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Chloroplast 16S rRNA sequences from different *Triticum* species

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ABSTRACT The wheats (*Triticum* spp.) form a polyploid series with diploid, tetraploid and hexaploid forms. The wild allotetraploid emmer wheat *T. turgidum* ssp. dicoccoides (AABB) arose by amphyploidy between the wild diploid wheat *T. urartu* (AA) and a diploid member of the *Aegilops* genus (BB). The origin of B genome is still a matter of debate. Hexaploid wheats (AABBDD) may have evolved by hybridisation between the AABB tetraploid as cytoplasm donor and the D genome diploid *Ae. taushii*. The source of the genomes in hexaploid wheats has been thoroughly investigated. Small subunit rRNAs are widely used for the evaluation of genetic diversity or relatedness between different species. In this work small subunit 16S rDNA sequences from chloroplasts of hexaploid *T. aestivum* possible ancestors were examined. Sequences of 16S rDNAs in the plastids of *Ae. taushii* (DD), *Ae. speltoides* (BB) and *T. dicoccum* (AABB) were determined. They share complete identity in their 16S rDNA sequence and differ from 16S rDNA of T. aestivum by a single position of the gene.

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KEY WORDS

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The wheats (*Triticum* spp.) form a polyploid series with diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) forms. The diploid wheats comprise a single genomic group with the genome formula AA (*T. monococcum*, *T. urartu*). The tetraploid emmer wheats are divided into two groups, those with the genome formula AABB (*T. turgidum*) and those with the genome formula AAGG (*T. timopheevi*). On evidence it appears that the wild allotetraploid emmer wheat *T. turgidum* ssp. dicoccoides (AABB) arose by amphyploidy between the wild diploid wheat *T. urartu* (AA) and a diploid member of the *Aegilops* genus (BB). The origin of B genome is still a matter of debate. Polyphyletic origin or divergent evolution of B genome from the donor species are hypothesized. On the basis of chondriome divergence *Ae. speltoides* seems to be the cytoplasm donor (female parent) of the tetraploid wheats (Wang et al. 2000).

A descendant of *T. turgidum* ssp. dicocoides, the *T. turgidum* ssp. dicoccon was probably the ancient tetraploid from which hexaploid wheats (AABBDD) may have evolved by hybridisation between the AABB tetraploid as cytoplasm donor and the D genome diploid *Ae. taushii*. (Bálint et al. 2000). The source of the genomes in hexaploid wheats has been thoroughly investigated by variations in isoenzymes (Dvorak et al. 1998), RLFP analysis (Liu and Tsunewaki 1991), and the rRNA gene non-transcribed spacer (Appels and Dvorak 1982). Small subunit rRNAs are also widely used for the evaluation of genetic diversity or relatedness between different species and the database comprises more than 20 000 complete or nearly complete bacterial, archeal, plastid and mitochondrial sequences (Wuyts et al. 2002).

In the bulk of higher plants the organelles exhibit maternal inheritance. Since hexaploid wheat cultivars may have arisen from different ancestors the aim of this work was to determine the small subunit rRNA sequences from chloroplasts of *T. aestivum* and its possible ancestors in order to reveal the origin of the plastids.

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Materials and Methods

DNA isolation and PCR amplification: Chloroplasts DNA was isolated from 2-3 weeks old seedlings of *T. dicoccum*, *Ae. speltoides*, *Ae. taushii* by plant DNAzol Reagent (GIBCO BRL) according to the manufacturer's instructions. PCR amplification was carried out as described by Henrion et al. (1992). The sequences of the oligonucleotide primers used for the PCR amplifications were:
(24F) 5'-ATGGAGAGTTCGATCCTGGC and
(1529R) 5'-GCGTGAAGAAGTGTCAAACC.

Sequencing: For direct sequencing PCR products were purified with Prep-A-Gene DNA Purification Kit (Biorad). For cycle sequencing ABI Prism BigDye Kit (Applied Biosystems) was used and the electrophoresis was executed on ABI PRISM 310 Genetic Analyser (Applied Biosystems) according to the manufacturer's instructions. Primers used for the direct sequencing were:
(F356) 5'-TTCCGCAATGGGCGAAAGC,
(F710) 5'-CCGACACTGACACTGAGAG,
(F1056) 5'-GTTAAGTCTCGCAACGAGC,
(R425) 5'-TCCTCAGATACCGTCAT.

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. Work is thus underway in our laboratory to determine the small subunit rRNA sequences from chloroplasts of *T. aestivum* and its possible ancestors in order to reveal the origin of the plastids and the possible cytoplasm donor

TGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCTTAACACATGCAAGTCGAACGGGAAGTGGTGTT
TCCAGTGGCGAACGGGTGAGTAACGCGTAAGAACCTGCCCTTGGGAGGGGAACAACAACTGGAAACGGTTGC
TAATACCCCGTAGGCTGAGGAGCAAAAGGAGAAATCCGCCCAAGGAGGGGCTCGCGTCTGATTAGCTAGTTGG
TGAGGTAATAGCTTACCAAGGCGATGATCAGTAGCTGGTCCGAGAGGATGATCAGCCACACTGGGACTGAGAC
ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATGC
CGCGTGGAGGTGGAAGGCCTACGGGTCGTCAACTTCTTTTCTCGGAGAAGAAACAATGACGGTATCTGAGGAA
TAAGCATCGGCTAACTCTGTGCCAGCAGCCGCGGTAAGACAGAGGATGCAAGCGTTATCCGGAATGATTGGGCG
TAAAGCGTCTGTAGGTGGCTTTTCAAGTCCGCCGTCAAATCCCAGGGCTCAACCCTGGACAGGCGGTGGAAAC
TACCAAGCTGGAGTACGGTAGGGGCAGAGGGAATTTCCGGTGGAGCGGTGAAATGCATTGAGATCGGAAAGA
ACACCAACGGCGAAAGCACTCTGCTGGGCCGACACTGACACTGAGAGACGAAAGCTAGGGGAGCAAATGGGA
TTAGAGACCCCAGTAGTCCTAGCCGTAAACGATGGATACTAGGTGCTGTGCGACTCGACCCGTGCAGTGCTGTA
GCTAACGCGTTAAGTATCCCGCCTGGGGAGTACGTTCGCAAGAATGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAAAGCGAAGAACCTTACCAGGGCTTGACATGCCGCGAAT
CCTCTTGAAAGAGAGGGGTGCCCTCGGGAACGCGGACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGCCGT
AAGGTGTTGGGTTAAGTCTCGCAACGAGCGCAACCCTCGTGTTTAGTTGCCACTATGAGTTTGGAACCCTGAA
CAGACCGCCGGTGTTAAGCCGGAGGAAGGAGAGGATGAGGCCAAGTCATCATGCCCCTTATGCCCTGGGCGAC
ACACGTGCTACAATGGGCGGGACAAAGGGTCGCGATCTCGCGAGGGTGAGCTAACTCCAAAAACCCGTCCTCA
GTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCAGGAATCGCTAGTAATCGCCGGTCAGCCATACGGCGG
TGAATCCGTTCCCGGGCCTTGTACACACCGCCCGTCACACTATAGGAGCTGGCCATGTTTGAAGTCATTACCCTT
AACCGTAAGGAGGGGGATGCCTAAGGCTAGGCTTGCGACTGGAGTGAAGTCGTAACAAGGTAGCCGTACTGGA
AGGTGCGGCTGGATCACCTCCTTT

Figure 1. The sequence of chloroplast 16S rDNA from *Ae. taushii*, *Ae. speltoides* and *T. dicoccum*. Differences from Chinese spring chloroplast 16S rDNA sequence are underlind in grey and the single nucleotide difference from hexaploid *T. aestivum* is underlined and bold.

female parents of present day hexaploid bread wheats. Sequence determination of the *T. aestivum* chloroplast rDNA was the first step towards achieving this aim. A 1482 bp region of 16S rDNA (AJ239003) was sequenced (Kovács et al. 1999).

In our present work the sequences of 16S rDNA of *Ae. taushii* (*Ae. squarrosa*) (DD), *Ae. speltoides* (BB) and *T. dicoccum* (AABB) were determined. They proved to be identical with our previously sequenced *T. aestivum* (AABBDD) chloroplast 16S rDNA except the position 1056 of the gene. The genome of *T. aestivum* chloroplast has also been solved recently (Ogihara et al 2002). Our sequences differed from the rDNA gene sequence (AB042240) of this plastome in two positions (Fig. 1). These are the positions 9 and 15 of the gene and 1 and 7 of our sequence, which begins at the position 9 of the complete gene.

The sequences of 16S rDNA of *Ae. taushii*, *Ae. speltoides*, *T. dicoccum* and *T. aestivum* also share almost complete identity with oat chloroplast rDNA. Differences were only found in positions 261 and 420 as compared to the oat chloroplast rDNA.

According to our results the cytoplasm donor female parent either seems to be identical in the ancestors examined, or the closely related ancestors are indiscernible at this level of highly conservative macromolecules. However, the list has not been completed yet and the determination of the 16S rDNA sequences from the other possible ancestors needs further examinations and is underway in our laboratory.

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