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Comparative assessment of the copy number of satellite repeats in the genome of Triticeae species

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Abstract. Satellite repeats are a significant component of the genome of Triticeae and play a crucial role in the speciation. They are a valuable tool for studying these processes. *Pseudoroegneria* species play a special role among grasses, as they are considered putative donors of the St-genome in many polyploid species. The aim of this study was to compare the copy number of satellite repeats in the genomes of Triticeae species. Quantitative real-time PCR was applied to determine the copy numbers of 22 newly discovered satellite repeats revealed in the whole-genome sequences of *Pseudoroegneria* species and one additional repeat previously identified in the genome of *Aegilops crassa*. The study focused on seven species of *Pseudoroegneria*, three species of *Thinopyrum*, *Elymus pendulinus*, *Ae. tauschii*, *Secale cereale*, and *Triticum aestivum*. Based on the copy number level and coefficients of variation, we identified three groups of repeats: those with low variability between species (medium-copy CL82), those with medium variability (low- and medium-copy CL67, CL3, CL185, CL119, CL192, CL89, CL115, CL95, CL168), and those with high coefficients of variation (CL190, CL184, CL300, CL128, CL207, CL69, CL220, CL101, CL262, CL186, CL134, CL251, CL244). CL69 exhibited a specific high copy number in all *Pseudoroegneria* species, while CL101 was found in both *Pseudoroegneria* and *Th. junceum*, CL244 in *Th. bessarabicum*, CL184 in *P. cognata* and *S. cereale*. CL95, CL128, CL168, CL186, CL207, and CL300 exhibited higher copy numbers in *P. cognata* compared to other species; CL3, CL95, CL115, CL119, CL190, CL220, CL207, and CL300 in *P. kosaninii*; CL89 in *P. libanotica*; CL134 in *P. geniculata*. Our assessment of the copy number of new satellite repeats in the St-genome and the analysis of their amplification specificity between species can contribute to the molecular-genetic and chromosome markers used for evolutionary, phylogenetic, and population studies of Triticeae species.
Key words: Triticeae; satellite repeats; qPCR; whole-genome sequencing.

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Сравнительная оценка копийности сателлитных повторов в геноме видов Triticeae

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Аннотация. Сателлитные повторы составляют значительную часть генома Пшеницевых, играя важную роль в видообразовании, что делает их ценным инструментом для изучения этих процессов. Особое место среди злаков занимают виды *Pseudoroegneria* – наиболее вероятные доноры St-генома у многих полиплоидов. Цель настоящего исследования состояла в сравнительной оценке копийности сателлитных повторов в геномах Triticeae. С помощью количественной полимеразной цепной реакции в реальном времени была установлена копийность 22 сателлитных повторов, выявленных в полногеномных нуклеотидных последовательностях видов *Pseudoroegneria*, и одного ранее опубликованного повтора, обнаруженного в геноме *Aegilops crassa*. Объектами анализа стали семь видов *Pseudoroegneria*, три вида *Thinopyrum*, *Elymus pendulinus*, *Ae. tauschii*, *Secale cereale* и *Triticum aestivum*. По уровню копийности и коэффициентам вариации нами выделено три группы повторов: с низким уровнем вариативности между видами (среднекопийный CL82), средним уровнем вариативности (низко- и среднекопийные CL67, CL3, CL185, CL119, CL192, CL89, CL115, CL95, CL168) и с высокими значениями коэффициента вариации (CL190, CL184, CL300, CL128, CL207, CL69, CL220, CL101, CL262, CL186, CL134, CL251, CL244). Повтор CL69 показал специфическую высокую копийность для всех видов *Pseudoroegneria*, CL101 – у *Pseudoroegneria* и *Th. junceum*, CL244 – у *Th. bessarabicum*, CL184 – у *P. cognata* и *S. cereale*. У *P. cognata* более высокую копийность, по сравнению с остальными видами, проявили повторы CL95, CL128, CL168,

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CL186, CL207, CL300; у *P. kosaninii* – CL3, CL95, CL115, CL119, CL190, CL220, CL207 и CL300; у *P. libanotica* – CL89; у *P. geniculata* – CL134. Проведенные нами оценка копийности сателлитных повторов, найденных в St-геноме, и анализ специфичности их амплификации между видами могут пополнить арсенал молекулярно-генетических и цитогенетических маркеров, используемых для эволюционных, филогенетических и популяционных исследований представителей трибы Пшеницевых.

Ключевые слова: Triticeae; сателлитные повторы; qPCR; полногеномное секвенирование.

Introduction

Triticeae is an economically important tribe of the Poaceae family, comprising approximately 500 species of annual and perennial herbaceous plants (NCBI database: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). Wheat, rye, barley, and fodder grasses are among the species of this tribe that play a significant role in providing food for humanity and have also become an integral part of animal diets (Hodkinson, 2018).

The interest in studying phylogenetic relationships within the Triticeae tribe is largely driven by the potential of wild wheat species to serve as valuable sources of economically important genes for the improvement of cultivated cereals. For example, *Thinopyrum* and *Dasypyrum* serve as gene donors for resistance to various diseases (Yang et al., 2005; Luo P.G. et al., 2009; Salina et al., 2015; Wang S. et al., 2019; Li L.F. et al., 2022; Guo et al., 2023). By crossing wheat and *Agropyron*, it is possible to increase head productivity (Zhang J. et al., 2016). Representatives of *Pseudoroegneria* are drought-resistant and are used as pasture grasses (Wu et al., 2023b).

The Triticeae tribe consists of approximately 100 annual and 400 perennial species, which carry one (in diploids) or several (in polyploids) of 13 genomes (Wang, Lu, 2014). Representatives of *Pseudoroegneria* carry the St-genome, and it is believed that this genus was the donor of the St-genome for *Elymus* and some *Thinopyrum* species (Mahelka et al., 2011; Dobryakova, 2017; Linc et al., 2017; Lei et al., 2018; Chen N. et al., 2020; Agafonov et al., 2021). Plants of the genus *Agropyron*, at all ploidy levels ($2n = 2x/4x/6x$), are distinguished by the presence of a P-genome (Zhang Y. et al., 2015). Genome J (=E) is mainly composed of diploids *Thinopyrum bessarabicum* (genome J = J^b) and *Th. elongatum* (genome E = J^e). The J-genome is evolutionarily close to the D-subgenome of common wheat, and the most likely donor of the D-subgenome is *Aegilops tauschii* (Baker et al., 2020). This may explain why the chromosomes of the D-subgenome in the introgressive lines of common wheat, developed with the aim of improving it through hybridization with *Thinopyrum*, show the highest frequency of introgressions from the J-genome (Chen Q. et al., 2001; Liu Z. et al., 2007; Cui et al., 2018).

At present, the origin, relationships, and proximity of genomes within the Triticeae tribe remain controversial and uncertain. The challenges associated with studying Triticeae genomes are primarily due to the significant differences between the polyploid subgenome and the ancestral genome of the diploid parent organism. These differences arise from the modifications that occur during evolution. Additionally, the diploid ancestor of the donor organism may have become extinct or has not yet been found (Jakob, Blattner, 2010; Liu Q.-L. et al., 2020; Sha et al., 2022). Perennial polyploid species, for example, *Th. intermedium* and *Th. ponticum*, may have an unbalanced genome or chromosomal translocations (Kruppa, Molnar-Lang, 2016; Liu Y. et al., 2023). This could be associated with the transition to vegetative reproduction, which does not involve sexual processes for seed formation and therefore does not require stable meiosis (Comai et al., 2005; Husband et al., 2013). The same species are characterized by recombinant subgenomes, the origin of which is still unclear (Wang R.R.C. et al., 2015; Liu Y. et al., 2023). Discussions continue regarding potential donors of the Y-subgenomes in *Elymus* and *Roegneria* (Yan et al., 2011; Liu Q.-L. et al., 2020; Wu et al., 2021), as well as the maternal form in the occurrence of *Thinopyrum*, *Roegneria*, *Elymus*, *Kengyilia*, and other polyploids (Mahelka et al., 2011; Luo X. et al., 2012; Zeng et al., 2012; Lei et al., 2018; Chen N. et al., 2020).

In addition, there is a problem of correlation between the species identification of a particular specimen based on botanical traits (often influenced by the environment) and that based on molecular genetic and cytogenetic characteristics (Wang, Lu, 2014; Al-Saghir, 2016; Rodionov, 2022). For example, this issue arises in *Elymus*, as demonstrated by studies conducted by A.V. Rodionov et al. (2019), V. Lucia et al. (2019), and L. Tan et al. (2021). Another example is the relationship between *Th. elongatum* and *Th. bessarabicum* that bear fairly similar genomes, but differ in botanical characteristics (Grewal et al., 2018; Dai et al., 2021; Chen C. et al., 2023). The problem is compounded by the fact that natural spontaneous hybrids are often found (Chen C. et al., 2022; Luo Y.C. et al., 2022; Wu et al., 2023a). The study of phylogenetic relationships deepens our understanding of evolutionary processes in plants and speciation, and helps to improve biosystematics. The acquired knowledge will enhance the efficiency of utilizing genetic resources from wild species by improving the understanding of their proximity to the genomes of cultivated crops and the potential for obtaining valuable introgressions.

The genome of Triticeae grasses is characterized by a large size, which complicates whole-genome deep sequencing and makes assembly difficult (Rabanus-Wallace, Stein, 2019). A significant portion of the Triticeae genome is composed of repetitive DNA, known as repeats. The repeatome include mobile elements, gene clusters (specifically the 5S and 45S rRNA genes), and satellite repeats (Dvořák, 2009; Shcherban et al., 2015; Gao et al., 2023; Vershinin et al., 2023).

Satellite repeats are tandem repeating non-coding sequences that exist as arrays of varying lengths in genetically silent heterochromatin regions (Badaeva, Salina, 2013). Satellite repeats are considered to be the most variable and rapidly evolving components. They can be species-specific or common to closely related species (Belyayev et al., 2019; Garrido-Ramos, 2021; Thakur et al., 2021). Comparative analysis of copy number, nucleotide sequences, and localization on the chromosomes serves as a tool for basic phylogenetic and evolutionary studies of plants, including Triticeae species (Anamthawat-Jónsson, Heslop-Harrison, 1993; Vershinin et

al., 1994; Kishii et al., 1999; Anamthawat-Jónsson et al., 2009; Han et al., 2017; Linc et al., 2017; Ruban, Badaeva, 2018; Said et al., 2018; Salina, Adonina, 2019; Dai et al., 2021; Wu et al., 2021; Chen C. et al., 2022; Kroupin et al., 2023; Shi et al., 2023). Satellite repeats have found practical use as PCR markers and chromosomal markers for identifying introgressions of alien genetic material containing valuable economic traits in the genomes of cultivated cereals (Li G. et al., 2016; Han et al., 2017; Liu L. et al., 2018; Chen J. et al., 2019).

Tangible progress has been made in the study of Triticeae genomes, owing to the invention of whole-genome sequencing methods and bioinformatic algorithms for analyzing the data obtained (Rabanus-Wallace, Stein, 2019; Gao et al., 2023). The rapid growth in the volume of information on genome-wide sequences of Triticeae has significantly accelerated and simplified the search for repetitive DNA that can be used as chromosomal markers (Du et al., 2017; Said et al., 2018; Tang et al., 2018; Chen J. et al., 2019; Kroupin et al., 2019a, 2022; Lang et al., 2019a; Liu Q.-L. et al., 2020; Wu et al., 2021, 2022). Due to the significant presence of repetitive DNA in the Triticeae genomes, information about satellite repeats can be obtained even through sequencing with low coverage. This greatly simplifies the process of searching for repeated sequences (Navajas-Perez, Paterson, 2009; Kroupin et al., 2019b; Šatovic-Vukšic, Plohl, 2023).

A well-established method for quantifying the number of copies of repetitive DNA, including satellite repeats, is quantitative real-time PCR (qPCR) (Harpke, Peterson, 2007; Navajas-Pérez et al., 2009; Baruch, Kashkush, 2012; Feliciello et al., 2015; Divashuk et al., 2016, 2019, 2022; Pereiera et al., 2018; Shams, Raskina, 2018). Compared to Southern blot or dot-blot hybridization on a membrane, or fluorescent *in situ* hybridization on chromosomes, qPCR has proven to be an easier-to-use, accurate, and effective method for assessing the copy number of the target sequence. This method allows researchers to identify the number of copies of satellite repeats in the genome and the variability between genomes (Kalendar et al., 2020; Pös et al., 2021).

In this study, the whole-genome sequencing of *Pseudoroegneria spicata*, *P. libanotica*, *P. tauri*, *P. geniculata*, *P. cognata*, and *P. kosaninii* revealed the presence of 22 satellite repeats. In order to comprehend the potential of using them as tools for evolutionary and phylogenetic studies of wild representatives of the Triticeae tribe, as well as for studying wide hybrids using molecular biology and cytogenetics methods, it is crucial to first determine the copy number of satellite repeats in the genomes of St-genome carriers. Therefore, we have chosen *Pseudoroegneria* species with different ploidy levels as our research subject.

To assess the specificity of satellite repeats for the St-genome, we included *Thinopyrum* species in the experiment, which contain the J-genome that is common among Triticeae. We also selected *Th. intermedium* with the J^rJ^{vs}St-genomic formula, serving as a carrier of the St-subgenome and St-specific repeats in the recombinant J^{vs}-genome. To explore the potential of utilizing the identified satellite repeats for the characterization of distant wheat and rye hybrids, *Triticum aestivum* and *Secale cereale* accessions were included in the study. In addition, due to the evolutionary proximity of the J- and D-genomes, we included the *Ae. tauschii* accession. *E. pendulinus* was also used, carrying both the St-subgenome targeted by our work and the Y-subgenome of unknown origin, which is common among *Elymus sensu lato* species. The experiment also included a satellite repeat of CL244, which we obtained as a result of analyzing the whole-genome nucleotide sequence of *Ae. crassa* (D^1X^{cr}), a carrier of the D-genome variant (Kroupin et al., 2022). Despite this, CL244 was not found in *Ae. tauschii*, showed a small number of hybridization sites on the chromosomes of *T. aestivum* and *Ae. crassa*, while on the chromosomes of the J^b-genome in *Th. bessarabicum*, bright signals were observed indicating a high level of its abundance.

Materials and methods

The Triticeae species with various genomic compositions, as listed in the Table, served as the material for our study.

The young leaves of the plants were frozen in liquid nitrogen. Genomic DNA was then isolated using the CTAB protocol (Rogers, Bendich, 1985). This DNA was used for subsequent sequencing and quantitative PCR (qPCR). The concentration and purity of the isolated DNA were tested using Qubit 4 (Thermo Fisher Scientific, USA) and electrophoresis in a 0.8 % agarose gel.

Shotgun sequencing libraries were synthesized using the Swift 2S Turbo DNA Library Kit (Swift Bioscience, USA) in accordance with the manufacturer's protocol. To assess the quality of the libraries, a test run was conducted on the MiSeq device (Illumina, Inc., USA). The libraries were then converted and sequenced using DNBSEQ-G400 on one track. The initial amount of DNA was 25 ng. The fragments were approximately 350 bp long and were indexed at both ends using the Swift 2S Turbo Unique Dual Indexing Kit (Swift Bioscience). Sequencing was performed on Illumina NextSeq (Illumina, Inc.), using the NextSeq 500/550 Mid Output Kit v.2.5 (Illumina, Inc.).

Bioinformatic analysis was conducted on the processing and assembly of the reads of nucleotide sequences that involved a sequence of satellite tandem repeats. The uniqueness of these sequences was evaluated in comparison to previously published methods described in P.Y. Kroupin et al. (2022). The primer sequences for the identified monomers of satellite repeats are provided in Supplementary Material 1[1].

Quantitative real-time PCR was conducted using DNA from the accessions listed in the Table as a template, with three replicates. The amplification was carried out using a CFX real-time amplifier system (Bio-Rad Laboratories, Inc., USA) and a reaction mixture of Real-Time PCR Mix containing the Eva Green fluorophore (Synthol Ltd., Russia) in accordance with the manufacturer's protocol. A single-copy gene, *VRN1*, was used as the reference gene. Primer concentration in mixtures consisted of 10 ng/µl, while DNA concentration was 0.4 ng/µl. Amplification was performed according to the following program: preincubation – 10 min at 95 °C; followed by 40 cycles: denaturation – 10 s at 95 °C; primer annealing – 30 s at 60 °C.

Statistical analysis, including the calculation of the average values of Cq, standard deviation, and the corresponding number of copies relative to the reference gene *VRN1*, was performed using Bio-Rad CFX and Manager 3.1 software. To

[1] Supplementary Materials 1 and 2 are available https://vavilov.elpub.ru/jour/manager/files/Suppl_Kroupin_Engl_27_8.xlsx

Plant material

Species	Accessions	Source	2*n*	Genome formula	Target of use (sequencing, qPCR)
P. spicata	PI 578855	GRIN[1]	14	StSt	Sequencing, qPCR
P. spicata	PI 236671	GRIN	28	StStStSt	Sequencing, qPCR
P. libanotica	PI 228389	GRIN	14	StSt	qPCR
P. libanotica	PI 330690	GRIN	14	StSt	Sequencing
P. tauri	PI 380652	GRIN	14	StSt	Sequencing, qPCR
P. geniculata	PI 670437	GRIN	28	StStStSt	Sequencing, qPCR
P. cognata	PI 670361	GRIN	28	StStStSt	Sequencing, qPCR
P. kosaninii	PI 237636	GRIN	56	StStStSt StStStSt	Sequencing, qPCR
Th. intermedium	PI 401200	GRIN	42	$J^{vs}J^{vs}J^{r}J^{r}$StSt	qPCR
Th. bessarabicum	PI 531711	GRIN	14	$J^{b}J^{b}$	qPCR
Th. junceum	PI 119604	GRIN	42	$J^{b}J^{b}J^{b}J^{b}J^{e}J^{e}$	qPCR
E. pendulinus	PI 639804	GRIN	28	StStYY	qPCR
Ae. tauschii	K-608	VIR[2]	14	DD	qPCR
S. cereale	cv. Bereginya	NGC[3]	14	RR	qPCR
T. aestivum	cv. Chinese Spring	–	42	BBAADD	qPCR

[1] GRIN – germplasm resources information network of the Agricultural Research Service of the US Department of Agriculture (USDA-ARS Germplasm Resources Information Network).
[2] VIR – N.I. Vavilov All-Russian Institute of Plant Genetic Resources.
[3] NGC – P.P. Lukyanenko National Grain Center.

assess the similarity of copy numbers among repeats, we have introduced the concept of "repeat copy number pattern", a set of copy number values for a specific repeat within the set of species being studied. To assess the similarity of copy number among the species under investigation, we have introduced the concept of "species copy number pattern", a set of values of the copy number of the satellite repeats being studied for a particular species.

Pearson's correlation coefficients (r) were calculated using Statistica 12 software (StatSoft, USA) to determine the relationship between repeat copy number patterns and species copy number patterns. The significance level was set at $p < 0.05$. Diagrams were constructed using the principal component analysis method for satellite repeats and the studied species. The diagrams were based on the data obtained from Statistica 12 software, which included information on the copy number of satellite repeats. The coefficient of variation of the satellite repeatability values between species was calculated using Microsoft Excel (USA).

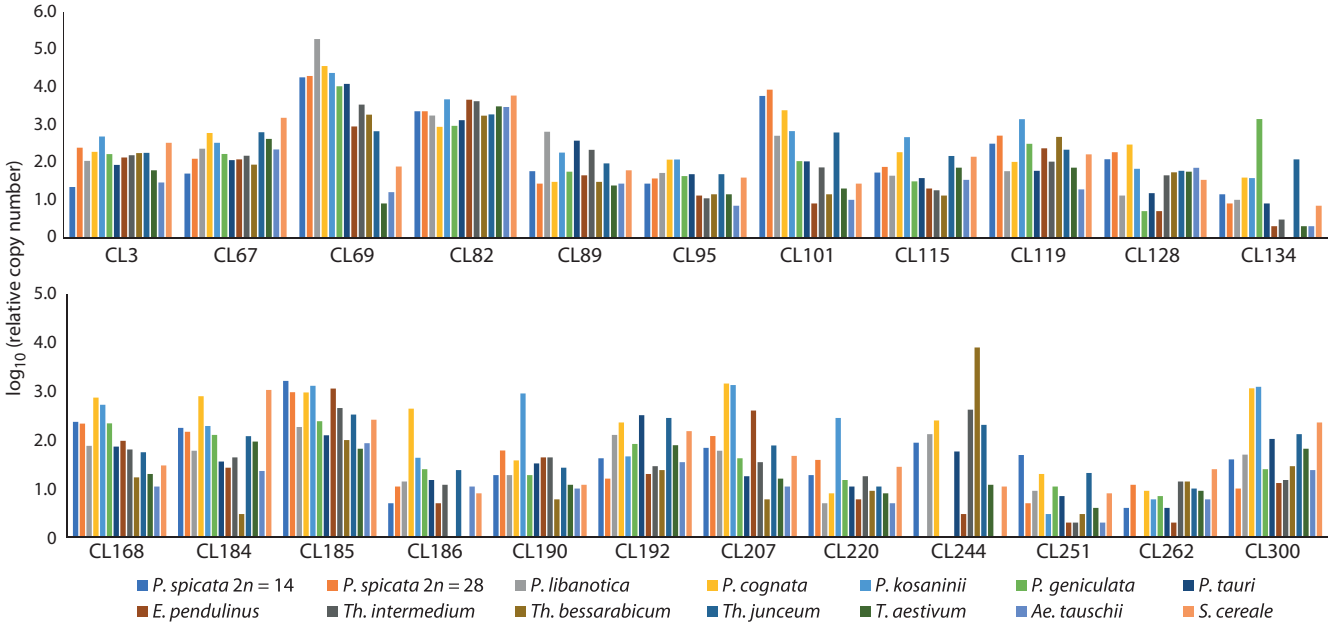
Results

Characteristics of identified satellite repeats

In the framework of the present study, a total of 22 satellite repeats were found in separate genome sequence assemblies. As a result of analyzing the nucleotide sequence of the diploid accession of the *P. spicata* (2*n* = 14) genome, 10 repeats were identified. These repeats include CL69, CL82, CL101, CL119, CL128, CL168, CL184, CL207, CL251, and CL262. Additionally, four repeats were found in the nucleotide sequence of the *P. tauri* genome (CL67, CL89, CL185, and CL192), as well as in the *P. kosaninii* genome (CL3, CL115, CL220, and CL300). Furthermore, one repeat was found in each of the genomes of *P. libanotica* (CL95), *P. geniculata* (CL134), *P. cognata* (CL186), and a tetraploid *P. spicata* (CL190).

The characteristics of the identified satellite including its length and the most similar sequences in the NCBI database are presented in Supplementary Material 1. After comparing the nucleotide sequences of the 22 repeats with those previously published in the NCBI, we did not find any matches for nine of them (CL69, CL89, CL95, CL168, CL185, CL207, CL251, CL262, CL300). For the remaining 13, the level of identity among similar published sequences ranged from 70 to 98 %. This indicates that they are different from previously published sequences (see Supplementary Material 1).

Two satellite repeats showed similarities to repeats found in common wheat: CL119 was similar to the pTa-465 clone (77 % identity), and CL101 was similar to the Spelt1-like subtelomeric repeat (80 % identity). Three repeats showed similarities to the following known satellites: CL220 to CL219, which was detected in the *Ae. crassa* genome (82 %), CL134 to CL97 from the *Th. bessarabicum* genome (71 %)



Relative copy number of the satellite repeats in the studied species of the Triticeae tribe expressed as a decimal logarithm.

and CL186 to ACRI_TR_CL80 from the *A. cristatum* genome (70 %). The other three repeats show similarities to microsatellites: CL128 has similaritiy to L15 identified in the *P. stipifolia* genome (84 %), CL190 shows similarity to P523 from the genome of *Ae. tauschii* (81 %), and CL82, to pTa-451 from common wheat (88 %). Four repeats we found showed similarity to the following mobile elements: CL184, which has a 98 % similarity to the *Cassandra* retrotransposon from the rye genome, CL67 and CL115, which have a 91 and 78 % similarity to retrotransposons from the barley genome *Cereba* and *Sandra5*, respectively, and CL192, which has a similarity to transposon XJ from the *Ae. tauschii* genome (70 %). The CL3 repeat was most similar to the E-gene-specific marker *Th. elongatum* 51-6 (79 %).

Assessment of the copy number of satellite repeats using qPCR

The data obtained on the relative copy numbers of 23 satellite repeats in 14 species, calculated in relation to the reference single-copy *VRN1* gene, are shown in Supplementary Material 2.1. All of the repeats we studied differed in terms of copy numbers and the coefficient of variation between the species. Since the order of the obtained copy numbers varied significantly, the results were presented in the form of a decimal logarithm for the convenience of comparing their abundance (see the Figure and Supplementary Material 2.2). Hereafter, we will simply refer to the decimal logarithm of relative copy number as "copy number". Since the copy number rate varied from 0 to 5, the repetitions were classified into the following groups based on their copy number: low (≤ 2), medium (> 2, < 4), and high (≥ 4). Since the coefficient of variation ranged from 0 to 0.6, we assumed that the variability was low when its value was less than 0.1, medium when it fell between 0.1 and 0.25, and high when it exceeded 0.25.

CL82 turned out to be the least variable repeat: its abundance was medium-closer to high and ranged from 2.9 to 3.8.

The medium coefficient of variation for the copy number of satellite repeats in all studied accessions (from 0.16 to 0.25) was observed in nine specific repeats, namely CL67, CL3, CL185, CL119, CL192, CL89, CL115, CL95, and CL168 (listed in ascending order based on their coefficient of variation). The average copy number values in *Pseudoroegneria* species were 2–11 % higher compared to the entire studied collection. However, CL67 had the highest copy number in the rye genome (3.2). CL89 showed the highest value in *P. libanotica* (2.8), CL3, CL119, and CL115 had the highest values in *P. kosaninii* (2.7, 3.1, and 2.7, respectively), CL95 had the highest value in both *P. cognata* and *P. kosaninii* (2.1), and CL168 had the highest value in *P. cognata* (2.9). The remaining repeats of this group were generally characterized by a low-to-medium level of copy number across all accessions. The minimum copy number was observed in CL192, ranging from 1.2 to 2.5, while the maximum copy number was found in CL185, ranging from 1.8 to 3.2.

A high level of variability was observed in the following 13 repeats: CL190, CL184, CL300, CL128, CL207, CL69, CL220, CL101, CL262, CL186, CL134, CL251, and CL244. The coefficient of variation ranged from 0.27 to 0.43. In this group, the following repeats can be distinguished: CL69 with a high copy number in *Pseudoroegneria* species (4.0–5.3), medium in *Thinopyrum* and *E. pendulinus* (2.8–3.0), and low in the rest; CL101 with a medium copy number in *Pseudoroegneria* (2.0–3.9) and *Th. junceum* (2.8), low in the rest; CL244, close-to-high in *Th. bessarabicum* (3.9), significantly varies in *Pseudoroegneria* species (0–2.4), and is medium to low in the others. CL184 is the highest in *P. cognata* (2.9) and *S. cereale* (3.0) compared to the others (0.5–2.3). Individual repeats showed the highest copy number in specific *Pseudo-*

roegneria species: CL128 and CL186 in *P. cognata* (2.5 and 2.6, respectively), CL190 and CL220 in *P. kosaninii* (2.5 and 3.0, respectively), CL134 in *P. geniculata* (3.1), CL207 and CL300 in *P. cognata* and *P. kosaninii* (ranging from 3.1 to 3.2). CL251 and CL262 were characterized by an overall low copy rate, ranging between 0.3–1.7 and 0–1.4, respectively.

Correlation analysis (see Supplementary Material 2.3) and principal component analysis (see Supplementary Material 2.4) were used to identify the following groups with similar repeat copy number patterns: 1) CL3, CL115, CL119, CL190, and CL220 ($r \geq 0.77$); 2) CL95, CL207, and CL300 ($r \geq 0.87$); 3) CL128, CL168, and CL186 ($r \geq 0.72$). Correlation analysis (see Supplementary Material 2.5) and principal component analysis (see Supplementary Material 2.6) revealed a high level of similarity in the species copy number patterns among the following groups: 1) *Pseudoroegneria* accessions ($r > 0.9$); 2) *E. pendulinus*, *Th. intermedium* and *Th. junceum* ($r > 0.8$); 3) rye, common wheat, *E. pendulinus*, *Th. junceum*, and *Ae. tauschii* ($r \geq 0.89$). The medium level of similarity in the species copy number pattern was observed between *Th. intermedium* and *Pseudoroegneria* ($r > 0.6$). The species copy number pattern in *Th. bessarabicum*, on average, showed the least similarity with other species.

Discussion

Satellite repeats constitute a significant portion of the Triticeae genome and play a crucial role in the formation and evolution of new species. As a consequence, they serve as valuable tools for analyzing these processes (Shcherban, 2015; Salina, Adonina, 2019; Vershinin et al., 2023). The search for new satellite repeats is necessary to understand the phylogenetic relationships and evolution of the Triticeae tribe, which is of significant importance to humans. One of the initial steps in determining the suitability of the identified satellite repeats as tools for such studies is to conduct a comparative assessment of their copy number in related species.

Some of the satellite repeats we found in the St-genome showed a similar copy number among the studied species. Homologs have been found in the genomes of wheat and barley, suggesting their common origin. CL82 and CL119 showed similarity to pTa-451 and pTa-465, respectively, which were identified in *T. aestivum* (Komura et al., 2013). CL67 is similar to the centromeric retrotransposon *Cereba* (Hudakova et al., 2001) and is conserved in Triticeae (Dvořák, 2009). Although CL3 is 79 % identical to the E-specific repeat 51-6, it did not show any specificity for *Thinopyrum* species in our study. This suggests that we have discovered an older and less genome-specific variant.

CL69 was distinguished by a high copy number in *Pseudoroegneria* accessions and a medium copy number in *Thinopyrum* and *E. pendulinus* species. This may indicate its occurrence before the divergence of the St- and J-genomes. CL101 has a medium copy number in *Pseudoroegneria* and *Th. junceum* species and could also occur in a common ancestor of the St- and J-genomes. Since CL101 is 80 % identical to the subtelomeric Spelt1-like repeat, it is likely that it may have a common origin with Spelt-1, which is common in *Triticum* and *Aegilops*. This repeat is characterized by significant variation in copy number between species (Pestsova et al., 1998; Ruban, Badaeva, 2018). The copy number of CL69 and CL101 in individual accessions is relatively high, reaching values of up to 3.9 and 5.3, respectively. This makes them suitable candidates for use as chromosomal markers in the FISH procedure. Further experiments using the FISH method will show whether these repeats can serve as chromosomal markers for identifying the St-subgenome in polyploid species, such as *E. pendulinus*, studying recombinant J^{vs}-genomes in intermediate wheatgrass and investigating chromosomal rearrangements in wide wheat hybrids.

The highest copy number value in *P. cognata* and *S. cereale* was demonstrated by CL184, which shows similarity to the *Cassandra* retrotransposon found in the rye genome. *Cassandra* is found in the genomes of many plant species and is characterized by significant differences in copy number between them (Kalendar et al., 2020). Since one of the mechanisms by which satellite repeats are propagated throughout the genome is through the movement of retroelements (Garrido-Ramos, 2021; Šatović-Vukšić, Plohl, 2023), it is possible that we have identified a repeat that has been retained as a consequence of *Cassandra* spreading along the ancestral lineage of the St- and R-genomes.

CL244, previously identified by us in the *Ae. crassa* genome, was characterized by a higher copy number in *Th. bessarabicum* than in common wheat, *Ae. crassa* and *Ae. tauschii* (Kroupin et al., 2022). In this study, the results were confirmed. However, at the same time, there was a significant variation in copy number between *Pseudoreogneria* species, which could be attributed to the elimination or accumulation of CL244 during speciation and subsequent evolution. CL244 has terminal localization on chromosome *Th. bessarabicum* (Kroupin et al., 2022), and can presumably accumulate or be eliminated in various species, similar to the terminal repeats of Spelt-1 and Spelt-52 in *Aegilops* and *Triticum* (Raskina et al., 2011; Ruban, Badaeva, 2018) or pSc200 and pSc250 in rye (Evtushenko et al., 2016).

CL220, which is specific to *P. kosaninii*, exhibited similarities with CL219, which we had previously identified in the *Ae. crassa* genome (Kroupin et al., 2022). CL186, specific to *P. cognata*, showed similarity to ACRI_CL80, which was identified in *A. cristatum* (P-genome) (Said et al., 2018). Both repeats probably arose before the divergence of Triticeae genomes from a common ancestor and accumulated in separate species at certain periods. Since CL219 and ACRI_CL80 were localized on separate chromosomes, it can be inferred that CL220 and CL186 will also exhibit chromosome-specific localization on the chromosomes of *P. kosaninii* and *P. cognata*, respectively.

We have identified repeats that vary in copy number between *Pseudoroegneria* species with varying levels of ploidy. For example, the octaploid *P. kosaninii* is characterized by a high copy number of CL115, CL190, and CL220, while the tetraploid *P. geniculata* has a high copy number of CL134. The observed differences in the abundance of the repeats may be attributed to polyploidization processes. This is because tandem repeats in the centromeric and terminal regions have a significant impact on chromosome recognition and divergence

during cell division. This is particularly relevant for homeological genomes in polyploid plants (March, 2019; Aguilar, Prieto, 2021). Such subgenome- and even chromosome-specific repetitive elements have been detected in polyploid species such as wheat, oats, and intermedium wheatgrass. These elements are apparently necessary for the differentiation of subgenomes during cell division (Shrama, Raina, 2005; Liu Z. et al., 2008; Divashuk et al., 2016; Lang et al., 2019b; Su et al., 2019).

Comparison of the repeat copy number patterns helped determine which of them have similar copy numbers among the studied accessions (see Supplementary Materials 2.3 and 2.4). CL3, CL115, CL119, CL190, and CL220 were grouped together because they exhibited the highest levels of copy number in *P. kosaninii*. CL95, CL207, and CL300 are more specific to *P. cognata* and *P. kosaninii*. In CL128, CL168, and CL186, the maximum copy number was observed in *P. cognata*. A comparison of the species copy number patterns allowed for a general understanding of which accessions are characterized by similar repeat copy numbers.

The overall clustering of *Pseudoroegneria* species (see Supplementary Materials 2.5 and 2.6) indicates that, in general, they exhibit similar copy numbers of repeats that are different from those of other species. The copy number pattern of *E. pendulinus* ($2n = 28$, StStYY) differed from that of *Pseudoroegneria*, suggesting that the St-specific repeats we discovered could be valuable for analyzing the St-subgenome of *E. pendulinus* and other *Elymus sensu lato* accessions. *Thinopyrum* and common wheat exhibited different copy number patterns. CL244 and CL69 can be utilized to identify wheat-wheatgrass hybrids and detect introgressions from all three studied wheatgrass species into the wheat genome. Similarly, CL134 and CL251 can be used for this purpose in *Th. junceum*.

L. Wang et al. (2017) found a repeat of St_2-80 in the genome of *P. libanotica*, hybridizing along the entire length of the St-(sub)genome chromosomes and only in the terminal regions of the E-, H-, P-, and Y-(sub)genomes. Q.-L. Liu et al. (2020) identified mobile elements in the genome of *P. stipifolia*, including dispersed repeats S13, S158, and S21, which showed varying intensity between the chromosomes of the St- and Y-subgenomes. They also found S5, which had a specific point localization and differed between the chromosomes of the St- and Y-subgenomes. D. Wu et al. (2022) created chromosomal markers STlib_96, STlib_98, and STlib_117 based on satellite repeats of *P. libanotica*. However, the possibility of using these markers for the analysis of allopolyploids with the St-genome is not reported.

Our assessment of the copy number of satellite repeats found in the St-genome and the determination of their amplification specificity between species can enhance the range of molecular genetic and cytogenetic markers utilized in studying the Triticeae tribe. The copy number of satellite repeats can vary significantly between species, populations, and even within them (Wang Q. et al., 2010; Belyaev, Raskina, 2013; Pollak et al., 2018; Tao et al., 2021). The satellite repeats identified in the present study can be useful, among other purposes, for population studies of *Pseudoroegneria* and Triticeae species.

Conclusion

Based on the data from whole-genome sequencing of *Pseudoroegneria* accessions, we identified 22 satellite repeats. In the genomes of 14 representatives of the Triticeae tribe, we determined their copy number, including CL244, which was previously discovered in *Ae. crassa*. As a result of the evaluation, the studied repeats were classified according to the level of abundance and variability between species. The satellite repeats identified in the present study can be used as molecular genetic markers for evolutionary, phylogenetic, and population studies of Triticeae. They also have the potential to serve as cytogenetic markers for *in situ* hybridization.

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