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Plant Gene Register

RNA Editing of the Mitochondrial *atpA/atp9* Co-Transcript of Triticale, Carrying the *timopheevi* Cytoplasmic Male Sterility Cytoplasm from Wheat[1]

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Subunits α and nine are components from two different portions of the mt H^+-ATP synthase enzyme, namely the soluble catalytic (F_1) and the membranous proton-conduction (F_0-ATPase) complexes. In wheat and triticale the genes for both subunits are located adjacently in the mt genome, irrespective of whether these lines are carrying the *aestivum, durum,* or *timopheevi* cytoplasmic male sterility cytoplasm. However, in the *timopheevi* mt genome the *atpA/atp9* cistron is part of a large DNA repeat and thus present in two different genomic environments. The repeat comprises at least 9 kb that are delimited 941 bp downstream of the *atp9* gene. The 2.7-kb triticale *atpA/atp9* sequence shows 100% homology with a *Triticum aestivum* sequence (Schulte et al., 1989), containing both reading frames, the intergenic region, and at least the first 400 bp upstream and the first 46 bp downstream of the *atpA/atp9* gene region.

Transcription of the *atpA/atp9* cistron in triticale gives rise to a co-transcript of 2.6 kb, which is posttranscriptionally modified by 5′ processing. To investigate whether further RNA modifications occur, we have analyzed this triticale transcript for RNA editing (Table I). To date, editing of an *atpA* transcript has been described in only two dicotyledonous species (Schuster et al., 1991; Senda et al., 1993). Overlapping cDNA clones covering the completely transcribed gene region were generated by reverse transcription PCR, and 10 individual clones were subsequently sequenced. In total, 17 RNA-editing sites could be identified, all of which represent cytidine to uridine transitions. The open reading frame of the *atpA* transcript carries five nonsilent and two silent RNA-editing sites. Equivalent alterations have been detected in *Oenothera* (amino acid 497) and sugar beet (amino acids 393 and 431), while all remaining sites are pre-edited in these two dicotyledonous species. Modified codons are located preferentially in the carboxy-terminal region of the protein, while silent RNA-editing sites are found exclusively at the amino terminus of the α subunit. Enzymatic studies with peptides derived by limited proteolysis from the α subunit suggest that the carboxy-terminal sequence is involved in regulation of ATPase activity (Tozawa et al., 1993).

Sites of partial RNA editing could be detected both in the leader sequence (30% edited transcripts at position −105) and in the *atpA* open reading frame (10, 90, and 90% edited transcripts at positions +393, +1490, and +1499, respectively). In the *atp9* open reading frame, eight sites of RNA

Table I. *Characteristics of the atpA and atp9 genes and their derived cDNA from triticale*

Organism:
- Triticale (× *Triticosecale* Wittmack; alloplasmic *Triticum durum* D30, *timopheevi* cytoplasm × *Secale cereale* L301).

Function:
- The *atpA* and *atp9* genes code for subunits of the soluble catalytic portion (F_1) and the membranous proton-conducting portion (F_0) of the mt ATP synthase complex, respectively.

Cloning Techniques:
- DNA cloning: A genomic library in EMBL4 (Mohr et al., 1993) was screened with an oligonucleotide specific for the *atpA* gene. Restriction fragments were subcloned into the pUC19 or pBluescript vectors.
- cDNA cloning: cDNA synthesis was performed using DNaseI-treated total RNA from triticale and five specific antisense oligonucleotides. Subsequent PCR amplification was done according to standard protocols using Replitherm (Biozym, Hameln, Germany) and six sets of specific primers. Sequences of overlapping DNA and cDNA clones were determined using dideoxy sequencing.

Features of the DNA Sequence:
- 100% sequence identity for both triticale *atpA/atp9* reading frames and their intergenic region is shared with the homologous *T. aestivum* region. Sequence homology between the *aestivum* and *timopheevi* genes is delimited 47 bp downstream of the *atp9* gene.

Editing Sites:
- *atpA:* C-to-U transitions at nucleotide positions −105, +30, +393, +971, +1178, +1292, +1490, +1499, +1550.
- *atp9:* C-to-U transitions at nucleotide positions +20, +81, +82, +134, +191, +205, +212, +223.

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Abbreviations: *atpA/atp9*, genes for subunits α and nine of the mitochondrial ATPase; mt, mitochondrial.

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editing were identified, one of which creates a stop codon and shortens the predicted polypeptide by six amino acids. We observed complete conservation when the triticale *atp9* cDNA sequence was compared with edited transcripts derived from corresponding maternal male-sterile *Triticum durum* (B. Laser, unpublished data) or from fertile *T. aestivum* (Bégu et al., 1990; Nowak and Kück, 1990). Although co-transcribed with the *atpA* gene, all editing sites of the *atp9* reading frame represent completely edited positions. These results are in contrast to published data (Araya et al., 1993), reporting incomplete editing of the *atp9* transcripts in a cytoplasmic male-sterile line of wheat. This partial RNA editing is most probably due to line-specific effects and is unlikely to be involved in the generation of the male-sterile phenotype.

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