном, *Elymus sibiricus*, ITS, *mat*K.

The genus *Hordeum* L. (tribe *Triticeae*) comprises about 32 species common in temperate and dry regions of Eurasia and the New World. The native range of *Hordeum jubatum* L. is Northern America and Eastern Asia. This species is highly adaptable to various environments particularly in disturbed areas. It is often found in grasslands and along roadsides, used as ornamental plant and it is considered invasive in many regions of Eurasia. The long-term observations in Russia (Tsvetkov, 2014) showed successful naturalization of *H. jubatum* in Western Siberia. The genetic basis for its wide dispersal in Eurasia remains unclear.

H. jubatum is mostly tetraploid with chromosome number 2*n* = 28. It has H-genome out of four basic genomes of *Hordeum* designated H, I, Xa, and Xu (Komatsuda et al., 1999). Moreover, there are two variants of rDNA clusters found in *Hordeum* species (Taketa et al., 2001) that results in the presence of different ITS sequences in the same individual which can complicate its phylogenetic resolution (Blattner, 2004). The complex and reticulate genetic relationship in the genus is explained by incomplete reproductive isolation between closely related species (Nishikawa et al., 2002). In Northern America, polyploidization and hybridization, along with different factors, were correlated with its range expansion. The other feature of *H. jubatum* is hybridization with different species of *Elymus*. The genus × *Elyhordeum* Mansf. is a hybrid of *Elymus* L. and *Hordeum*. Species of this genus are native to subarctic and temperate regions of the Northern Hemisphere (Tzvelev, Probatova, 2019). One of its hybrids, × *Elyhordeum arcuatum* W. W. Mitch. et H. J. Hodgs (*Elymus sibiricus* L. × *H. jubatum*), is recorded in Alaska (Subarctic America, Northern America) and in the Magadan Region (the North-East of Russia). In this work we studied genetic features of *H. jubatum* and *E. arcuatum* to better understand their origin and phylogenetic relationship in Eurasian part of the range.

Analysed specimens were stored in herbarium (IRK) of Siberian Institute of Plant Physiology and Biochemistry (Irkutsk, Russia) and collected from 1970 to 2018. Specimens of *H. jubatum* were collected in the Irkutsk, Omsk, Krasnoyarsk, and Novosibirsk Regions, the Republics of Buryatia, Tuva and Chukotka Autonomous Okrug and specimens of *E. arcuatum* were from the Magadan Region. DNA was extracted from dry leaves with the kit C-8896 (Vector-Best, Novosibirsk, Russia). The nuclear ITS and chloroplast *mat*K regions were analysed. Primers and conditions for PCR reactions and Sanger sequencing were the same as previously described (Kulakova et al., 2023). Molecular cloning with TA vector was used to obtain ITS sequences. BLAST search (BLAST URL: https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to find nearest homologues. Sequences were aligned and edited in BioEdit 7.0.5.3 (Hall, 1999).

Two variants of ITS sequences of *Hordeum* were identified in all studied specimens of *H. jubatum* that proved its allotetraploid origin. The same two variants of ITS were found in GenBank database in specimens from North America (Utah, USA and Mexico). Only one of these two variants can be found in GenBank for *H. jubatum* from Estonia, Kazakhstan and Korea that may be due to its dominance in these samples. Both ITS variants were clearly separated by > 2 % nucleotide differences. From ITS data no difference between *H. jubatum* from Siberia, Far East and North America was found. The *mat*K gene was identical in all studied samples. This gene is highly conservative and did not allow us to discriminate *H. jubatum* from other species of the genus.

For × *E. arcuatum*, we studied three specimens. For each, 6–10 clones were used for the analysis. The set of sequences including those of *E. sibiricus*-specific (identical to the *E. sibiricus* voucher CCDB-18344-G7 from Canada) and both ITS variants of *H. jubatum* were obtained. From the *mat*K gene analysis, *H. jubatum*-specific sequences were present in × *E. arcuatum* samples. Identification of *H. jubatum* chloroplast gene (*mat*K) indicated the maternal inheritance of chloroplast DNA. This provided data that *H. jubatum* was a maternal parent in studied samples of this hybrid species.

The complex evolutionary history of nuclear ITS does not provide enough data to infer its phylogenetic relationships but a deeper analysis of ITS variants of samples from different parts of its range might give new ideas on these subject. The more variable chloroplast markers, except the data on maternal parent in this hybrids, can be used to obtain the phylogeographic data of *H. jubatum* in Eurasia.

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