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RNA editing in CMS wheat: Influence of nuclear background leads to differential editing on *orf 256*

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Abstract

RNA editing and cytoplasmic male sterility are two important and correlated phenomenons in mitochondria of higher plants. We investigated the occurrence of RNA editing in *orf 256,* which is implicated in causing male sterility in wheat in two different CMS lines carrying different cytoplasms along with their fertility restored lines. This study provides the first preliminary report of RNA editing in *orf 256* with greater frequency observed in a fertility-restored line compared to male sterile line carrying the *T.timopheevi* cytoplasm. In order to investigate whether a similar process occurs in a line carrying the *T.araraticum* cytoplasm, it was observed that the male sterile line showed a complete lack of editing in *orf 256* whereas in the restored hybrids there was an increase in frequency of editing. Editing sites were also found conserved in both the hybrids at 381 position of C residue indicating the non random nature of editing especially under different nuclear backgrounds. Thus RNA editing might be involved in either causing male sterility or restoration of fertility in these two systems suggesting that a common mechanism may exist in these two different cytoplasms.

Key words: Cytoplasmic male sterility, wheat, RNA editing, codon, *orf 256*

Introduction

Cytoplasmic male sterility-fertility system is used in heterosis breeding to produce hybrids. Cytoplasmic male sterility (CMS) is a maternally inherited phenotype characterized by the inability of a plant to produce functional pollen. When mitochondrial DNA undergoes rearrangement (natural or in a somatic fusion process) to generate novel (ORFs) or chimeric genes that produce abnormal polypeptides, which may interfere with normal functioning of mitochondria [1], then cytoplasmic male sterility (CMS) arises. However, the nuclear restoration of fertility leads to either down regulation or suppression of expression of these genes. These restorer genes may act at different stages i.e., at DNA level, transcription initiation, RNA editing, translation or post translation. In wheat, even when both parents are fertile [2], the CMS trait results from incompatibility between *Triticum timopheevi* Zhuk. mitochondria and *T. aestivum* L. nucleus. Plants are restored to fertility by introducing nuclear restoration genes derived from *T. timopheevi* [3]. Mitochondrial DNA from *Triticum timopheevi* has a chimeric gene, *orf* 256 [4]. This gene is co- transcribed with *coxI* in cytoplasmic male sterile plants and produces a 7-kDa protein, which is not produced in fertile or fertility-restored plants [5].

RNA editing is a post-transcriptional process involving the partial change of C residues into U the process whereby messenger RNA is modified from the sequence of the corresponding DNA template [6]. These C to U changes lead to the synthesis of proteins different from that of predicted gene. It is also reported that the consequences of the RNA editing process are either the modification of the coded information for some amino acids or the generation of new initiation and/or termination codons. In organisms where RNA editing is active the protein sequence predicted from the gene may be different from that of the mRNA translated protein. In addition, they also aid in evolutionary process by restoring the conserved codon identities [7]. As we know that RNA editing is generally found in mitochondrial genome and to a lesser extent in the chloroplasts of higher plants, most of the mRNA in wheat mitochondria as well as higher plants is edited [8]. The *T-urf* in maize that causes CMS is an exception and it consists mostly of 26sRNA sequences that are usually not edited [9]. In wheat, Song and Hedgcoth [5] reported that *orf 256* transcripts from CMS and fertility restored plants are additional exceptions and undergo no editing. Nevertheless, no clear relationship between the *in vivo* observed spontaneous CMS and a lower level of RNA editing can be established [10].

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However, it has been reported that complete editing of an *atp6* gene may restore the fertility in CMS lines of rice [11]. In sorghum, Howad and Kempken [12] have shown that loss of *atp6* RNA editing contributes to or causes cytoplasmic male sterility. The effects of nuclear background and tissue specificity on RNA editing of the mitochondrial ATP subunits in fertile and CMS wheat has also been reported earlier [10]. Although partially edited transcripts were found in all cytoplasm, a large number of partially edited clones were present in CMS cytoplasm. In the euplasmic *T.timopheevi* all clones were fully edited. Thus it proved that RNA editing efficiency can be affected by tissue and nuclear background. All this show a correlation between RNA editing and CMS and the present study aims at investigating the possible editing changes in *orf 256* in *T.timopheevi and T araraticum* cytoplasms.

Materials and methods

Material of study comprised of two hybrids *viz*., TMS 20/2338 x 2988R and *arraticum* CMS 2022 x 2995R and their corresponding CMS lines *viz*., TMS 20/2338 and *araraticum* CMS/2022. These hybrids were developed at Division of Genetics, Indian Agricultural Research Institute, New Delhi. The cytoplasmic source of the 1st hybrid is *T. timopheevi* and for the second is *T. araraticum* which is closely related to *T. timopheevi* [13].

DNA and RNA were isolated from the flower buds of these lines as they are rich in mitochondrial. DNA was isolated according to the standard CTAB method and RNA was isolated using TRIZOL reagent (Invitrogen). Amplification of both DNA and RNA was carried out using primer designed from mitochondrial gene *orf 256* that is responsible for male sterility. The primers were designed internal to the coding region (*orf 256* F: CCCGAAACTTGGTTTAGTA, R: CTTCTAAGATCCTCCGACT) for amplification of 840 bp whereas the entire length of *orf* 256 spans 936 bp [5]. Amplification of DNA through PCR was done at 95°C for 3 min, 95°C-30 sec, 55°C for 30 sec and 72°C for a minute x 35 cycles, followed by an incubation at 72°C for 5 min using the high fidelity *Taq* polymerase from MBI Fermentas. The PCR products were purified by gel extraction kit QIAEX II (Qiagen, USA) and cloned into pGEM-T Easy Vector (Promega). Three positive clones were picked from each line and sequenced. Sequencing was done with the Megabace 1000 sequencer (Pharmacia-GE) using the Dynamic ET Terminator cycle sequencing kit (Amersham Pharmacia**)**. RT-PCR was done using the QIAGEN one step RT-PCR kit at 42°C for 45 min, 95°C for 15 min, followed by the above mentioned cycling conditions. The sequences from different clones was then merged using the software MULTALIN and high quality consensus sequence was obtained for each genomic and cDNA fragments. Sequencing was partial for all cDNA clones but genomic clones were sequenced completely. These genomic sequences were then compared to the reported sequence of *orf 256* in the NCBI database (Acc. no 10332).

Results and discussion

C to U transitions in plant mitochondrial mRNA leads to amino acid changes as well as to the creation of new initiation or termination codons. To study the occurrence of RNA editing in the *orf* 256, primers were designed to amplify both genomic and mRNA sequences. High fidelity *Taq* polymerase was used in PCR reactions to minimize amplification errors. An 840 bp amplicon was amplified from the 4 i.e. (1). CMS line TMS 20/2338, (2), fertility restored hybrid TMS 20/2338 x 2988R (3), *araraticum* CMS/2022A and (4), fertility restored hybrid araraticum/CMS 2022x2995R (Fig. 1). The RT-PCR also resulted in the same size amplification although a slightly smaller cDNA was detected in lines 3 and 4 (Fig. 1).

The cDNA sequence was aligned individually with *orf 256* genomic sequence using the software LALIGN. The scoring matrix was Blossum62 and gap penalty kept as default at -4. The cDNA sequence upon comparison with *orf 256* in the four lines indicated a low level of C to A changes (Table 1). A total of 5 editing changes were detected in the line 1 over a span of around 547 bp of high quality sequence. For the restored hybrid i.e. line 2, the editing changes were 8 over a span of around 278 bp of sequence. In line 3 i.e. *T. araraticum* CMS, no editing changes were detected in the partial transcript of around 356 bases whereas in the fertility restored hybrid (line 4) the editing changes appeared in 6 C residues in a 698 bp long cDNA sequence. Some editing sites were found conserved in both hybrids at 381 position of 'C' residue (Fig. 2b, 2d).

The production of chimeric protein, extensive recombination without creation of new *orf*s, mitochondrial DNA deletions and eventually a decrease or lack of RNA editing may be some of the multiple causes of the CMS phenotype by lowering the capacity of the mitochondria to furnish energy to the cell [14]. In wheat, Song and Hedgcoth [15] reported that *orf 256* transcripts from CMS and fertility restored plants are exceptions to the rule that most mRNA in wheat mitochondria is edited. In contrast to the previous report

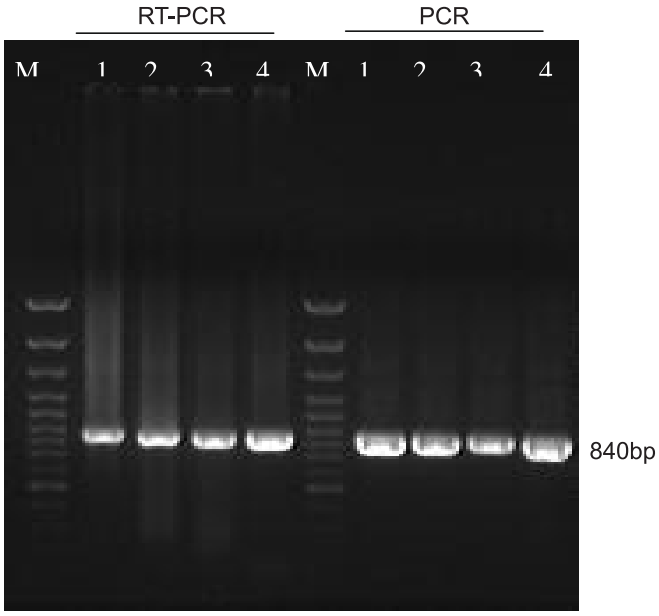

Fig. 1. RT-PCR and PCR amplification of *orf 256* in RNA and DNA isolated from bud tissues of CMS20/2338 (Lane 1), fertility restored hybrid CMS20/2338 x 2988R (Lane 2), *T.araraticum* CMS/2022(Lane 3) and fertility restored hybrid *T. araraticum* CMS/ 2022 x 2995R (Lane 4). M: 100 bp ladder (MBI Fermentas)

that *orf 256* is influenced by different nuclear backgrounds, we did not get any transcript size alterations in *orf 256* on 2995R and 2988R background of fertility restorers (data not shown). However, it was observed that both CMS lines and fertility-restored lines underwent differential RNA editing in different nuclear backgrounds. It was observed that the CMS line of *T. timopheevi* had 5 editing sites compared to 8 sites in its hybrid. In the case of *T. araraticum* CMS which is a close relative of *T. timopheevi* no editing sites were detected where as its restored hybrid showed 6 editing sites. Absence of editing of *orf 256* in *T. araraticum* cytoplasm might indicate the differential nature of *orf 256* editing in diverse cytoplasms. Similar behaviour showed by the restored lines also might indicate the role of editing in restoration of fertility with its frequency particularly high in fertility-restored hybrids. Such results were also observed in Sorghum [12] where mitochondrial *atp6* editing was strongly reduced in anthers of the male sterile lines whereas normal RNA editing was observed in transcripts of fertile lines. Loss of *atp6* editing contributed to or caused male sterility in *S. bicolor*. Kurek *et al*. [10] also reported about the effects of nuclear background and tissue specificity on RNA editing of the mitochondrial ATP subunits in fertile and CMS wheat. Editing frequency also vary in mature as well as

Table 1. RNA editing changes in cytoplasmic male sterile and hybrid lines

S.No.	Name of line	Sequence length	Position of 'C' residue	No. of 'C' residue edited
1.	*T. timopheevi* CMS (TMS20/2338A)	547	283 319 462 604 621	5
2.	Fertility restored hybridCMS20/2338 x 2988R	278	343 364 **381*** 419 439 445 477 483	8
3.	*T. araraticum* CMS arari CMS/2022A	355	None	-
4.	Fertility restored hybrid arari CMS 2022 x 2995R	698	304 **381*** 428 471 728 748	6

*Indicate the conserved editing 'C' residue

precursor transcripts in *nad3* and *rps12* genes in wheat mitochondria [16] indicating that RNA editing efficiency could be affected by tissue and nuclear background.These post transcriptional processing events may critically impair the coding region leading to a start or stop codon, but more often creating an internal codon with strong functional significance. Editing may also lead to creation of alternate forms of the polypeptide having an evolutionary effect on the mitochondrial genome.

PPR (pentatricopeptide) repeat motif genes have been reported to restore fertility in *Petunia, Brassica* and rice by their action on target *orf* [17-20]. Except in maize all other fertility restorer genes identified from plants code for a PPR protein [21]. It has been reported that such PPR proteins are also essential for RNA editing in chloroplast [22] with which higher plants can manage several hundreds of editing events in organelles. [23-25]. In our study, the editing position was found to be conserved in both hybrids at 381 positions. This is not unexpected as it has been reported that certain C residues have a higher tendency to undergo editing as compared to others. Generally these do not result in

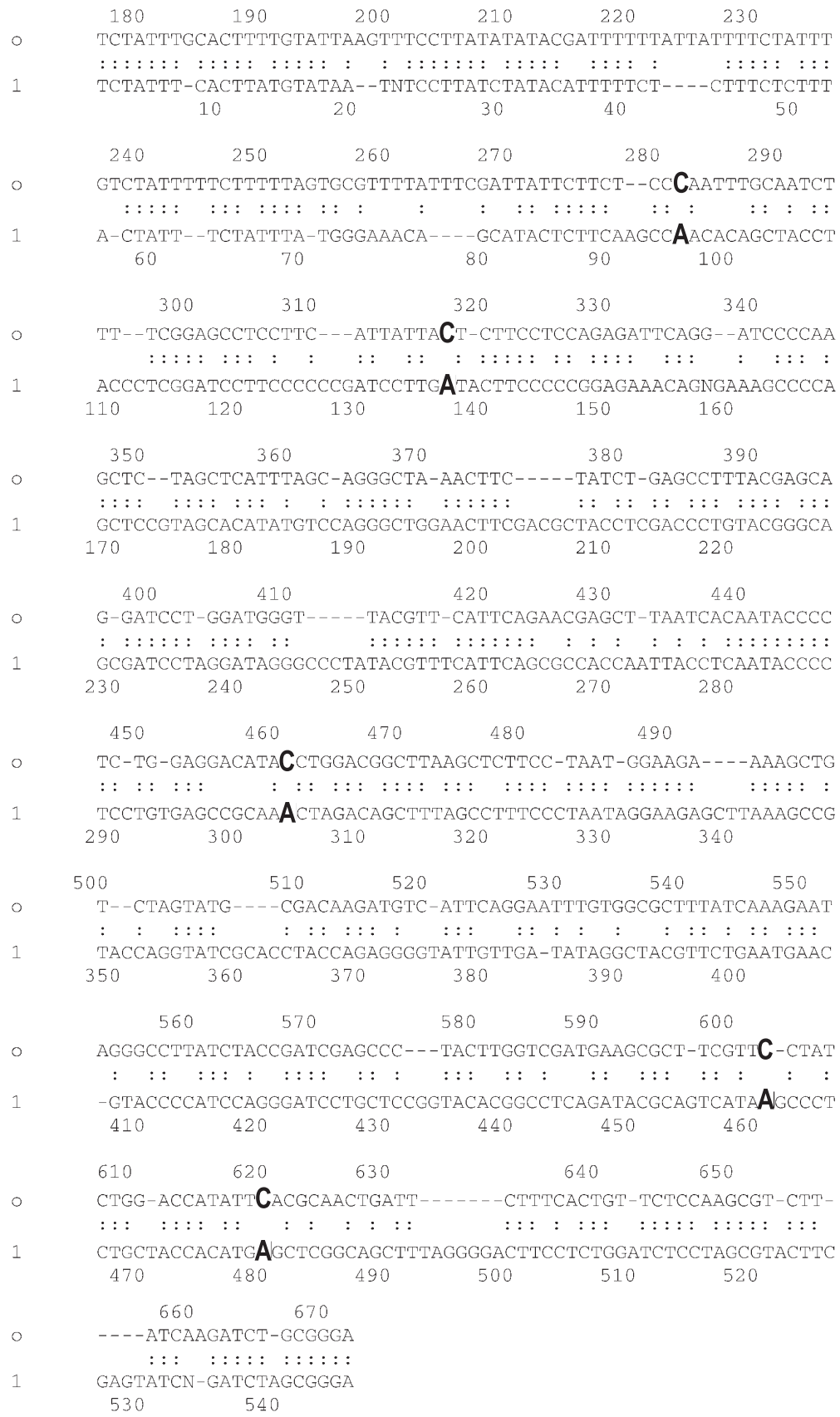

Fig. 2A

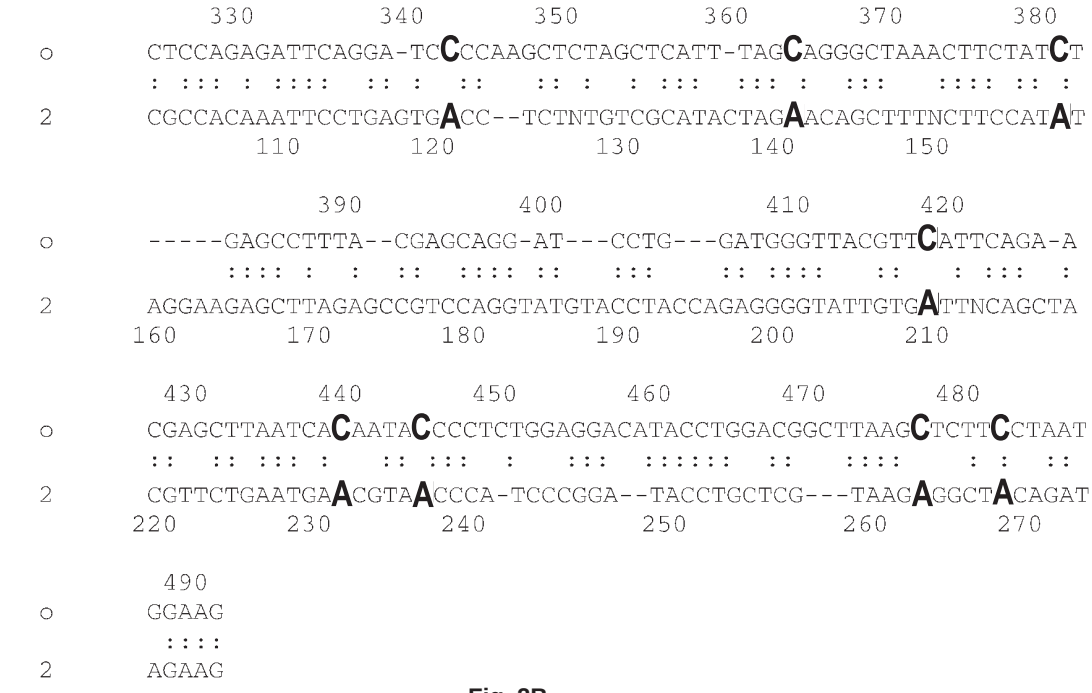

Fig. 2B

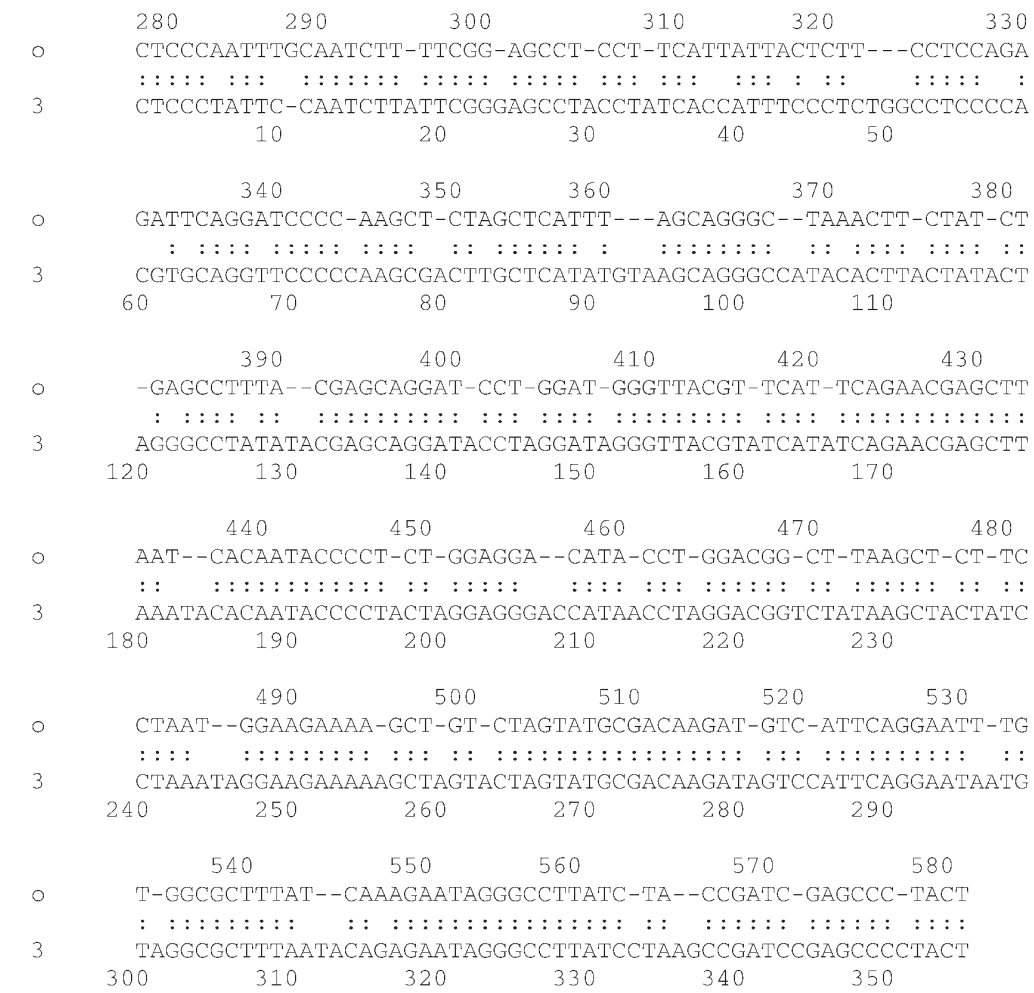

Fig. 2C

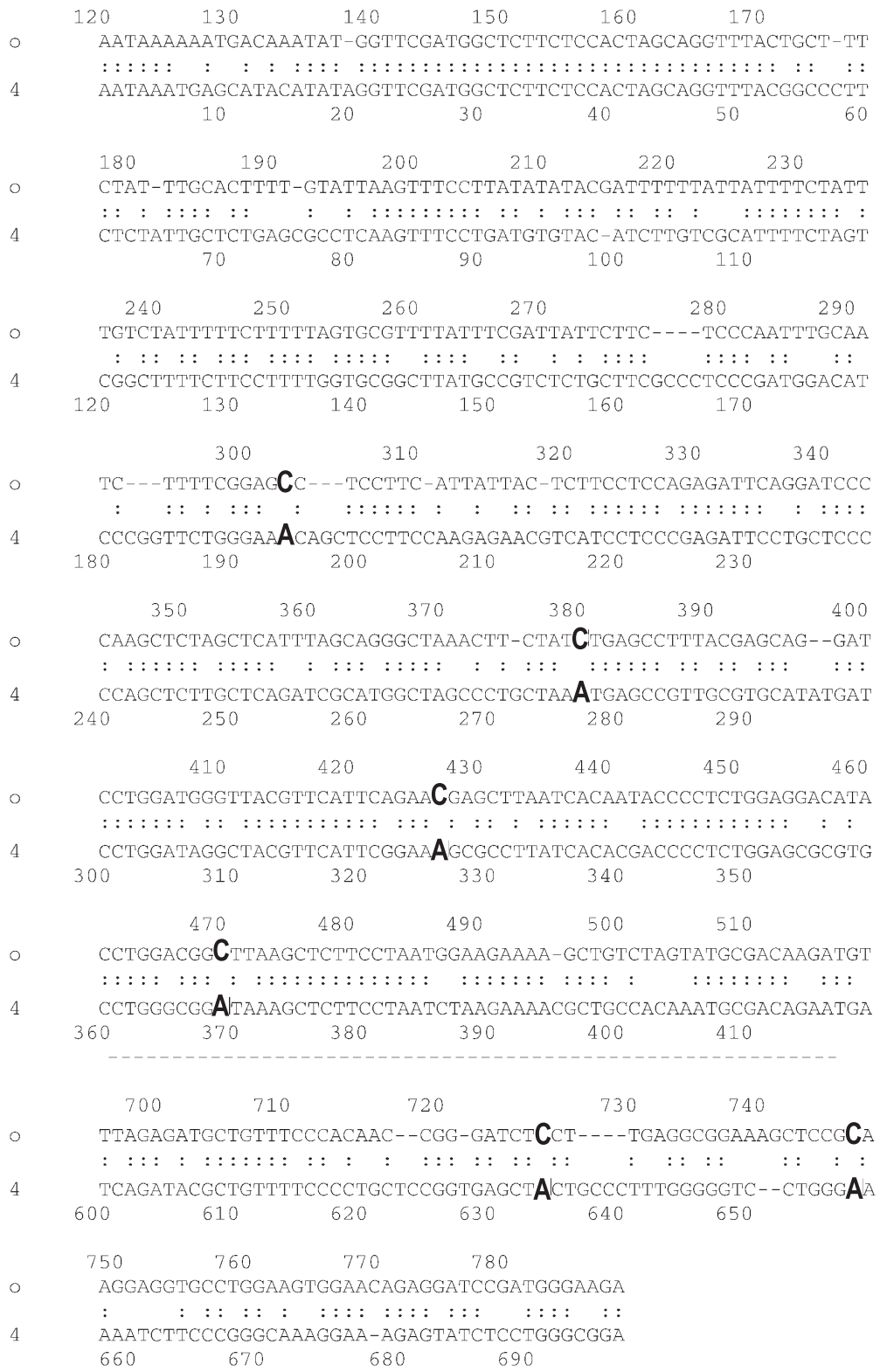

Fig. 2D

Fig. 2. (A-D): Comparison of the sequences between (A)*orf 256* genomic(O) and cDNA of CMS line CMS20/2338A9 (1), (B) *orf 256* genomic (O) and cDNA of fertility restored hybrid CMS20/2338A x 2988R (2), (C) *orf 256* genomic (O) and cDNA of male sterile line *T. araraticum* CMS2022A9 (3), (D) *orf 256* genomic (O) and cDNA of fertility restored hybrid CMS2022 x 2995R (4); Bold letters indicate site of editing changes

any significant codon changes so as to keep the polypeptide functional [26]. Since we did not have the full sequences it remains to be seen whether the editing changes are resulting in altered proteins and its status in pre transcripts and vegetative tissues.

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References

1. **Hanson M. R. and Bentolila S.** 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell., **16**: Suppl: S154-69.
2. **Leaver C. J., Isaac P. G., Small I. D., Bailey-Serres J., Liddell A. D. and Haw-kesford M. J.** 1988. Mitochondrial genome diversity and cytoplasmic male sterility in higher plants. Philos Trans R. Soc. L and B, **319**: 165-176.
3. **Mann S. S., Lucken K. A. and Bravo J. M.** 1984. Genetic analyses of. male-fertility restoration in wheat. Crop Sci., **24**: 17-20.
4. **Rathburn H., Song J. and Hedgcoth C.** 1993. Cytoplasmic male sterility and fertility restoration in wheat are not associated with rearrangements of mitochondrial DNA in the gene regions for cob, coxII, or coxI. Plant Mol Biol., **21**: 195-201.
5. **Song J. and Hedgcoth C.** 1994. Influence of nuclear background on transcription of a chimeric gene (*orf 256*) and coxI in fertile and cytoplasmic male sterile wheats. Genome, **37**: 203-9.
6. **Araya A., Blanc V., Begu D., Crabier F., Mouras A. and Litvak S.** 1995. RNA editing in wheat mitochondria. Biochimie., **77**: 87-91.
7. **Knoop V**. 2004. The mitochondrial DNA of Plants: Peculiarities in Phylogenetic Perspective**.** Current Genetics, **46**: 123-139.
8. **Covello P. S. and Gray M. W.** 1989. RNA editing in plant mitochondria. Nature, **19:** 341(6243): 662-6.
9. **Ward G. C. and Levings C. S.** 1991. The protein-encoding gene T-urf13 is not edited in maize mitochondria. Plant Mol Biol., **17**: 1083-8.
10. **Kurek I., Ezra D., Begu D., Erel N., Litvak S. and Breiman A.** 1997. Studies on the effects of nuclear background and tissue specificity on RNA editing of the mitochondrial ATP synthatase subunits a, 6 and 9 in fertile and cytoplasmic male sterile (CMS) wheat. Theor. Appl. Genet., **95**: 1305-1311.
11. **Iwabuchi M., Kyozuka J. and Shimamat K.** 1993. Processing followed by complete editing of an altered mitochondrial *atp6* RNA restores fertility of cytoplasmic mazle sterile rice. EMBO J., **12**: 1437-1446.
12. **Howad W. and Kempken F.** 1997. Cell type-specific loss of *atp6* RNA editing in cytoplasmic male sterile *sorghum bicolor*. Proc. Natl. Acad. Sci. U.S.A., Sep. 30, **94**: 11090-5.
13. **Tomar S. M. S. and Anbalagan S.** 2004. Charachterization of cytoplasmic male sterile lines in wheat (*Triticum aestivum* L.). Indian J. Genet., **64**: 196-200.
14. **Schnable P.S. Wise R.P.** 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. Trends Plant Sci., **3**: 175-179.
15. **Hedgcoth C., el-Shehawi A. M., Wei P., Clarkson M. and Tamalis D.** 2002. A chimeric open reading frame associated with cytoplasmic male sterility in alloplasmic wheat with *Triticum timopheevi* mitochondria is present in several *Triticum* and *Aegilops* species, barley, and rye. Current Genetics, **41**: 357-65.
16. **Gualberto J. M., Bonnard G., Lamattina L. and Grienenberger J. M.** 1991. Expression of the wheat mitochondrial nad3-rps12 transcription unit: correlation between editing and mRNA maturation. Plant Cell., **3**: 1109-20.
17. **Bentolila S., Alfonso A. A. and Hanson M. R.** 2002. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc. Natl. Acad. Sci. U.S.A., **6:** 99(16): 10887-92.
18. **Desloire S., Gherbi H., Laloui W., Marhadour S., Clouet V., Cattolico L., Falentin C., Giancola S., Renard M., Budar F., Small I., Caboche M., Delourme R. and Bendahmane A.** 2003. Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family. EMBO Rep., **4**: 588-94.
19. **Kazama T. and Toriyama K.** 2003. A pentatricopeptide repeat-containing gene that promotes the processing of aberrant atp6 RNA of cytoplasmic male-sterile rice. FEBS Letters, **544**: 99-102.
20. **Wang Z., Zou Y., Li X., Zhang Q., Chen L., Wu H., Su D., Chen Y., Guo J., Luo D., Long Y., Zhong Y. and Liu Y.G.** 2006. Cytoplasmic male sterility of rice with boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. Plant Cell., **18**: 676-87.
21. **Saha D., Prasad A. M. and Srinivasan R.** 2007. Pentatricopeptide repeat proteins and their emerging roles in plants. Plant Physiol. Biochem., **45**: 521-34.
22. **Kotera E., Tasaka M. and Shikanai T.** 2005. A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. Nature, **433**: 326-30.
23. **Wise R. P. and Pring D. R.** 2002. Nuclear-mediated mitochondrial gene regulation and male fertility in higher plants: Light at the end of the tunnel. Proc. Natl. Acad. Sci., U.S.A., **99**: 10887-92.
24. **Mackenzie S. A.** 2005. The influence of mitochondrial genetics on crop breeding strategies. Plant Breeding Reviews, **25**: 115–138.
25. **Small I. D. and Peeters N.** 2000. The PPR motif- a TPR- related motif prevalent in plant organellar proteins. Trends Biochem. Sci., **25**: 46-47.
26. **Handa H.** 2003. The complete nucleiotide sequence and RNA editing content of mitochondrial genome of rapeseed (*B. napus* L.), comparative analysis of the mitochondrial genomes of rapeseed and *Arabidopsis thaliana*. Nucl. Acids Res., **31**: 5907-5916.