ANALYSIS OF MITOCHONDRIAL GENES AND THE ASSOCIATION WITH CYTOPLASMIC MALE STERILITY- RNA EDITING

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Abstract

Cytoplasmic male sterility (CMS) is a maternally inherited trait in more than 150 higher plants, resulting in failure to produce functional pollen. The CMS system is widely used in hybrid breeding to produce F1 hybrid crops to utilize heterosis, i.e., hybrid vigor. CMS is caused by the dysfunction of mitochondrial DNA genes encoding for ATP subunits (atp) or cytochrome oxidases (cox). RNA editing, one of the post-transcriptional processes leading to one to more than one C-to-U change in nucleotide sequences of RNA for restoration of protein functionality, is also implicated in some CMS systems. However, the molecular mechanism of CMS in cotton is currently unknown. The objective of this study was to detect RNA editing sites in mitochondrial genes, atp1, 4, 6, 8 and 9, and cox1, 2 and 3 of a isogenic 'three line' system and to compare editing efficiency differences between the lines. The three lines consisted of an A line- CMS (with CMS-D8 cytoplasm and non-functional recessive restorer rf2 gene), a B line-maintainer (with Upland cotton AD1cytoplasm and non-functional recessive restorer rf2 gene), and an R line-restorer (with CMS-D8 cytoplasm and functional dominant restorer Rf2 gene). The full lengths of two CMS candidate genes, atp6 and cox2 were sequenced. PCR and RT-PCR were conducted using mitochondrial gene specific primers from the three lines followed by cloning and sequencing. Fifty-five C-to-U and two U-to-C RNA editing sites were identified in the eight mitochondrial genes. The majority of amino acid changes due to RNA editing caused alternation of hydrophilicity to hydrophobicity. Twelve editing efficiency differences between CMS-D8 and its restorer line were identified in all the sequenced mitochondrial genes except for atp1 including one 3' UTR region of atp6, which were attributed to the restorer gene Rf2. A comparison in the degree of editing in atp6 transcripts between the CMS line and the restorer line suggested that the existence of a restorer fertility gene may intensely modulate the RNA editing efficiency. A few nucleotide replacements and indels were also identified between CMS-D8 and AD1 cytoplasms, which could be utilized in developing molecular markers for distinguishing between the two cytoplasms by a simple PCR. This study provides information on RNA editing and its alterations associated with cytoplasm and nuclear restorer gene in cotton for the first time. However, association between RNA editing and CMS in mitochondrial genes studied, i.e., atp1, 4, 6, 8 and 9, and cox1, 2 and 3 is still not clear at present.