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Studies on chloroplast and nuclear rDNA in hexaploid bread wheat and its relatives

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ABSTRACT Though allohexaploid bread wheat (*Triticum aestivum*) is grown on more acreage than any other cereal crop, its evolutionary history and origin of its three genomes have not been cleared up in every detail. The wheats form a polyploid series with diploid, tetraploid and hexaploid forms. Hexaploid wheats (AABBDD) may have evolved by hybridization between the AABB tetraploid as cytoplasm donor and the D genome diploid *Aegilops tauschii*. The origin of B genome is still a matter of debate. In the present study sequences of chloroplast and nuclear rDNA regions were used for giving new data to contribute to our knowledge on evolution of wheat. Analysis of cloned nrITS sequences of *T. aestivum* showed that 1) more than one ITS sequence type can be derived from the same sample, 2) a rye chromosome element formerly introgressed into wheat genome can be detected and 3) a partial segment of ITS similar to that of einkorn wheats (AA genome) can be identified. These results give evidences for ancient and recent introgressions and could help us to identify all of the three genomes of hexaploid bread wheat.

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KEY WORDS

wheat
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The wheat genera *Aegilops* and *Triticum* form a polyploid series of diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) species. The hexaploid bread wheat (*T. aestivum*, AABBDD) originated from hybridization between the wild diploid donor of the D genome, *Ae. tauschii* (2n = 2x = 14, DD) and the early domesticated tetraploid *T. turgidum* ssp. *dicoccum* (2n = 4x = 28, AABB). Since allohexaploid bread wheat is grown on more acreage than any other cereal crop, it is in the foreground of the scientific research. Nonetheless, its origin and evolutionary history have not been cleared up in every detail, for example the origin of B genome is still a matter of debate. Polyphyletic origin and/or divergent evolution of B genome are hypothesized.

In the last decade numerous molecular markers and techniques were used for studies on origin, evolution and relationships in the wheat group: chloroplast and nuclear microsatellite markers (Lelley et al. 2000 and Ishii et al. 2001, respectively), chromosome-specific low-copy DNA (Liu et al. 2003), nuclear genes (Blake et al. 2004; Caldwell et al. 2004) and nrITS (Wang et al. 2000) among others.

In the present study sequences of three loci were used: chloroplast (cp) 16S rRNA genes, spacer regions between the cp23S and 5S rRNA genes and nuclear ribosomal ITS (nrITS). The main goal of the work was to give new data to contribute to our knowledge on evolution of the three genomes of hexaploid bread wheat.

Materials and Methods

Plant materials *Triticum aestivum* Mv15, *T. turgidum* ssp. *dicoccum* MvGB 300, *T. urartu* MvGB 110, *T. monococcum* MvGB 515, *Aegilops tauschii* MvGB 363, *Ae. speltoides* MvGB 621were sourced from Agricultural Research Institute of the Hungarian Academy of Sciences (Martonvásár, Hungary). Samples *T. aestivum Pioneer*, *Secale cereale*, *Poa pratensis* (as an outgroup in trees) were used as reference or outgroup sequences from other works.

For conditions of DNA extraction, PCR amplification, sequencing and sequence analysis see Rudnóy et al. 2002, 2004. ITS PCR products derived from *T. aestivum* Mv15 were ligated and transformed using the pGEM–T Easy Vector System II (Promega). Transformed cells were spread on ampicillin LB-agar and white colonies were directly screened by PCR for the presence of inserts of the expected size.

Results and Discussion

The origin of the genomes in hexaploid wheats has thoroughly been examined; however, the source of genomes, especially the donor of B genome is still a matter of debate.

16S rRNA genes of *T. aestivum, T. turgidum, Ae. speltoides* and *Ae. tauschii* were formerly studied in our laboratory (Rudnóy et al. 2002) and identical sequences or only insignificant differences were found. As the spacers are usually more variable compared to genes, the cp ribosomal spacer region between the 23S and 5S rRNA genes was chosen for sequence analysis. However, this cpITS region is proved to be at least as conservative as the genes: no differences were found among the cpITS sequences of *T. aestivum, T. turgidum, Ae. speltoides* and *Ae. tauschii*.

Better resolution was achieved using nrITS (Rudnóy et

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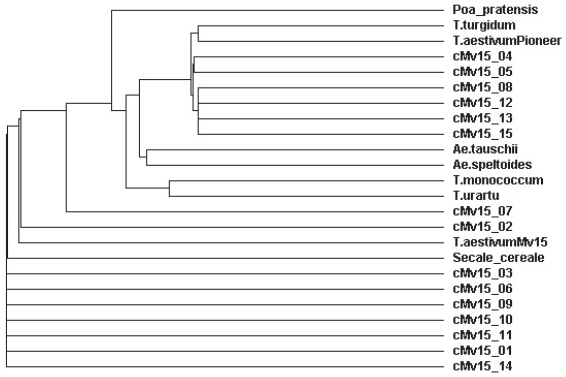

Figure 1. Cladogram of the species studied, cloned ITS sequences of *T. aestivum* Mv15, *T. aestivum* Pioneer and *Secale cereale* as references and *Poa pratensis* as an outgroup. The tree was constructed by the ClustalW program (default parameters). Wheat–like clones of Mv15 ITS are clustered with *T. aestivum* Pioneer and *T. turgidum* while rye–like ones with Mv15 and *Secale cereale*.

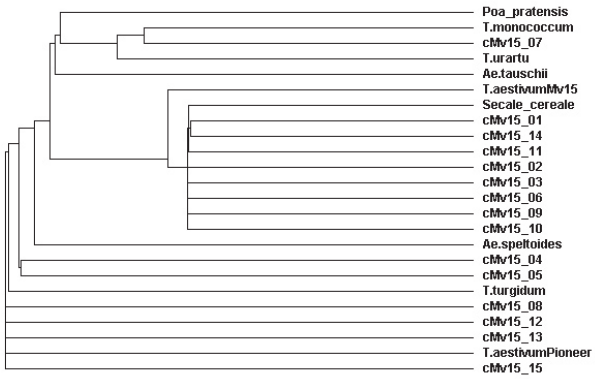

Figure 2. Cladogram of ITS1 sequences of species studied, Mv15 clones, *T. aestivum* Pioneer and *Secale cereale* as references and *Poa pratensis* as an outgroup. The tree was constructed by the ClustalW program (default parameters). Position of cloneMv15_07 is different compared to Figure 1: it is significantly similar to ITS1 of einkorn wheats (*T. urartu* and *T. monococcum*).

al. 2004), which is one of the most popular sequences for recent evolution studies. However, some open questions have remained: (1) ITS sequence derived from *T. aestivum* strain Mv15 containing an additional rye chromosome element (1RS) showed significant similarity to not only other wheat ITS sequences but also to rye ITS. Accordingly, it seems to be a chimeric sequence. (2) The results confirmed *Ae. tauschii* (presumptive D genome donor) and *T. urartu* (presumptive A genome donor) to be close relatives of hexaploid wheat but did not appoint one or more species as probable donor(s) of genome B.

Comparison of sequence distance data of wheat and its relatives does not provide adequate inferences. Getting inner information by cloning directly from three genomes of the hexaploid wheat could be a novel approach. Chimeric characteristics of Mv15 ITS sequence raised the issue of mixed feature of sequences derived from wheat, *i.e.* an allopolyploid plant containing different genetic series. If concerted evolution has failed to homogenize the paralogous sequences, cloning could resolve the problem.

ITS sequences were obtained from 15 clones of Mv15. The cloned sequences could be divided into two groups: wheat (*T. aestivum*) –like and rye–like ITS (Fig. 1).

However, we found a cloned ITS sequence (namely cloneMv15_07) having mixed structure: its second half, *i.e.* ITS2 region is rye–like but its ITS1 spacer is very similar to ITS1 sequences of einkorn wheat species, *T. urartu* and *T. monococcum* (Fig. 2).

The A genome of the hexaploid wheat is originated from the einkorn lineage so it can be a residue of the A genome donor species involved in the former polyploidization. By analysis of cloned ITS sequences we found genetic traces of recent and ancient introgression events: ITS1 sequence similar to diploid A genome species and entire ITS sequences similar to or almost identical to rye ITS.

Further refinement of this procedure, *i.e.* sampling and sample preparing, PCR parameters and cloning conditions is our future project. This method could provide molecular evidence for accurate identification of progenitors of the three genomes including the uncertain donor(s) of B genome.

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