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DIFFERENCES IN PLOIDY LEVEL AND GENOME CONSTITUTION REVEALED BY CYTOGENETIC ANALYSIS OF *PSEUDOROEGNERIA* GERMPLASM ACCESSIONS: CASE STUDY

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Pseudoroegneria is a genus in Triticeae (Poaceae) consisting of diploid, auto- and allopolyploid species that are built around St genome. Total genomic DNA of St genome is used in molecular assays for fundamental and applied studies. It is important to verify whether an accession belongs to the species shown in the catalogue or label. The study of plant material from Germplasm Resources Information Network (GRIN) have shown that the P. spicata accession PI 635993 is a diploid (2n=14), the P. spicata accession PI 537371 is a tetraploid (2n=28), the P. strigosa accessions PI 639805 and PI 595164 are hexaploids (2n=42). Genomic in situ hybridization revealed that each of the polyploids PI 537371 and PI 639805 possesses one St genome and one or two unidentified genomes, respectively.

Key words: Pseudoroegneria, ploidy level, chromosome number, germplasm, genomic in situ hybridization.

Pseudoroegneria is a genus in Triticeae (Poaceae) consisting of nearly 15 species (for review see the information source [20]). Genus is built around one genome designated St and consists of diploid (2n=14) and tetraploid (2n=28) species. Among others diploid species are represented by *P. strigosa* (M. Bieb.) Á. Löve (St) and *P. spicata* (Pursh) A. Löve. Polyploids are autotetraploids (StSt) or near-autotetraploids (St_1St_2). St genome is one of the basic genomes in perennial *Triticeae* and is believed to be a candidate donor genome of the species in Douglasdeweya (StP), Roegneria (StY), Elytrigia (ESt), Thinopyrum (JJsSt), Elymus (StH, StYH and StYW), Kengyilia (StYP), and Pascopyrum (StHNsXm) [20].

Species carrying St subgenome is an important gene pool for genetic improvement of cereal crops. In particular, *Thinopyrum intermedium*, *Th. ponticum* and Elymus species are used for wheat and barley breeding for developing resistance to biotic and abiotic stresses via intergeneric hybridization [5, 9, 20]. At the same time, a great majority of the St-bearing wild grasses possessing valuable traits such as resistance to pests, fungal and viral diseases, tolerance to salinity and drought are still to be assessed and used in breeding process.

The phylogenetic relationship and taxonomic position of Pseudoroegneria and its relative species are still in dispute [22, 24]. The study of the phylogenetic relationship between wild Triticeae grasses is of greatest importance. Firstly, it provides the informa-

tion on genetic differentiation, polyploidization and evolution trajectories of Triticeae species [10, 11, 24]. Secondly, the knowledge of genomic constitution and homeology degree between the chromosomes of wild grass and cultivated species may help to estimate the success of transfer of valuable traits that occurs via chromosome pairing at meiosis [5, 15]. Finally, the proper identification of alien chromosome introgression facilitates the application of the genetic stocks in breeding and the development of commercial cultivars [17].

The complex of approaches is used for distinguishing Pseudoroegneria-related species and the establishment of their evolutional relationships. The morphological features of grasses (leaves, glumes, spikelets, rachis and others) in certain cases can hardly be applied as reliable introgeneric distinctions in the closely related species where such features differ very insignificantly [24]. The genomic constitution suggested by Löve [12] and Dewey [3] as the main taxonomical criteria consequently has been widely accepted. The widely used technology for determining the genomic constitution is genomic *in situ* hybridization (GISH) using labeled total DNA of St-genome [2, 7, 15]. GISH procedure is applied to analyze the contribution of Pseudoroegneria genome into genomic constitution of investigated polyploids. Molecular approaches involve the comparison of polymorphism in internal transcribed spacer (ITS) region of nuclear ribosomal DNA [11, 13, 14, 22]; granule-bound starch synthase gene [15], plastid-specific genes [11, 23, 24], the repetitive sequences [10, 16, 19] and others.

In evolutionary and/or breeding studies the Pseudoroegneria germplasm is used as a source of St-genome DNA for GISH or as a source of St-derived nuclear and plastid genes. The basic prerequisite successful study is the use of properly identified plant material of Pseudoroegneria and its genomic constitution. The botanical identification of Pseudoroegneria species is difficult and germplasm bank accessions needs to be analyzed by application of different approaches.

The aim of this work was to determine the chromosome number and to identify St genome in two accessions of *P. strigosa* and two accessions *P. spicata* obtained from the Germplasm Resources Information Network (GRIN).

Materials and methods

Two accessions of *P. strigosa* (PI 595164, PI 639805) and two accessions *P. spicata* (PI 635993, PI 537371) were used in this study. Seeds of the accessions were kindly provided by the Germplasm Resources Information Network (GRIN) of the United States Department of Agriculture (USDA). The accessions numbers and geographical origin of the accessions are listed in the Table 1.

Table 1

List of Pseudoroegneria species

Species	Accession No	Origin
P. strigosa ssp. aegilopoides	PI 595164	Xinjiang, China
P. strigosa	PI 639805	Mongolia
P. spicata	PI 635993	Washington, United States
P. spicata	PI 537371	Idaho, United States

Protocols for chromosome preparation, *in situ* hybridization and signal detection were described previously [6, 8].

Results and Discussion

The chromosome number was established for four presumably diploid Pseudoroegneria accessions ordered via GRIN: two accessions of *P. spicata* (PI 635993, PI 537371) and two accessions of *P. strigosa* (PI 595164, PI 639805. Only the *P. spicata* accession PI 635993 was a diploid one having 14 chromosomes (Fig.1a). The *P. spicata* accession PI 537371 had 28 chromosomes (tetraploid; Fig. 2a). The *P. strigosa* accessions PI 595164 and PI 639805 had 42 chromosomes (hexaploids; Fig. 1b and 2b).

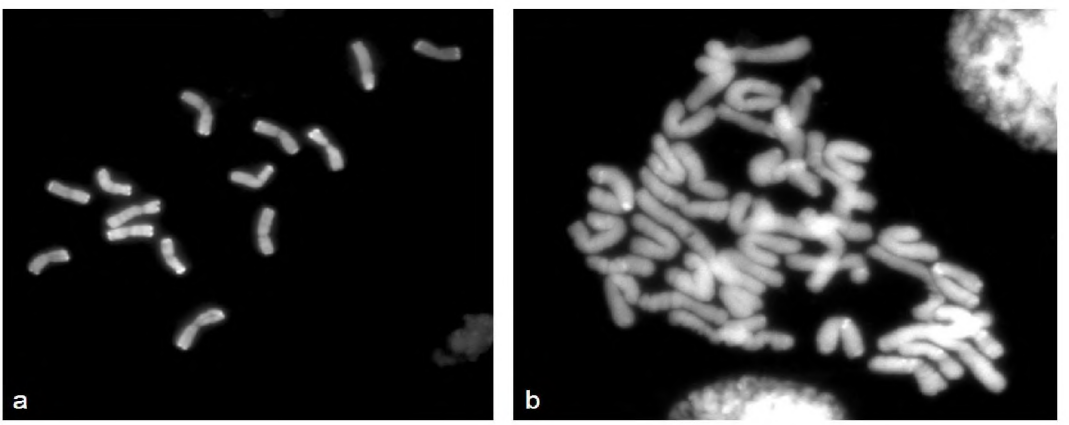

Fig. 1. Mitosis metaphase chromosomes of *P. spicata* accession PI 635993 (a) and *P. strigosa* accession PI 595164 (b)

Thus, our analysis showed that only one out of four *Pseudoroegneria* accessions was diploid. Particular attention should be paid to the *P. strigosa* accession PI 595164, as it is listed as a diploid species in the table of the used materials [23]. In our study it was shown that it possesses 42 chromosomes. Such discrepancy may be due to the fact that our PI 595164 was a spontaneous auto- or allopolyploid, to heterogeneity of the accession or technical issues.

Pseudoroegneria species is represented by diploids (St) and auto- (StSt) and/or near autotetraploids (St_1St_2) [20]. If a genomic constitution of particular St carrying accession is diploid or even autopolyploid, it may be useful in different molecular and cytogenetic assays: preparing genomic DNA probes, cloning genes, molecular phylogenetic analysis, and other purposes. Difficulties may arise in the estimation of phenotypic response to biotic and abiotic stresses, as diploids and polyploids show different resistance [21]. Besides, using such accession can result in discrepancy in the data of molecular phylogenetic analyses.

Therefore, to establish auto or allopolyploid nature of the studied accessions we performed genomic *in situ* hybridization (GISH) on the tetraploid accession PI 537371 and hexaploid accession PI 639805 with total genomic DNA of the *P. spicata* accession PI 635993 (14 chromosomes) as a probe to identify St genome. GISH revealed that the studied

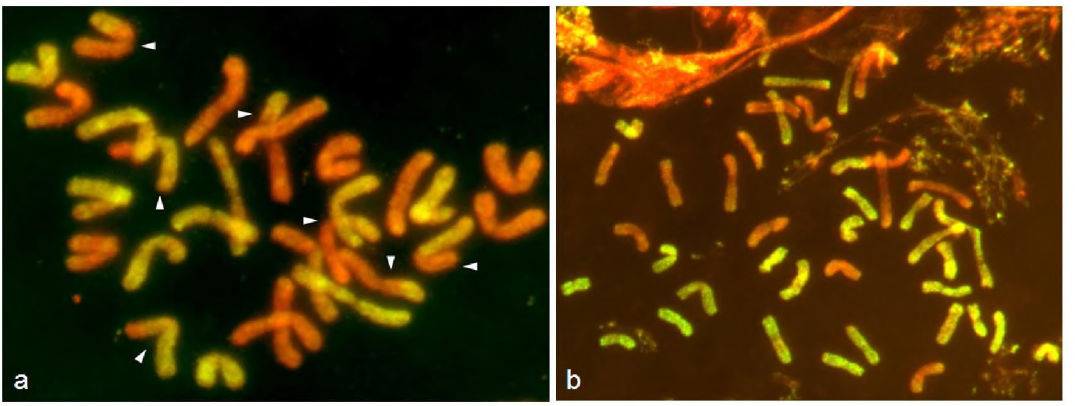

Fig. 2. GISH on the root-tip cells at mitotic metaphase in *P. spicata* PI 537371 (a) and *P. strigosa* PI 639805 (b). Yellow or greenish yellow fluorescence with FITC corresponds to sites of St-probe hybridization, red fluorescence with propidium iodide counterstain corresponds to sites blocked with DNA of bread wheat. The translocated chromosomes are indicated by arrowheads

accessions are allopolyploids and not autopolyploids (Fig. 2). The accession PI 537371 is an allotetraploid (2n=28) (Fig. 2a). The St-probe hybridized with 10 chromosomes, which, therefore, belong to St genome. The St-signal was absent on 11 chromosomes, which means that they belong to another unidentified genome. Seven chromosomes possessed the St-signal on their separate regions, i.e. they resulted from the translocation/recombination between chromosomes of St and the unidentified genomes (Fig. 2a, shown with arrows). The translocations may have occurred in either somatic cells or at the formation of unreduced 2n gametes in the initial interspecific hybrid [4]. The formation of such gametes was described in interspecific hybrids of the tribe *Triticeae* [18].

GISH revealed that the accession PI 639805 is an allohexaploid (2n=42) (Fig. 2b): the St-probe hybridized with 28 chromosomes; 14 did not show St-signal, consequently, they belong to the unidentified genome (Fig. 2b).

In conclusion, we showed that among the four analyzed accessions only PI 635993 was found to be a diploid one. The other three accessions were polyploid, in particular, allopolyploids, as was revealed by GISH. These polyploids may be either misidentified or confused with other species [1] or were treated using previous rules of taxonomy [20] or even evolved as a spontaneous hybrid. In order to avoid false results using the accessions of wild grasses for breeding purposes or for molecular and cytogenetic studies of phylogenetic relationship and taxonomic position of Pseudoroegneria and its relative species it is necessary to carry out preliminary cytological assays (at least chromosomes counting) to verify whether the accession belongs to the species shown in the catalogue or label. This procedure is important even when the seeds are obtained from seed banks or for accessions previously validated in other studies unless they are obtained directly by the researchers themselves.

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ЦИТОГЕНЕТИЧЕСКИЙ АНАЛИЗ УРОВНЯ ПЛОИДНОСТИ И ГЕНОМНОЙ КОНСТИТУЦИИ У ОБРАЗЦОВ *PSEUDOROEGNERIA* ИЗ ГЕНЕТИЧЕСКИХ БАНКОВ СЕМЯН

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Псевдорогнерия (Pseudoroegneria) относится к трибе Пшеницевых семейства Злаки (Poaceae: Triticeae). В ее состав входят диплоидные, авто- и аллополиплоидные виды, несущие St геном. Тотальная геномная ДНК St генома используется в молекулярных анализах при проведении фундаментальных и прикладных исследований. При этом необходимо проверять, соответствует ли видовая идентичность используемого образца указанной в каталоге или на этикетке. Исследование растительного материала, заказанного в системе Germplasm Resources Information Network (GRIN), установило, что образец P. spicata PI 635993 является диплоидом (2n=14), образец P. spicata PI 537371 – тетраплоидом (2n=28), образцы P. strigosa PI 639805 и PI 595164 – гексаплоидами (2n=42). Анализ с помощью геномной гибридизации in situ показал, что полиплоиды PI 537371 и PI 639805 в дополнение к St геному несут соответственно один и два неидентифицированных генома.

Key words: Pseudoroegneria, псевдорогнерия, число хромосом, банк семян, геномная гибридизация in situ.

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