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SHORT COMMUNICATIONS

Sequencing and Annotation of the Chloroplast Genome of *Triticum militinae*—A "Natural Mutant" of Tetraploid Wheat *Triticum timopheevii* Zhuk.

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Abstract—*Triticum militinae* Zhuk. et Migusch., a tetraploid wheat with the GAA genome, is considered a natural naked mutant of *Triticum timopheevii* Zhuk. Previously, the karyotype and crossing characteristics of this wheat were examined. To clarify the origin and relationships of *T. militinae* with other representatives of the wheat family, analysis of its chloroplast genome, which remained unexplored, is of great interest. In the present study, for the first time, sequencing and annotation of the complete chloroplast genome of *T. militinae*, the size of which was found to be 135898 bp, was conducted. The plastome of this wheat is composed of two inverted repeats, each of 21552 bp in length, the small single-copy (SSC) region of 12791 bp, and the large single-copy (LSC) region of 80003 bp. The chloroplast genome of *T. militinae* contains 132 annotated structural genes, of which 85 genes are protein-coding, 31 are tRNA genes, and four genes code for rRNA.

Keywords: wheat, *Triticum militinae*, *Triticum timopheevii*, chloroplast genome, sequencing

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Tetraploid wheat *Triticum militinae* Zhuk. et Migusch. (the Militina's wheat) is considered a natural mutant of the tetraploid wheat *Triticum timopheevii* Zhuk. The process of discovery of the Militina's wheat from the moment of "segregation" of a certain mutant in *T. timopheevii* crops in 1950 until the report on a new species called *T. militinae* Zhuk. et Migusch. in 1969, took almost two decades [1]. However, the origin of this species remained mysterious. The results from our previous study provided conclusion that this could be some kind of a hybrid of the Persian and Timopheev's wheat [2]. A similar suggestion was made earlier by N.A. Navruzbekov, who showed that *T. militinae* displayed similarities with *T. persicum* ssp. *fuliginosum* and that the F_1 progeny from the cross of the latter with *T. timopheevii* formed dense black spikes, similar to those of the Militina's wheat [3]. N.A. Navruzbekov concluded that *T. militinae* was a product of introgression of a part of the genome of *T. persicum* into the genome and cytoplasm of *T. timopheevii*, and considered it a product of crossing of these two species. However, which of these species acted as the maternal form of *T. militinae* can be determined by analyzing the chloroplast genome sequences of these wheats. Since no information on the plastome of *T. militinae* was available so far, the objective of this study was sequencing and annotation of the chloroplast genome of the Militina's wheat.

The seeds of *T. militinae* var. *albimilitinae* k-59942 (Belarus) were provided by the Federal Research Center All-Russian Institute of Plant Genetic Resources (VIR, St. Petersburg). The plants were germinated in a greenhouse at 21°C for three weeks. Then, about 20 g of green leaves were collected. Next, these leaves were placed in cold (4°C) and darkness for 48 h to reduce the starch content.

Chloroplast DNA was extracted from the leaves using the sucrose gradient-based method [4] with some modifications. For example, at the stage of isolating chloroplast DNA from intact chloroplasts, Triton X-100 in a concentration of 0.15% to the final volume of specimens was used, and then the phenol—chloroform extraction method was used [5]. Homogenization of the leaves was carried out using a mortar and pestle in liquid nitrogen.

At the initial step of sequencing, quality control of the isolated chloroplast DNA was carried out. For this purpose, DNA concentration was measured on a NanoDrop OneC spectrophotometer (Thermo Fisher Scientific, United States) and the A260/280 and A260/230 ratios were assessed. The amount of DNA

was measured using fluorimetric method with the SpectraQ BR kit (Raissol, Russia) and a Qubit 4.0 fluorimeter (Thermo Fisher Scientific). DNA libraries were prepared using the shotgun technique with the SG GM Plus kit (Raissol). Sequencing was performed using 200 ng of chloroplast DNA. At the first step of preparation, enzymatic fragmentation of chloroplast DNA was carried out to a fragment size of 350 bp with simultaneous end repair. At the second step, the adapters were ligated and the reaction product was purified using Smart beads (Raissol) magnetic particles. At the third step, index PCR and purification of the reaction product using Smart beads (Raissol) magnetic particles were carried out. Quality control of the resulting DNA library was carried out using a Qsep400 bioanalyzer (Bioptic, Taiwan). Fluorimetric measurement of DNA library concentration was performed using a SpectraQ BR kit (Raissol) and a Qubit 4.0 fluorimeter (Thermo Fisher Scientific). DNA libraries were pooled and sequenced using the FASTASeq 300 Sequencing Kit V1.0 100 M reads/flow cell on a Genolab M sequencer (GeneMind, China). Sequencing mode: 2 × 150 bp. The base format files were demultiplexed on a Genolab M instrument to obtain files with the fastq extension. A total of 13 million reads were obtained. Sequence reads were cleaned using the Trimmomatic v0.22 program [6]. Reads containing unattributable "N" nucleotides were trimmed. The plastome was assembled using the NOVOwrap program [7] using the chloroplast genome sequence of *T. timopheevii* (KJ614408) as a reference. The complete chloroplast genome of *T. militinae* was annotated using the Chloroplast Genome Annotation, Visualization, Analysis, and GenBank Submission 2 (CPGAVAS2) resource (http://47.96.249.172:16019/analyzer/home) [8]. The circular map of the chloroplast genome was visualized using the OGDRAW program (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) [9].

The chloroplast genome of *T. militinae* var. *albimilitinae* was found to be 135898 bp in size. The length of the inverted repeat region was 21552 bp, the length of SSC region was 12791 bp, and the length of LSC region was 80003 bp. The GC content in the whole plastid genome of *T. militinae* was 38.3%; in the SSC region it was 32.19%, and in the LSC region, 36.28%. In the IR region of *T. militinae*, the GC content reached 43.91%.

In the Militina's wheat, a total of 132 structural genes were annotated, including 85 protein-coding genes, 31 tRNA genes, and four rRNA genes. Seven protein-coding genes (*rps19*, *rpl2, rpl23, ndhB, rps7, rps12, rps15*), eight tRNA genes (*trnH-GUG*, *trnM-CAU*, *trnL-CAA*, *trnV-GAC*, *trnT-CGU*, *trnA-UGC*, *trnR-ACG*, *trnN-GUU*), and four rRNA genes (*rRNA4.5*, *rRNA23*, *rRNA16*, and *rRNA5*) were duplicated as part of IR region. Moreover, nine protein-coding genes and one tRNA gene were found in the SSC region, and 69 protein-coding genes and 22 tRNA genes were found in the LSC region. In addition, among 132 genes, 14 had one intron (*trnK-UUU, rps16, trnS-CGA, atpF, trnV-UAC, petB, petD, rpl16, rpl2, ndhB, trnI-GAU, trnA-UGC, ndhA, trnL-UAA*) and one gene (*ycf3*) had two introns. The *trnK-UUU* gene had the largest intron (2558 bp), within which there was another gene, *matK*. The chloroplast genome of *T. militinae* also contains a trans-spliced *rps12* gene. All obtained results are presented in Fig. 1 in the form of a circular structure obtained using the OGDRAW software program. Different color blocks reflect the belonging of genes to certain functional groups.

The origin of the Militina's wheat is of great interest. Most often it is considered a mutant of the Timopheev's wheat. At the same time, sequencing of the chloroplast genome of this species and its comparison with other similar wheat genomes shows that *T. militinae* is most likely not a mutant, but a hybrid, which we paid special attention to in our previous study [2]. However, for final conclusions, additional studies of the chloroplast genomes of other wheats, especially of *T. persicum*, are required. The nucleotide sequence of the chloroplast genome of *T. militinae* was deposited in GenBank and is available under the accession number OR936057.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

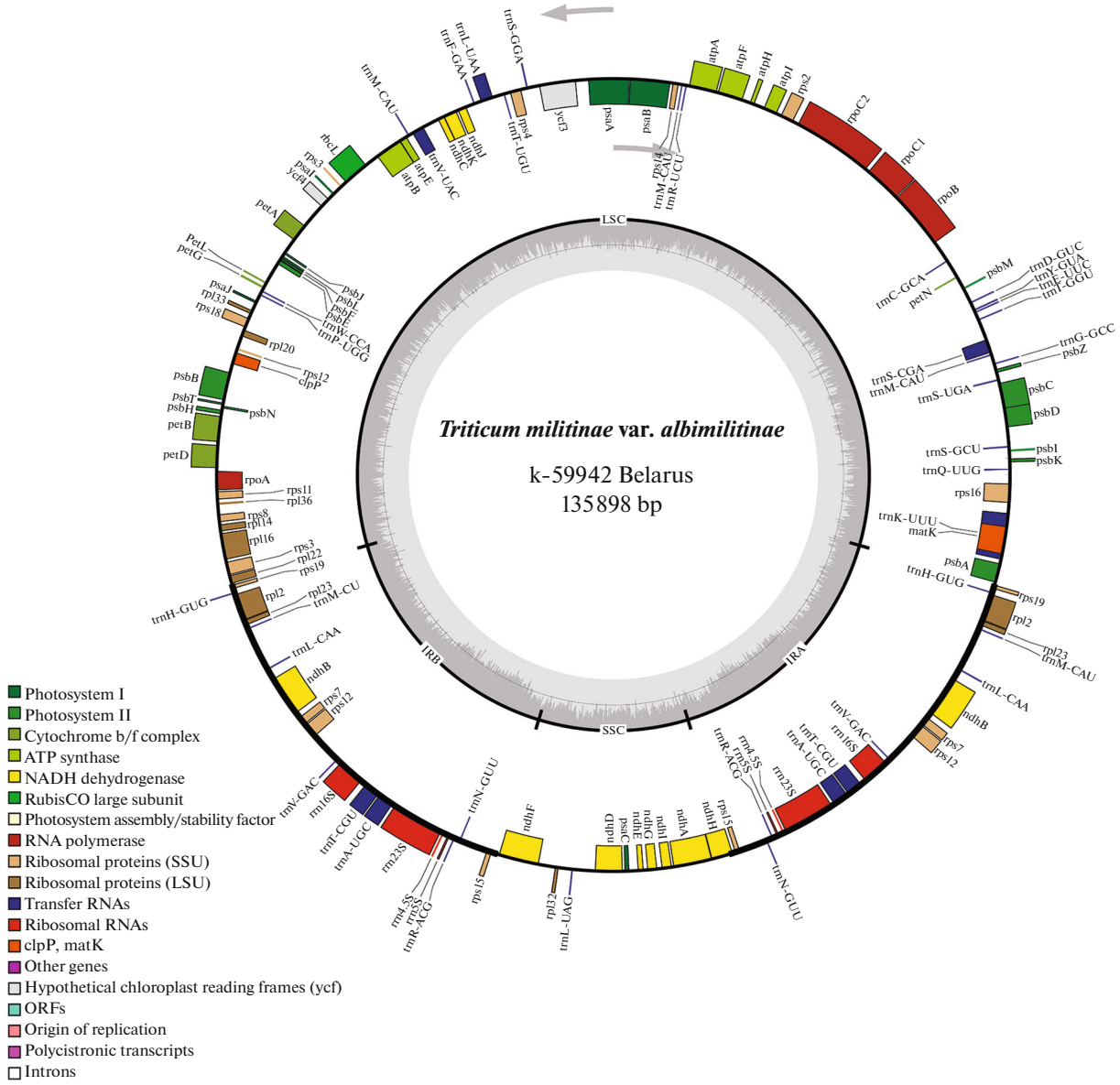

Fig. 1. Circular representation of the chloroplast genome of *T. militinae* var. *albimilitinae* k-59942. The plastome was visualized using the OGDRAW resource. Genes are shown in different colors; the area in the inner gray circle indicates the percentage of GC content. IRA, inverted repeat region A; IRB, inverted repeat region B. Genes located outside the outer circle are transcribed clockwise, and those inside are transcribed counterclockwise.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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