Tissue-related differences in editing efficiencies of newly discovered *atp8* polymorphisms in CMS-S maize

Terry L Kamps[1] and Christine D Chase[2]
[1]New Jersey City University, Jersey City, NJ 07305
[2]University of Florida, Gainesville, FL 32611

Communicating author email: tkamps@njcu.edu

Background:

A key strategy to meeting the agriculture needs of a rapidly growing population combined with declining arable land is to take advantage of the general superiority and increased yield associated with hybrid plants (ECKARDT 2006). A variety of methods to generate hybrids include mechanical emasculation, genic male sterility (ZHANG *et al.* 2013; RHEE *et al.* 2015), self-incompatibility (LUNDQVIST 1994; LUDWIG *et al.* 2013), cytoplasmic male sterility (CMS)(CHASE 2007; HANSON 2004; HU *et al.* 2014; TOUZET and H. MEYER 2014; HORN *et al.* 2014; HAVEY 2004), and chemical hybridization agents (CHENG *et al.* 2013; PARODI and GAJU 2009). CMS is the maternally inherited inability of plants to produce viable pollen by either pollen abortion or the abnormal development of male reproductive organs. CMS is significant in providing a method to produce on an agricultural scale the hybrid seeds used to grow uniform, high yielding crops of rice, maize, sorghum, onion, sunflower, carrot, and rape seed (ECKARDT 2006). In addition to their application in producing hybrid seed, CMS mutants are useful for investigations concerning the genetics of mitochondrial biogenesis during male gametogenesis—a critical function in all plant reproduction.

CMS has been documented to occur widely in flowering plants(LASER and LERSTEN 1972)and results from a specific incompatibility between nuclear and mitochondrial genomes occurring during male gametogenesis. In most well studied CMS systems the mitochondrial loci responsible for male sterility are chimeric open reading frames (*orf*). These *orfs* usually arise from mitochondrial genome rearrangements and frequently include partial sequences from a known mitochondrial gene fused with partial sequence of another gene or non-coding sequence (HANSON and BENTOLILA 2004; KUBO and NEWTON 2008; CHASE 2007). Nuclear encoded restorer-of-fertility (*Rf*) genes counteract the deleterious expression of these mitochondrial loci during pollen development. These *Rf* genes provide the basis for making the CMS trait central to agricultural scale hybrid seed production. Although *Rf* genes are known in many agronomically important species (GABAY-LAUGHNAN *et al.* 2004; LI 2005; WANG *et al.* 2006; LIU *et al.* 2013; CASTILLO *et al.* 2014; YAMAGISHI and BHAT 2014), how they functionally affect mitochondria is not well understood

The three major CMS cytotypes of maize, CMS-T, CMS-C, and CMS-S, were originally distinguished by their *Rf* genes and restoration mode(BECKETT 1971). In CMS-S, the *Rf* genotype of pollen determines their post meiotic development. Male fertility in CMS-S is a unique system because restoration is determined by gametophytically expressed *Rf* genes (BECKETT 1971; GABAY-LAUGHNAN *et al.* 2004; GABAY-LAUGHNAN *et al.* 2009; SCHALLENBERG-RÜDINGER *et al.* 2013). Thus abortion or full development of each pollen grain is dependent on the allele of the *Rf* gene that it carries (KAMPS *et al.* 1996; WEN *et al.* 2003). The maize CMS-S cytoplasm was first characterized by the *Rf3* gene (BECKETT 1971)and the restoring allele is dominant (KAMPS *et al.* 1996). Microspores of CMS-S plants carrying the *Rf3* allele complete their developmental program to produce fertile trinucleate starch-filled pollen (KAMPS *et al.* 1996). In normal, non-sterile inducing cytoplasms, the *Rf3* alleles have no observable effect on pollen development and plants are fully male fertile regardless of the *Rf3* genotype. In contrast microspore development in CMS-S plants harboring the recessive *rf3* allele suddenly abort after the first mitotic division (LEE *et al.* 1980).

The *Rf3* gene was initially mapped by a chromosome translocation strategy to maize 2L (LAUGHNAN and GABAY 1978). RFLP experiments later positioned the locus 4.3 cM distal to the *whp1* locus and 6.4 cM proximal to the bnl17.14 marker (KAMPS and CHASE 1997). Additional studies have narrowed this region and identified loci encoding pentatricopeptide repeat (PPR) proteins as candidates for *Rf3* (ZHANG and ZHENG 2008; LANGEWISCH 2012).

Cloned *Rf* genes from several plant species are members of the large family of PPR proteins found in plants (QIN *et al.* 2014; BENTOLILA *et al.* 2002a; DAHAN and MIREAU 2013). PPR proteins are sequence specific binding proteins involved in multiple aspects of RNA processing (YIN *et al.* 2013; RÜDINGER *et al.* 2012; TAKENAKA *et al.* 2014; YAGI *et al.* 2014; FUJII and SMALL 2011; SHIKANAI 2006). RNA editing and endonucleolytic processing within mitochondria by proteins possessing a

PPR motif are two potential mechanisms by which *Rf* genes can counteract the deleterious expression of the mitochondrial locus conditioning pollen abortion. Examples of altered processing of polycistronic sequences have been described for the CMS causing *pcf* locus of Petunia (BENTOLILA *et al.* 2002b) and the *orf224/atp6* locus for the pol CMS of *Brassica napus* (MENASSA *et al.* 1999). An alternative mechanism for fertility restoration by post-transcriptional processing was demonstrated with *Sorghum bicolor* IS1112C male sterile cytoplasm by the specific increase in RNA editing of *atp6* transcripts from anthers (HOWAD and KEMPKEN 1997). Both transcript shortening and editing appear to play a role in conferring the CMS phenotype in rice WA-CMS(DAS *et al.* 2010).

Whole genome sequencing analyses of the three major CMS cytotypes of maize have shown that their mitochondrial genomes are highly rearranged relative to each other (ALLEN *et al.* 2007; CLIFTON *et al.* 2004). The dynamic nature of these rearrangements has produced unique chimeric loci in each of the cytoplasms. Early molecular investigations of CMS-S maize associated the mitochondrial chimeric *orf355-orf77* locus with male sterility induction (ZABALA *et al.* 1997). Subsequent studies have shown strong correlations for the *orf355-orf77* and their flanking regions with male fertility restoration by *Rf* genes or by novel rearrangements of this region that result in cytorevertants (GALLAGHER *et al.* 2002; MATERA *et al.* 2011; GABAY-LAUGHNAN *et al.* 2004; WEN and CHASE 1999a). High expression levels of its 1.6-kb transcript were detected during microsporogenesis in contrast to low levels occurring in non-tassel tissues. This 1.6-kb transcript is also reduced or absent during microsporogenesis of male fertile cytorevertant lines (MATERA *et al.* 2011). However, the possibility that other mitochondrial loci are either a direct cause or contribute to CMS-S induced pollen abortion is not precluded, particularly since a toxic polypeptide encoded by the *orf355/orf77* locus has yet to be identified. A systematic investigation to determine if they have a contributing or causative role in CMS-S has not been done.

The *atp8* gene, also known as *orfB* (HEAZLEWOOD *et al.* 2003) encodes a small protein which is a subunit of the F_o component of the F_1F_o-ATPsynthase (SABAR *et al.* 2003). Several CMS are reported to be determined by the *atp8* gene (DAS *et al.* 2010) or chimeric *orfs* containing partial *atp8* (YANG *et al.* 2009; HANSON and BENTOLILA 2004) sequence. Alterations in post-transcriptional processing of *atp8* have been implicated as causal for WA-CMS male sterility in rice (DAS *et al.* 2010; CHAKRABORTY *et al.* 2015). The coding sequence of *atp8* in *Zea* species and cytotypes share 100 percent identity as shown by ClustalW (http://www.genome.jp/tools/clustalw/) alignment analysis of their GenBank sequences (DQ490951.2, DQ645536.1, AY506529.1, NC_007982.1, NC_008332.1, NC_008333.1, NC_008331.1, and DQ490953.1). Therefore it would be expected that differences in post-transcriptional processing would be required if the *atp8* gene contributed to, or caused, CMS-S in maize. The *atp8* transcripts from immature ear and uni-nucleate microspore tissues of normal male fertile and non-restored CMS-S lines were compared for editing. The following report demonstrates how six bases were found to be edited with efficiencies that appear to be mostly tissue specific. Differences in protein accumulation were also observed during pollen development.

Materials and Methods:

Plant materials: Mo17-S is male sterile due to the CMS-S type male sterile inducing cytoplasm and non-restoring *rf3/rf3* nuclear genotype. Mo17-N has a normal cytoplasm with the *rf3/rf3* nuclear genotype and is male fertile maintainer line for Mo17-S. Uni-nucleate microspores (msp) were isolated from pre-emergent tassels. Later pollen developmental stages were obtained from post-emergent tassels and sucrose gradient density separation as described by WEN and CHASE 1999b. Immature ears were collected at the pre-silking stage of development.

RNA and sequencing analysis: Total cellular RNA was extracted from microspores and immature ears in the presence of Trizol Reagent (Life Technologies Inc., Gaithersburg, MD) as described by WEN and CHASE 1999b. DNA was removed with the DNA-free DNase treatment and removal kit (Ambion, Austin, Tex.). Primers CC296 5'-GAGGGTTGGTTTGATTGGAA and CC297 5'-GGCAAGGATCCTCAGTCCTA used for first-strand cDNA synthesis, subsequent PCR reactions, and forward and reverse sequencing reactions were designed from the published upstream and downstream sequences flanking the *atp8* coding sequence. First-strand cDNA synthesis was performed with the CC296 primer and the SuperScript® III First-Strand Synthesis System (Invitrogen) according to the manufacturer's protocol. PCR to amplify product for sequencing used 1.5 ul of first-strand cDNA per 50 ul reactions. Amplified products were gel electrophoresed for purification. DNA was extracted from gel slices with the DNA Clean & Concentrator™-25 kit (Zymo Research, Irvine, CA). Sanger DNA sequencing was performed by the Interdisciplinary Center for Biotechnology Research at the University of Florida. Resulting chromatograms were visualized by the BioEdit software program (Ibis Biosciences, Carlsbad, CA) to identify

partial RNA editing events. All RNA edited bases were identified by ClustalW alignments of sequenced products compared to maize CMS-S and NB *atp8* genomic sequences obtained from Genbank (accession numbers DQ490951.2 and AY506529.1 respectively).

Protein analysis: Mitochondria were purified from CMS-S (S) or normal (N) cytoplasm tissues by means of Percoll density gradient centrifugation: ear, immature ears; msp, uni-nucleate microspores; CP, collapsed pollen; BCP, bi-cellular pollen; MP, mature pollen. 7.5 ug of each mitochondrial sample was fractionated by gel electrophoresis, transferred to nitrocellulose, decorated with primary antibody against ATP8 and detected by a secondary antibody coupled to horseradish peroxidase (HRP).

Results:

Protein accumulation: Tissue and cytoplasmic specific differences in ATP8 accumulation were observed (Figure 1). The protein was abundant and of similar quantity in ear tissue and mature pollen from Mo17-N plants. Developmentally staged pollen showed that ATP8 is absent from the uni-nucleate microspores and bi-cellular pollen that can be collected from the CMS-S plants prior to pollen collapse. In contrast, ATP8 was detected as a weak signal from the microspores obtained from Mo17-N plants and it increased in accumulation at each stage with similar amounts occurring in mature pollen as is seen in the immature ear tissue.

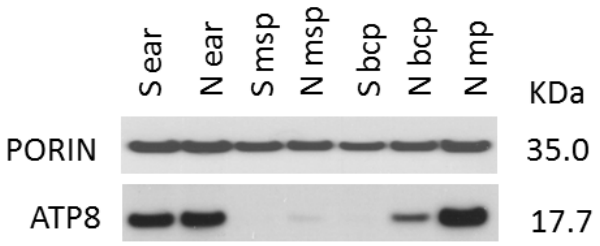

Figure 1. Protein accumulation in immature ears and developmentally staged pollen from maize normal (N) and CMS-S (S) cytotypes: ear, immature ear; msp, uni-nucleate microspores; bcp, bi-cellular pollen; and mp, mature pollen. The Mo17 nuclear background is non-restoring, therefore no S mp are produced.

RNA editing: The four sources of cDNA: Mo17-N ear and microspores; Mo17-S ear and microspores, were each sequenced in both directions. All resulting sequences were compared to each other and the published genomic sequences of the *atp8* coding region by the ClustalW multiple sequence alignment tool. The analysis revealed six C – T edited sites in all transcripts with four occurring within the first half of the gene (Table 1). Positions and changes to the amino acid sequence are shown graphically in Figure 2. Five of the six edits involve leucine. Two of the edits change the codon from leucine to phenylalanine, and two additional edits change a codon to leucine. One edit in a leucine codon is silent. Interestingly the first and second base of the amino acid at position 146 near the carboxy terminus of the protein are edited. If only the first base is edited the result will be a serine residue in place of the genomic coded proline. Editing of the second base, regardless of editing at the first base, results in a codon for leucine.

Examination of the sequencing chromatographs revealed apparent differences in editing efficiencies (Table 1). Efficiencies were similar for each base within a tissue. In general, editing efficiencies were reduced in microspores as compared to immature ear. Notable exceptions to this generality are the less efficient editing of CMS-S microspores transcripts as compared to N microspores for the base at position 200 and the first and second base of the double edited codon near the carboxy terminus of the protein.

Table 1. Editing of *atp8* transcripts from microspore and immature ears. Editing efficiency was estimated as a percentage by the relative height of C and T peaks observed on the sequencing chromatograms. Edited bases are bolded and underlined.

cDNA bp	Codon Change	Amino Acid Change	Amino Acid Position	Estimated Percent Ratio C > T Edits Immature Ear N	Estimated Percent Ratio C > T Edits Immature Ear CMS-S	Estimated Percent Ratio C > T Edits Microspores N	Estimated Percent Ratio C > T Edits Microspores CMS-S
58	**C**TC > **T**TC	L > F	20	0 : 100	0 : 100	30 : 70	30 : 70
76	**C**TC > **T**TC	L > F	25	55 : 45	55 : 45	90 : 10	90 : 10
123	CT**C** > CT**T**	L > L	41	0 : 100	0 : 100	40 : 60	55 : 45
200	T**C**G > T**T**G	S > L	67	0 : 100	0 : 100	0 : 100	15 : 85
436	**C**CA > **T**CA	P > S	146	30 : 70	30 : 70	45 : 55	60 : 40
437	C**C**A > C**T**A	P > L	146	0 : 100	0 : 100	5 : 95	20 : 80
436 & 437	**CC**A > **TT**A	P > L	146				

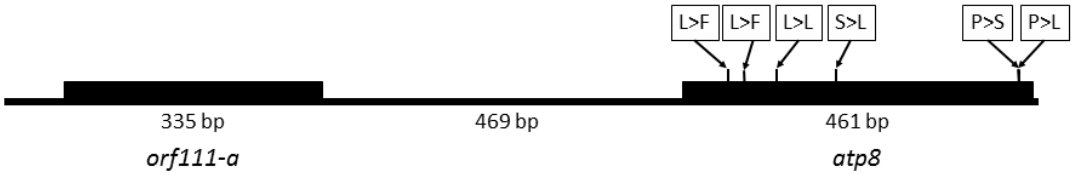

Figure 2. Map showing the maize N and CMS-S mitochondrial genome organization of the region containing the *atp8* locus. Boxes above the atp8 gene show amino acid changes due to RNA editing: L, leucine; F, phenylalanine; S, serine; P, proline.

Discussion:

The gametophytic mode of fertility restoration of maize CMS-S allows for unique opportunities to investigate nuclear-mitochondrial interactions and mitochondrial biogenesis during male gametogenesis. Research to identify the mitochondrial locus conferring the CMS-S phenotype has largely focused on the chimeric *orf355/orf77* locus (ZABALA *et al.* 1997; WEN and CHASE 1999a; b; GALLAGHER *et al.* 2002; GABAY-LAUGHNAN *et al.* 2004; MATERA *et al.* 2011), but other loci, including *atp8*, have been implicated.

Subunits of the ATP synthase complex and RNA editing have been shown to be the determining factors of some CMS systems (HANSON and BENTOLILA 2004; KUBOA *et al.* 2011; HORN *et al.* 2014; HU *et al.* 2014). Sequences corresponding to the *atp8* gene are reported to be involved in conferring CMS to several plant species. The *atp8* gene encodes a proteolipid, integral membrane protein (DEVENISH *et al.* 2000) composing part of the F_0 component of the F_1F_0-ATP synthase (DEVENISH *et al.* 2000; MUELLER 2000; HEAZLEWOOD *et al.* 2003; SABAR *et al.* 2003). ATP8 is believed to be involved in the assembly and the F_0 component (MUELLER 2000). Devendish et al (2000) concluded that roles for ATP synthase may, through conformational changes, include determining its activity. The importance of ATP8 to pollen function is indicated by its high quantity in the mature pollen (Fig. 1). Failure to detect ATP8 protein in developmentally staged microspores from non-restored Mo17-S shortly before pollen abortion would be consistent with an impaired ability to assemble functional ATP synthase at this critical time in development. These results suggest a functional role for *atp8* in CMS-S.

The *atp8* genomic sequences do not differ between normal and the CMS-S cytoplasms (CLIFTON *et al.* 2004; ALLEN *et al.* 2007). Post-transcriptional processing is a common feature of mitochondrial loci and has been investigated in CMS systems as the mechanism for male fertility restoration by nuclear encoded *Rf* genes. This idea is consistent with the finding that products of many of the cloned *Rf* genes are PPR proteins targeted for the mitochondria (HANSON and BENTOLILA 2004; CHASE 2007). Overexpression in transgenic plants of an unedited transcripts including the *atp8* gene has

also been shown to induce male sterility with fertility being restored by RNAi-mediated silencing of the transgene (CHAKRABORTY *et al.* 2015). We have begun our investigations on the possible contribution of ATP8 in conditioning CMS-S induced pollen abortion by characterizing RNA editing in tissues unaffected by the cytoplasm and in uni-nucleate microspores, the pollen developmental stage occurring just prior to observable effects by the cytoplasm. Results showed that four of the six identified sites were fully edited in the immature ear tissue regardless of cytoplasm (Table 1). Editing at base pairs 76 and 146 with an estimated less than 80% efficiency are considered partially edited sites (MOWER and PALMER 2006). Since the edit at base pair 76 changes the codon but not hydrophobicity of the amino acids, this may be a functionally insignificant edit. The edit at base pair 436, the first position in the codon, has two possible outcomes depending on the edit at the second position in the codon. Since the second base is fully edited in immature ear tissue the only outcome would appear to be an amino acid change from proline to leucine, which may be a common alteration (SUZUKI *et al.* 2013).

Interestingly editing efficiency of *atp8* transcripts from microspores were generally reduced at all six positions (Table 1). The edit to change the codon from hydrophilic serine to hydrophobic leucine was complete in normal microspores but reduced to nearly meeting the criteria for a partial edit ((MOWER and PALMER 2006) in the CMS-S microspores, which contrasts with full editing of transcripts from immature ear. Although editing efficiency was reduced in microspores relative to immature ears for base pair 437 determining the proline to leucine amino acid change, it appeared to be more reduced for CMS-S microspores. The significance of the differences in observed editing efficiencies is unclear with the current data. The reduction in editing efficiency, particularly at base pairs 200 and 437 may be sufficient to result in the differences in observed steady state levels of ATP8. They could also be artifacts of differences in RNA preparation of two different tissue types. Differences in microspores may be due to the initiation of programming of pollen abortion. Our future investigations will attempt to address this question by characterizing editing of CMS-S microspores obtained from Mo17-S plants with the *Rf3/Rf3* genotype.

Acknowledgements:

This research was supported by USDA CSREES NRI awards 2005-35301-15 and 00-35300-9409 and by the National Science Foundation award IOS-0816782.

Literature Cited:

ALLEN J. O., FAURON C. M., MINX P., ROARK L., ODDIRAJU S., GUAN N. L., MEYER L., SUN H., KIM K., WANG C., DU F., XU D., GIBSON M., CIFRESE J., CLIFTON S. W., NEWTON K. J., 2007 Comparisons among two fertile and three male-sterile mitochondrial genomes of maize. Genetics **177**: 1173–1192.

BECKETT J. B., 1971 Classification of male-sterile cytoplasms in maize (Zea mays L.). Crop Sci. **11**: 724–727.

BENTOLILA S., ALFONSO A. a, HANSON M. R., 2002a A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc. Natl. Acad. Sci. U. S. A. **99**: 10887–10892.

BENTOLILA S., ALFONSO A. a, HANSON M. R., 2002b A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc. Natl. Acad. Sci. U. S. A. **99**: 10887–10892.

CASTILLO A., ATIENZA S. G., MARTIN a. C., 2014 Fertility of CMS wheat is restored by two Rf loci located on a recombined acrocentric chromosome. J. Exp. Bot. **65**: 6667–6677.

CHAKRABORTY A., MIRTA J., BHATTACHARYYA J., PRADHAN S., SIKDAR N., DAS S., CHAKRABORTY S., KUMAR S., LAKHANPAUL S., SEN S. K., 2015 Transgenic expression of an unedited mitochondrial orfB gene product from wild abortive (WA) cytoplasm of rice (Oryza sativa L.) generated male sterility in fertile rice lines. Planta **241**: 1463–1479.

CHASE C. D., 2007 Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions. Trends Genet. **23**: 81–90.

CHENG Y., WANG Q., LI Z., CUI J., HU S., ZHAO H., CHEN M., 2013 Cytological and comparative proteomic analyses on male sterility in Brassica napus L. induced by the chemical hybridization agent monosulphuron ester sodium. PLoS One **8**.

CLIFTON S. W., MINX P., FAURON C. M., GIBSON M., ALLEN J. O., SUN H., THOMPSON M., BARBAZUK W. B., KANUGANTI S., TAYLOE C., MEYER L., WILSON R. K., NEWTON K. J., 2004 Sequence and Comparative Analysis of the Maize NB Mitochondrial Genome. Plant Physiol. **136**: 3486–3503.

DAHAN J., MIREAU H., 2013 The Rf and Rf-like PPR in higher plants, a fast-evolving subclass of PPR genes. RNA Biol. **10**: 1469–1476.

DAS S., SEN S., CHAKRABORTY A., CHAKRABORTI P., MAITI M. K., BASU A., BASU D., SEN S. K., 2010 An unedited 1.1 kb mitochondrial orfB gene transcript in the wild abortive cytoplasmic male sterility (WA-CMS) system of Oryza sativa L. subsp. indica. BMC Plant Biol. **10**: 39.

DEVENISH R. J., PRESCOTT M., ROUCOU X., NAGLEY P., 2000 Insights into ATP synthase assembly and function through the molecular genetic manipulation of subunits of the yeast mitochondrial enzyme complex. Biochim. Biophys. Acta **1458**: 428–442.

ECKARDT N. A., 2006 Cytoplasmic Male Sterility and Fertility Restoration. Plant Cell **18**: 515–517.

FUJII S., SMALL I., 2011 The evolution of RNA editing and pentatricopeptide repeat genes. New Phytol. **191**: 37–47.

GABAY-LAUGHNAN S., CHASE C. D., ORTEGA V. M., ZHAO L., 2004 Molecular-Genetic Chracterization of CMS-S Restorer-of-Fertility Alleles Identified in Mexican Maize and Teosinte. Genetics **166**: 959–970.

GABAY-LAUGHNAN S., CHASE C. D., ORTEGA V. M., ZHAO L., 2004 Molecular-Genetic Characterization of CMS-S Restorer-of-Fertility Alleles Identified in Mexican Maize and Teosinte. Genetics **166**: 959–970.

GABAY-LAUGHNAN S., KUZMIN E. V., MONROE J., ROARK L., NEWTON K. J., 2009 Characterization of a Novel Thermosensitive Restorer of Fertility for Cytoplasmic Male Sterility in Maize. Genetics **182**: 91–103.

GALLAGHER L. J., BETZ S. K., CHASE C. D., 2002 Motochondrial RNA editing truncates a chimeric open reading frame associated with S male-sterility in maize. Curr. Genet. **42**: 179–184.

HANSON M. R., BENTOLILA S., 2004 Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell **16** 154–S169.

HAVEY M., 2004 The use of cytoplasmic male sterility for hybrid production. In: Daniell H, Chase C (Eds.), *Molecular Biology and Biotechnology of Plant Organelles*, Springer, pp. 623–634.

HEAZLEWOOD J. L., WHELAN J., MILLAR A. H., 2003 The products of the mitochondrial orf25 and orfB genes are FO components in the plant F1FO ATP synthase. FEBS Lett. **540**: 201–5.

HORN R., GUPTA K. J., COLOMBO N., 2014 Mitochondrion role in molecular basis of cytoplasmic male sterility. Mitochondrion **19**: 198–205.

HOWAD W., KEMPKEN F., 1997 Cell type-specific loss of atp6 RNA editing in cytoplasmic male sterile Sorghum bicolor. Proc. Natl. Acad. Sci. U. S. A. **94**: 11090–11095.

HU J., HUANG W., HUANG Q., QIN X., YU C., WANG L., LI S., ZHU R., ZHU Y., 2014 Mitochondria and cytoplasmic male sterility in plants. Mitochondrion **19**: 282–288.

KAMPS T. L., MCCARTY D. R., CHASE C. D., 1996 Gametophyte genetics in Zea mays L.: Dominance of a restoration-of-fertility allele (Rf3) in diploid pollen. Genetics **142**: 1001–1007.

KAMPS T. L., CHASE C. D., 1997 RFLP mapping of the maize gametophytic restorer-of-fertility locus (rf3) and aberrant pollen transmission of the nonrestoring rf3 allele. Theor. Appl. Genet. **95**: 525–531.

KUBO T., NEWTON K. J., 2008 Angiosperm mitochondrial genomes and mutations. Mitochondrion **8**: 5–14.

KUBOA T., KITAZAKIA K., MATSUNAGAA M., KAGAMIA H., MIKAMIA T., 2011 Male Sterility-Inducing Mitochondrial Genomes: How Do They Differ? CRC. Crit. Rev. Plant Sci. **30**: 378–400.

LANGEWISCH T., 2012 Localization of the Rf3 restorer-of-fertility gene for maize S-type cytoplasmic male sterility. Thesis. Retrieved from https://mospace.umsystem.edu/xmlui/handle/10355/41914

LASER K., LERSTEN N., 1972 Anatomy and Cytology of Microsporgenesis in Cytoplasmic Male Sterile Angiosperms. Bot. Revies **38**: 425–454.

LAUGHNAN J. R., GABAY S. J., 1978 Nuclear and cytoplasmic mutations to fertility in S male-steril maize. In: Walden DB (Ed.), *Maize Breeding and Genetics*, John Wiley & Sons, New York, pp. 427–446.

LEE S. J., EARLE E. D., GRACEN V. E., 1980 The cytology of pollen abortion in S cytoplasmic male-sterile corn anthers. Am. J. Bot. **67**: 237–245.

LI S., 2005 Distribution of Fertility-restorer Genes for Wild-abortive and Honglian CMS Lines of Rice in the AA Genome Species of Genus Oryza. Ann. Bot. **96**: 461–466.

LIU Z., WANG D., FENG J., SEILER G. J., CAI X., JAN C.-C., 2013 Diversifying sunflower germplasm by integration and mapping of a novel male fertility restoration gene. Genetics **193**: 727–37.

LUDWIG S., ROBERTSON a., RICH T. C. G., DJORDJEVIC M., CEROVIC R., HOUSTON L., HARRIS S. a., HISCOCK S. J., 2013 Breeding systems, hybridization and continuing evolution in Avon Gorge Sorbus. Ann. Bot. **111**: 563–575.

LUNDQVIST A., 1994 The self-incompatibility system in Ranunculus repens (Ranunculaceae). Hereditas **120**: 151–157.

MATERA J. T., MONROE J., SMELSER W., GABAY-LAUGHNAN S., NEWTON K. J., 2011 Unique changes in mitochondrial genomes associated with reversions of S-type cytoplasmic male sterility in Maizemar. PLoS One **6**: 1–12.

MENASSA R., L'HOMME Y., BROWN G. G., 1999 Post-transcriptional and developmental regulation of a CMS-associated mitochondrial gene region by a nuclear restorer gene. Plant J. **17**: 491–499.

MOWER J. P., PALMER J. D., 2006 Patterns of partial RNA editing in mitochondrial genes of Beta vulgaris. Mol. Genet. Genomics **276**: 285–93.

MUELLER D. M., 2000 Partial Assembly of the Yeast Mitochondrial ATP Synthase 1. J. Bioenerg. Biomembr. **32**: 391–400.

PARODI P. C., GAJU M. a., 2009 Male sterility induced by the chemical hybridizing agent clofencet on wheat, Triticum aestivum and T. turgidum var. durum. Cienc. e Investig. Agrar. **36**: 267–276.

QIN X., WARGUCHUK R., ARNAL N., GABORIEAU L., MIREAU H., BROWN G. G., 2014 In vivo functional analysis of a nuclear restorer PPR protein. BMC Plant Biol. **14**: 313.

RHEE S.-J., SEO M., JANG Y.-J., CHO S., LEE G. P., 2015 Transcriptome profiling of differentially expressed genes in floral buds and flowers of male sterile and fertile lines in watermelon. BMC Genomics **16**: 914.

RÜDINGER M., VOLKMAR U., LENZ H., GROTH-MALONEK M., KNOOP V., 2012 Nuclear DYW-type PPR gene families diversify with increasing RNA editing frequencies in liverwort and moss mitochondria. J. Mol. Evol. **74**: 37–51.

SABAR M., GAGLIARDI D., BALK J., LEAVER C. J., 2003 ORFB is a subunit of F1F(O)-ATP synthase: insight into the basis of cytoplasmic male sterility in sunflower. EMBO Rep. **4**: 381–386.

SCHALLENBERG-RÜDINGER M., KINDGREN P., ZEHRMANN A., SMALL I., KNOOP V., 2013 A DYW-protein knockout in Physcomitrella affects two closely spaced mitochondrial editing sites and causes a severe developmental phenotype. Plant J. **76**: 420–432.

SHIKANAI T., 2006 RNA editing in plant organelles: Machinery, physiological function and evolution. Cell. Mol. Life Sci. **63**: 698–708.

SUZUKI H., YU J., NESS S. a., O'CONNELL M. a., ZHANG J., 2013 RNA editing events in mitochondrial genes by ultra-deep sequencing methods: A comparison of cytoplasmic male sterile, fertile and restored genotypes in cotton. Mol. Genet. Genomics **288**: 445–457.

TAKENAKA M., VERBITSKIY D., ZEHRMANN A., HÄRTEL B., BAYER-CSÁSZÁR E., GLASS F., BRENNICKE A., 2014 RNA editing in plant mitochondria -Connecting RNA target sequences and acting proteins. Mitochondrion **19**: 191–197.

TOUZET P., H. MEYER E., 2014 Cytoplasmic male sterility and mitochondrial metabolism in plants. Mitochondrion **19**: 166–171.

WANG Z., ZOU Y., LI X., ZHANG Q., 2006 Cytoplasmic Male Sterility of Rice with Boro II Cytoplasm Is Caused by a Cyto ... Agriculture.

WEN L., CHASE C. D., 1999a Pleiotropic effects of a nuclear restorer-of-fertility locus on mitochondrial transcripts in male-fertile and S male-sterile maize. Curr. Genet. **35**: 521–526.

WEN L.-Y., CHASE C. D., 1999b Mitochondrial gene expression in developing male gametophytes of male-fertile and S male-sterile maize. Sex. Plant Reprod. **11**: 323–330.

WEN L., RUESCH K. L., ORTEGA V. M., KAMPS T. L., GABAY-LAUGHNAN S., CHASE C. D., 2003 A nuclear restorer-of-fertility mutation disrupts accumulation of mitochondrial ATP synthase subunit alpha in developing pollen of S male-sterile maize. Genetics **165**: 771–9.

YAGI Y., NAKAMURA T., SMALL I., 2014 The potential for manipulating RNA with pentatricopeptide repeat proteins. Plant J. **78**: 772–782.

YAMAGISHI H., BHAT S. R., 2014 Cytoplasmic male sterility in Brassicaceae crops. Breed. Sci. **64**: 38–47.

YANG J. H., ZHANG M. F., YU J. Q., 2009 Mitochondrial nad2 gene is co-transcripted with CMS-associated orfB gene in cytoplasmic male-sterile stem mustard (Brassica juncea). Mol. Biol. Rep. **36**: 345–351.

YIN P., LI Q., YAN C., LIU Y., LIU J., YU F., WANG Z., LONG J., HE J., WANG H.-W., WANG J., ZHU J.-K., SHI Y., YAN N., 2013 Structural basis for the modular recognition of single-stranded RNA by PPR proteins. Nature **504**: 168–71.

ZABALA G., GABAY-LAUGHNAN S., LAUGHNAN J. R., 1997 The nuclear gene Rf3 affects the expression of the mitochondrial chimeric sequence R implicated in S-type male sterility in maize. Genetics **147**: 847–860.

ZHANG Z., ZHENG Y., 2008 Identification of candidate genes associated with fertility restoration in maize S cytoplasmic male sterility. Plant Mol. Biol. Report. **26**: 60–71.

ZHANG H., XU C., HE Y., ZONG J., YANG X., SI H., SUN Z., HU J., LIANG W., ZHANG D., 2013 Mutation in CSA creates a new photoperiod-sensitive genic male sterile line applicable for hybrid rice seed production. Proc. Natl. Acad. Sci. U. S. A. **110**: 76–81.