Molecular basis of cytoplasmic male sterility and fertility restoration in rice

Kinya Toriyama*

Graduate School of Agricultural Science, Tohoku University, 468-1 Aramaki Aza Aoba, Aoba-ku, Sendai, Miyagi 980-8572, Japan

*E-mail: torikin@tohoku.ac.jp  Tel: +81-22-757-4231  Fax: +81-22-757-4232

Received May 8, 2021; accepted June 7, 2021 (Edited by S. Arimura)

Abstract  Cytoplasmic male sterility (CMS) is a maternally inherited trait that causes dysfunctions in pollen and anther development. CMS is caused by the interaction between nuclear and mitochondrial genomes. A product of a CMS-causing gene encoded by the mitochondrial genome affects mitochondrial function and the regulation of nuclear genes, leading to male sterility. In contrast, the RESTORER OF FERTILITY (Rf) gene in the nuclear genome suppresses the expression of the CMS-causing gene and restores male fertility. An alloplasmic CMS line is often bred as a result of nuclear substitution, which causes the removal of functional Rf genes and allows the expression of a CMS-causing gene in mitochondria. The CMS/Rf system is an excellent model for understanding the genetic interactions and cooperative functions of mitochondrial and nuclear genomes in plants, and is also an agronomically important trait for hybrid seed production. In this review article, pollen and anther phenotypes of CMS, CMS-associated mitochondrial genes, Rf genes, and the mechanism that causes pollen abortion and its agronomical application for rice are described.

Key words: cytoplasmic male sterility, fertility restoration, mitochondria, Rf gene, rice.

Introduction

Cytoplasmic male sterile plants are often obtained by successive backcrossing between distantly related species or sub-species, which induces nuclear substitution (Figure 1). For example, a combination of cytoplasm (mitochondria) of wild rice (Oryza rufipogon Griff.) and nucleus of cultivated rice (Oryza sativa L.) triggers imbalanced interaction between mitochondrial and nuclear genomes, and thus often causes cytoplasmic male sterility (CMS). This is termed alloplasmic CMS. CMS is also found in wild rice populations (Lin and Yuan 1980). CMS is regulated by the interaction between a mitochondrial CMS-causing gene and a nuclear gene, called the RESTORER OF FERTILITY (Rf) gene. As shown in Figure 1, compatible interaction between a mitochondrial CMS-causing gene and a nuclear Rf gene does not cause male sterility in wild rice. Nuclear substitution via successive backcrossing of cultivated rice results in the removal of a functional Rf gene and allows the expression of a CMS-causing gene in mitochondria; this is detrimental to the mitochondria and results in male sterility. On the other hand, the introduction of the Rf gene from a cytoplasmic donor plant into the CMS plant restores fertility, because the Rf gene suppresses the accumulation of CMS-causing proteins. Alloplasmic CMS lines and isonuclear restorer lines are useful for investigating the molecular basis of CMS/Rf systems (Chen and Liu 2014; Hanson and Bentolila 2004, for reviews of general species).

History and origin of CMS in rice

A cytoplasm inducing CMS of rice was first observed in the W1 strain of weedy rice (Oryza sativa L. f. spontanea in the original literature) by scientists at Tohoku University, Japan (Katsuo and Mizushima 1958). It was named Chinese wild-type CMS (CW-CMS), and the cytoplasm donor of CW-CMS has been described as O. rufipogon (Kinoshita 1997). Extensive studies of CMS rice have been conducted at Ryukyu University, Japan by Shinjyo, for which CMS lines were obtained from an indica cultivar Chinsurah Boro II and 62 accessions of wild rice (O. rufipogon Griff.) after backcrossing with the japonica cultivar Taichung 65 (Shinjo 1984; Shinjyo 1969, 1975). The cytoplasms of some Japanese cultivars

Abbreviations: CDS, coding sequence; CMS, cytoplasmic male sterility; CRISPR/Cas9, clustered regularly interspaced short palindromic repeat/CRISPR-associated endonuclease 9; PPR, pentatricopeptide repeat; RMS, RETROGRADE-REGULATED MALE STERILITY; Rf, RESTORER OF FERTILITY; SNP, single nucleotide polymorphism; T65, Taichung 65; TALEN, Transcription activator-like effector nuclease.

This article can be found at http://www.jspcmb.jp/
Published online September 18, 2021

Copyright © 2021 Japanese Society for Plant Biotechnology
The origins of CMS lines, on which extensive molecular studies have been conducted, are summarized in Table 1 and briefly described below. The Boro-Taichung 65 type CMS (BT-CMS) and Lead Rice type CMS (LD-CMS) lines were derived from indica rice varieties (O. sativa L.) Chinsurah Boro II and Lead Rice, respectively (Shinjyo 1969; Watanabe 1971). The wild-abortive type CMS (WA-CMS), Hong Lian-type CMS (HL-CMS), RT98-type CMS (RT98-CMS), RT102-type CMS (RT102-CMS), and Dongxiang-type CMS (D1-CMS) lines originate from wild rice (O. rufipogon Griff.) (Lin and Yuan 1980; Motomura et al. 2001, 2003; Rao 1988; Xie et al. 2018). The cytoplasmic donors of the WA-CMS and HL-CMS lines were found on Hainan Island (Li et al. 2007). The RT98-CMS and RT102-CMS lines originate from the Indian wild rice accessions W1109 and W1125, respectively (Motomura et al. 2001, 2003).

The origins of CMS lines, on which extensive molecular studies have been conducted, are summarized in Table 1 and briefly described below. The Boro-Taichung 65 type CMS (BT-CMS) and Lead Rice type CMS (LD-CMS) lines were derived from indica rice varieties (O. sativa L.) Chinsurah Boro II and Lead Rice, respectively (Shinjyo 1969; Watanabe 1971). The wild-abortive type CMS (WA-CMS), Hong Lian-type CMS (HL-CMS), RT98-type CMS (RT98-CMS), RT102-type CMS (RT102-CMS), and Dongxiang-type CMS (D1-CMS) lines originate from wild rice (O. rufipogon Griff.) (Lin and Yuan 1980; Motomura et al. 2001, 2003; Rao 1988; Xie et al. 2018). The cytoplasmic donors of the WA-CMS and HL-CMS lines were found on Hainan Island (Li et al. 2007). The RT98-CMS and RT102-CMS lines originate from the Indian wild rice accessions W1109 and W1125, respectively (Motomura et al. 2001, 2003). The origin of the CW-CMS line is described above. Gene symbols of the CMS cytoplasm defined by the

Table 1. Types and characteristics of cytoplasmic male sterility (CMS).

| CMS type | Cytoplasm source | Accession no. of whole mitochondrial genome | Morphology of pollen* | Abortive stage | CMS-associated gene (accession no.) | Fertility restorer genes** (accession no.) |
|----------|-----------------|--------------------------------------------|----------------------|----------------|-------------------------------------|------------------------------------------|
| BT       | O. sativa ssp. indica (Chinsurah Boro II) | AP017385, AP017386 | Lightly stained; spherical | Tricellular pollen | orf79 (D14339) | Rf1/Rf1a/PPR791 (AB110016, AB112808, AB110443, DQ311053), Rf1b/PPR506 (DQ311054) |
| CW       | O. rufipogon (W1) | AP011076 | Stained; round but no germination | Germination | CW-orf307 (in AP011076) | Rf17 (=retrograde-regulated male sterility) (AB481199) |
| D1       | O. rufipogon (Dongxiang wild rice) | KY486275 | No pollen | Early uninucleate microspore | orf182 (in KY486275) | NA |
| HL       | O. rufipogon (Hong Lian wild rice) | NA | Unstained; spherical | Bicellular pollen | orfH79 (LR794118) | Rf5 (=Rf1a) (MN592703) Rf6/PPR894 (MN592707) |
| LD       | O. sativa ssp. indica (Lead rice) | AP011077 | Lightly stained; spherical | Tricellular pollen | L-orf79 (AB254027) | Rf2 (glycine-rich protein) (AB583697) |
| RT98     | O. rufipogon (W1109) | AP012527 | Stained; round but no germination | Germination | orf113 (in AP012527) | Rf98/PPR762 (LC131122) |
| RT102    | O. rufipogon (W1125) | AP012528 | Unstained; irregular withered, and stained; round but no germination | Early uninucleate microspore, and germination | orf352 (in AP012528) | NA |
| TA       | O. sativa ssp. indica (Tadukan) | LC595639, LC592696 | Stained; round (Indehiscent anthers) | Anther dehiscent | orf312 (in LC592696) | NA |
| WA       | O. rufipogon (Wild abortive) | JF281154 | Unstained; irregular withered | Early uninucleate microspore | WA352 (JX131325) | Rf4/PPR782a (AB900792, KJ680248) |

* Pollen stainability with I₂-KI are indicated. **The names of the PPR genes are based on the number of encoded amino acids.
Committee of Gene Symbolization, Nomenclature and Linkage (CGSNL) of the Rice Genetics Cooperative are found in the integrated rice research database, Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/).

**Anther and pollen phenotypes**

The morphology of anthers and pollen grains at the flowering stage differs among CMS types (Table 1). Figure 2 shows pollen grains of various alloplasmic CMS lines with the nucleus of Taichung 65, along with a course of developmental stages of microspores/pollen (uninucleate microspore stage, bicellular pollen stage, and tricellular pollen stage (mature stage)). As described below, the variation in the timing of pollen abortion suggests that each mitochondrial genome determines the fate of pollen.

The mature pollen grains of wild-type Taichung 65 are darkly stained with iodine-potassium iodide (I₂-KI) solution, showing full starch accumulation. The anthers of the WA-CMS line are slender and milky white, and the pollen grains are shrunken, empty of starch, and unstainable with an I₂-KI solution (Figure 2). The microspores abort at the uninucleate microspore stage (Table 1). The pollen grains of the BT-CMS line are globular, small in size, and slightly accumulate starch (Figure 2). They abort at the tricellular pollen stage. The starch accumulation in the pollen grains of the LD-CMS line is slightly more abundant than that of the BT-CMS line, but still less than that of wild-type Taichung 65. The mature pollen grains of the CW-CMS and RT98-CMS lines are morphologically normal under optical microscopy (Figure 2), but lack germination ability.

RT102-CMS plants produce both unstained shrunken and stained spherical pollen grains lacking germination ability (Figure 2).

The anthers of D1-CMS plants have been reported to be small, transparent, and shrunken, lacking pollen grains. Retarded degradation of the tapetum has been reported to result in non-pollen-type abortion (Xie et al. 2018). Pollen grains of an HL-type CMS line (YTA) have been reported to be spherical, and scarcely accumulate starch, and pollen abortion occurs at the bicellular pollen stage (Li et al. 2007).

In addition, a CMS line resulting from difficulty in anther dehiscence has been reported in backcrossed progenies of the indica cultivar Tadukan (a cytoplasm donor) and Japanese cultivars Norin 8 and Taichung 65 (Kitamura 1962; Takatsuka et al. 2021). This CMS line was named TA-type CMS (Kinoshita 1997).

**Mitochondrial CMS-associated genes**

A candidate CMS-causing gene in mitochondria is often called a CMS-associated gene, as a causal relationship between CMS and a mitochondrial candidate gene has not yet been proven in most cases. CMS-associated genes have been mainly identified by whole-genome sequencing of mitochondria to search for unique open reading frames (orfs) compared with those of a standard cultivar such as Nipponbare, followed by northern blot screening of the orf genes to compare the transcript profiles of CMS and fertility-restored iso-cytoplasmic lines, which are expected to be modified by the presence of a fertility restorer gene.

Whole mitochondrial genome sequences have been reported for japonica cultivars Nipponbare (GenBank accession numbers BA000029; DQ167400) and PA64S (photoperiod- and thermo-sensitive genic male sterile line used as a maternal parent of super hybrid rice Liangyoupeijiu; DQ167807); indica cultivars 93–11 (paternal parent of Liangyoupeijiu; DQ167399), IR6888B (maintainer line for WA-CMS; JF281153), and Shuhui498 (restorer line for WA-CMS; CP018169); CMS lines carrying indica cytoplasm, BT-CMS, LD-CMS, and TA-CMS; and CMS lines carrying O. rufipogon cytoplasm, WA-CMS, CW-CMS, RT98-CMS, RT102-CMS, and D1-CMS. The GenBank accession numbers for the mitochondrial genomes of these CMS lines are listed in Table 1.

CMS-associated genes often show chimeric structures with a part of a known mitochondrial gene, and encode proteins with transmembrane domains. A CMS-associated gene is generally named orfN, where N is the number of amino acids encoded. CMS-associated genes are listed with GenBank accession numbers in Table 1 and are described below.

The CMS-associated gene for BT-CMS is named orf79.
because it encodes 79 amino acids (Akagi et al. 1994; Iwabuchi et al. 1993). Orf79 and its sequence variants, orfH79 and L-orf79, have been reported in HL-CMS (He et al. 2020; Li et al. 2008; Yi et al. 2002) and LD-CMS (Itabashi et al. 2009), respectively. The CDS of orfH79 and L-orf79 contain five and one nucleotide changes, respectively, compared with that of orf79 of BT-CMS, which lead to four and one amino acid substitutions, respectively. The structure of orf79 is chimeric with cox1 encoding cytochrome c oxidase subunit I and an origin-unknown sequence (Figure 3). The orf79 gene is located downstream of the atp6 gene, which encodes subunit 6 of ATP synthase, and is co-transcribed with atp6 as 2.0 kb transcripts, whereas two transcripts of 1.5 kb (atp6) and 0.45 kb (orf79) are generated by RNA processing in the presence of a fertility restorer gene, Rf1. The amount of ORF79 protein in LD-CMS rice is reported to be a twentieth of the amount in BT-CMS rice (Kazama et al. 2016). This difference in ORF79 protein levels likely accounts for the mild and severe pollen defects in LD-CMS and BT-CMS rice, respectively. ORFH79 of HL-CMS, which is encoded by a sequence variant of orf79, has been reported to interact with P61, a subunit of the cytochrome bc1 complex (mitochondrial electron transport chain complex III; Figure 4), resulting in energy production dysfunction and oxidative stress in mitochondria, which leads to abnormal pollen development (Wang et al. 2013). The multicentric origin and diversification of atp6-orf79-like structures in O. rufipogon and O. sativa suggest that their divergence may have been beneficial to their survival during evolution (He et al. 2020).

A known CMS-associated gene for WA-CMS is WA352, which encodes 352 amino acids (Luo et al. 2013; Tang et al. 2017a). WA352 and its sequence variants (also called orf352) have been reported in other CMS types such as Dissi (D-CMS), Dwarf abortive (DA-CMS), Gambiaca (GA-CMS), Indonesia paddy rice (ID-CMS), K52 (K-CMS) (Luo et al. 2013), and RT102 (Okazaki et al. 2013). The nucleotide sequences of orf352 of RT102-CMS and WA352 show five nucleotide differences, which cause four amino acid substitutions. WA352/orf352 has a chimeric structure composed of parts of three function-unknown genes present in the Nipponbare mitochondrial genome, namely, orf284, orf224, and orf288, each encoding a hypothetical protein (GenBank protein IDs AAZ99359.1, AAZ99350.1, and AAZ99379.1, respectively), and an origin-unknown sequence (Luo et al. 2013; Okazaki et al. 2013), as shown in Figure 3. It is co-transcribed with rpl5, which encodes ribosomal protein large subunit 5. The WA352/orf352-containing transcripts are reduced or cleaved in the fertility restorer line for WA-CMS and RT102-CMS, respectively. WA352 protein has been reported to accumulate at the anther tapetum at the meiotic stage, and the C-terminal half of WA352, which is almost identical to a part of ORF288, interacts with subunit 11 (COX11) of cytochrome c oxidase (mitochondrial electron transport chain complex IV; Figure 4), resulting in reactive oxygen species (ROS) burst and tapetal programmed cell death (PCD), and consequently, sporophytic male sterility (Luo et al. 2013).
orf288, and the errors in the upstream region of no. BA000028 has been shown to contain sequencing
2010). It is notable that orf288 has been renamed (GenBank protein ID BAI67968.1), is composed of the 5′
CMS, Tadukan mitochondria. A CMS-associated gene of CW-
3), and also carries a promoter region of half of orf288
line (Xie et al. 2018).
The orf288 gene might also play an important role in the evolution of other CMS genes, because a CMS-
orf312 gene is a CMS-causative gene. Based on the
orf79 through recombination of homologous regions. Restoration of the fertility of orf79-depleted plants
mitoTALEN as a powerful tool to certify CMS-causative genes
It is unknown whether CMS-associated genes are really
causative genes in most cases, because gain-of-function
mitoTALEN, to knock out the CMS-associated gene, orf79, in BT-CMS rice (Kazama et al. 2019). TALENs are
gene with a mitochondrial targeting signal sequence into the nuclear genome of a wild-type plant, and to
observe whether transformation with the mitochondrial
candidate gene confers male sterility (Kojima et al. 2010;
Luo et al. 2013; Peng et al. 2010; Wang et al. 2006). This
approach might not rule out the hidden pitfall where
delivery of a foreign protein into an improper location
of mitochondria might cause mitochondrial dysfunction.
Recently, we demonstrated loss-of-function analysis employing mitochondria-targeted TALEN, named
mitoTALEN, to knock out the CMS-associated gene, orf79, in BT-CMS rice (Kazama et al. 2019). TALENs are
genome-editing tools that are generally composed of two
parts (left TALEN and right TALEN), each containing
a DNA-binding domain and a nuclease domain. The
mitoTALEN vector was designed to deliver TALEN protein into mitochondria by adding a mitochondrial-
targeting signal sequence. It was integrated into the
nuclear genome via Agrobacterium-mediated gene
transfer. Knocking out the mitochondrial orf79 gene
in BT-CMS rice by mitoTALEN caused deletion of orf79 through recombination of homologous regions.
Fertility restoration
The modes of fertility restoration are categorized into two
types, gametophytic and sporophytic, based on the action
of the Rf gene. In the sporophytic type, the pollen fertility
of the F1 plant between a CMS line and a restorer line
is determined by the genotype (Rfrf) of the sporophyte
(the tapetum), resulting in all the pollen (both Rf and
rf pollen) being fertile and participating in fertilization.
Thus, a quarter of F₂ plants are expected to be sterile (rfrf). The tapetum is the innermost layer of the anther wall and supplies nutrients and constituents of the pollen wall for developing microspores. In the gametophytic type, the genotype of individual pollen grains determines their fertility. Therefore, rf pollen without a functional restorer gene in the F₁ plant are aborted, and all F₂ plants are expected to be fertile (RfRf or Rfrf).

Sporophytic restoration has been reported for two major Rf genes of WA-CMS: Rf3 on chromosome 1 and Rf4 on chromosome 10 (Sattari et al. 2007; Suresh et al. 2012). Fertility restoration efficiency has been reported as follows: Rf3Rf3/Rf4Rf4 > rfrf/Rf4Rf4 > Rf3Rf3/rf4 (Katara et al. 2017). Several minor quantitative trait loci (QTLs) contributing to weak fertility restoration have also been reported. The gametophytic mode of fertility restoration in BT-CMS, LD-CMS, and CW-CMS is governed by single Rf genes, Rf1, Rf2, and Rf17, respectively (Fujii and Toriyama 2009; Shinjyo 1975). Two Rf genes, Rf5 and Rf6, have been reported in HL-CMS. F₁ hybrids carrying either Rf5 or Rf6 have been reported to display 50% normal pollen grains, whereas those harboring both Rf5 and Rf6 generate 75% fertile pollen (Huang et al. 2012).

Molecular cloning has been reported for Rf1/Rf1a and Rf1b of BT-CMS (Akagi et al. 2004; Kazama and Toriyama 2003; Komori et al. 2004; Wang et al. 2006); Rf2 of LD-CMS (Itabashi et al. 2011); Rf4 of WA-CMS (Kazama and Toriyama 2014; Tang et al. 2014), Rf5 (= Rf1), and Rf6 of HL-CMS (Huang et al. 2015); Rf17 of CW-CMS (Fujii and Toriyama 2009); and Rf98 of RT98-CMS (Igarashi et al. 2016). Cloned Rf genes and their GenBank accession numbers are listed in Table 1.

### Rf genes encoding PPR protein

Except for Rf2 and Rf17, all the other Rf genes cloned in rice have been shown to encode pentatricopeptide repeat (PPR) proteins. Some Rf genes of other plant species, such as petunia Rf (Bentolila et al. 2002) and radish Rf6/Rfk1 (Brown et al. 2003; Desloire et al. 2003; Koizuka et al. 2003), also encode PPR proteins. The names of PPR genes are often based on the number of encoded amino acids. A PPR protein contains tandem repeats of degenerate 35 amino acid units, termed the PPR motif. PPR proteins generally bind to RNA and are involved in RNA processing, including RNA editing, RNA cleavage, RNA stabilization, and translational activation in mitochondria and plastids (Schmitz-Linneweber and Small 2008). Each PPR motif recognizes one nucleotide (Figure 5). The nucleotide-specifying residues have been reported to be determined by 3 amino acids in each PPR motif, namely, amino acids at locations 1, 4, and “ii” of each PFAM-defined PPR motif, where “ii” indicates the amino acid located 2 residues before the first amino acid of the next PPR motif (Yagi et al. 2013). Recently, revised numbering of the PPR motif, that is, counting from the first amino acid of the first helix, has been proposed, and the 5th and 35th amino acids (corresponding to 4 and 11 in the method of Yagi et al. 2013) have been reported to be primarily involved in nucleotide recognition (Cheng et al. 2016). The precise prediction of a binding nucleotide, however, has not yet been achieved for every PPR motif. There are 491 PPR genes in rice (Chen et al. 2018; O’Toole et al. 2008). Half the PPR genes are predicted to be targeted toward mitochondria, and a quarter may localize in chloroplasts. A comparison of nucleotide sequences of PPR genes and known Rf genes

![Figure 5. Schematic structure of RF1 protein carrying 18 PPR motifs and predicted binding site in intergenic region of atp6-orf79 RNA. Two amino acids proposed to be involved in nucleotide recognition are indicated in each PPR motif. The binding site was predicted based on the RNA recognition code of Melonek et al. (2016) and Yan et al. (2019). Nucleotides that match with the prediction are indicated by stars. Site of RNA cleavage promoted by RF1 is indicated.](image-url)
has identified a group of Rf-PPR-like (RFL) sequences and selection patterns in the evolution of RFL genes resulting from an arms race between the nuclear and mitochondrial genomes (Fujii et al. 2011; Melonek et al. 2016).

The Rf1 gene (also called Rf1a) encodes a 791 amino acid PPR protein with a mitochondrial-targeting signal sequence and 18 repeats of the PPR motif (Akagi et al. 2004; Kazama and Toriyama 2003; Komori et al. 2004; Wang et al. 2006). The Rf1 protein has been shown to bind to the intergenic region of atp6-orf79 RNA and promote RNA processing (Kazama et al. 2008). In BT-CMS mitochondria, orf79 is co-transcribed with atp6, which encodes subunit 6 of ATP synthase as a 2.0 kb RNA sequence, whereas 1.5 kb RNA containing atp6 and 0.45 kb RNA containing orf79 are generated through RNA cleavage by the action of Rf1 52 nucleotides upstream from the start codon of orf79 (Kazama et al. 2008; Wang et al. 2006) (Figure 5). Based on the available RNA recognition code for each PPR motif (Melonek et al. 2016; Yan et al. 2019), the Rf1 protein is predicted to bind to 5′-GAUGUU?GAYCCCGUUCCU-3′, which approximately matches the RNA sequence corresponding to 31 bp upstream of the cleavage site in the intergenic region between atp6 and orf79 RNA (Figure 5). The processed orf79 RNA is not translated, and hence there is no accumulation of ORF79 protein, and consequently resulting in restoration of fertility. Rf1 reportedly plays an additional role in promoting the RNA editing of atp6 mRNA (Wang et al. 2006). The rfi allele in Nipponbare (Locus ID in Rice Annotation Project Database (RAP-DB; Kawahara et al. 2013; Sakai et al. 2013) Os10g0497432 encodes a truncated putative protein of 266 amino acids due to a frame-shift mutation (Akagi et al. 2004; Kazama and Toriyama 2003; Komori et al. 2004; Wang et al. 2006). The Rf1b gene for BT-CMS encodes a 506 amino acid PPR protein with 11 PPR motifs. Rf1B blocks ORF79 production via the post-transcriptional degradation of orf79-containing transcripts (Wang et al. 2006). The non-functional rf1b allele (Os10g0499500 in RAP-DB) has been attributed to the A1235G mutation, which causes the amino acid substitution Asn412Ser (Wang et al. 2006).

The Rf5 gene, identified as an Rf gene for HL-CMS, is identical to Rf1/Rf1a and encodes a 791 amino acid protein (Hu et al. 2012). Rf5 functions through an association with a glycine-rich protein containing the RNA recognition motif GRP162 (Os12g063200 in RAP-DB). GRP162 has been reported to physically interact with Rf5 and bind to atp6-orfH79 transcripts via the RNA recognition motif of GRP162. Both Rf5 and GRP162 are components of a restoration of fertility complex (RFC; 400 to 500 kDa in size) that cleaves the atp6-orfH79 transcripts (Hu et al. 2012). The RFC in HL-CMS is also reportedly composed of RFC subunit 3, a DUF1620-containing and WD40-like repeat protein (Os05g0230600 in RAP-DB), as a scaffold protein for the assembly of the complex (Qin et al. 2016). The RFC is expected to contain an additional unknown factor that possesses endoribonuclease activity for RNA processing. Another Rf gene for HL-CMS, Rf6, encodes an 894 amino acid protein with 20 PPR motifs, including a duplication of the 3rd to 5th PPR motifs, which are absent in r6 (Os08g0110200 in RAP-DB) (Huang et al. 2015). Rf6 has been reported to physically associate with mitochondrially localized hexokinase 6 (OsHXK6; Os01g0742500 in RAP-DB) and promote the processing of the atp6-orfH79 RNA 12 nucleotides (at the G residue) downstream of the start codon of orfH79 to restore HL-CMS fertility (Huang et al. 2015).

The Rf4 gene for WA-CMS encodes a 782 amino acid protein containing 18 repeats of the PPR motif (Kazama and Toriyama 2014; Tang et al. 2014). Rf4 functions in the reduction of WA352-containing transcripts, and thereby, prevention of accumulation of WA352 protein, resulting in fertility restoration. A non-restorer line, Nipponbare, contains a putative allele of Rf4 (Os10g0495200 in RAP-DB), whose amino acid sequence shows 95% identity to that of Rf4. Amino acids crucial for the function of Rf4 have not been reported. Molecular cloning of another major Rf gene for WA-CMS, Rf3, has also not been reported.

The Rf98 gene for RT98-CMS encodes a 762 amino acid protein with 18 PPR motifs. Although Rf98 has been shown to be responsible for partial restoration of fertility, the effect of RF98 on mitochondrial RNA is unknown (Igarashi et al. 2016). Rf1/Rf1a/Rf5, Rf1b, Rf4, and Rf98 are present near the rfi locus of chromosome 10 (Os10g0497432 in RAP-DB). Seven other Rf-like PPR genes, which show a high degree of similarity, have been identified in the restorer line, RT98C, which is known to restore various CMS types (Igarashi et al. 2016). Some of the Rf-like PPR genes in this cluster likely interact with different mitochondrial transcripts, including orf352 and orf79, in a gene-for-gene fashion. This gene clustering suggests that the members of this Rf-like PPR gene cluster have been recruited as suppressors of newborn mitochondrial CMS-associated genes and have become new Rf genes for different CMS systems (Igarashi et al. 2016). Rapid progress in studies on the RNA recognition code for the PPR motif will enable the prediction of the target RNA of each Rf-like PPR gene in the near future.

**Rf genes encoding non-PPR proteins**

Rf genes other than those encoding PPR proteins have also been reported in rice and other plant species (Bohra et al. 2016; Chen and Liu 2014; Kim and Zhang 2018, for reviews of various crops).
The Rf2 gene for LD-CMS encodes a mitochondrial glycine-rich protein consisting of 152 amino acids (Itabashi et al. 2011). The Rf2 gene promotes degradation of atp6-orf79 mRNA in LD-CMS and BT-CMS (Itabashi et al. 2009; Kazama et al. 2016). RF2 has been shown to be effective in reducing the accumulation of ORF79 to almost 0% and 25%, resulting in complete and weak restoration of fertility in LD-CMS and BT-CMS rice, respectively, indicating that the level of ORF79 protein determines pollen abortion (Kazama et al. 2016). RF2-interacting candidate factors have been reported; one of them encodes a mitochondrially targeted ubiquitin domain-containing protein (Fujii et al. 2014). It is unknown how RF2 protein reduces atp6-orf79 RNA.

The replacement of isoleucine with threonine at the 78th amino acid of the RF2 protein has been considered the cause of functional loss in the rf2 allele (Os02g0274000 in RAP-DB) (Itabashi et al. 2011).

Fertility of CW-CMS lines is gametophytically restored by a single nuclear gene, Rf17, which has been identified to be a reduced expression allele of the RETROGRADE-REGULATED MALE STERILITY (RMS) gene (Fujii and Toriyama 2009). RMS encodes a 178 amino acid mitochondrial targeting protein containing a part of an acyl-carrier protein synthase-like domain (Os04g0475900 in RAP-DB) (Itabashi et al. 2011). The Rf17 allele or rf17 allele does not affect the expression profile of the CMS-associated gene, CW-orf307. The nucleotide sequence of the coding region is identical in the restorer line (Rf17Rf17) and the CW-CMS line (rf17rf17), but a single nucleotide polymorphism (SNP) is present in the promoter region 2,286 bp upstream of the start codon of RMS.

A model of CMS occurrence/fertility restoration mechanism in CW-CMS is presented in Figure 6. CW-orf307 in CW-mitochondria is considered to be responsible for the production of a certain signal from the mitochondria to the nucleus (called retrograde signal; Fujii and Toriyama 2008) to enhance the expression of the RMS allele (=rf17 allele) in the nucleus of the CW-CMS line, leading to male sterility, whereas the rms allele (=Rf17 allele) in the fertility restorer line might not perceive the retrograde signal because of a mutation in its promoter region, resulting in reduced RMS expression, and thus, fertility restoration (Fujii and Toriyama 2009).

To prove that RMS is directly involved in restoring the fertility of CW-CMS, mutations have been introduced into the coding sequence (CDS) and the promoter of RMS using CRISPR/Cas9. Fertility was restored in the genome-edited CMS plants with reduced expression of RMS when the mutation was introduced in its promoter region (Suketomo et al. 2020). These results demonstrated that fertility was restored by reduced expression of RMS, providing a new method to create artificial fertility restorer lines for agronomical use.

**CMS/Rf system for hybrid rice breeding**

A CMS line, a maintainer line, and a fertility restorer line are often used for hybrid rice breeding, known as a three-line breeding method. The seeds of the CMS lines are multiplied by crossing a CMS line and a maintainer line. F1 hybrid seeds are produced by crossing a CMS line and a restorer line. The resulting F1 hybrid plants are fertile because the Rf gene is provided by the restorer line. It is notable that plants with a heterozygous Rf gene (Rfrf) set seeds normally, although the gametophytic mode of fertility restoration reduces the rate of viable pollen to 50% in F1 plants. Hybrid rice cultivars have an average yield advantage of 15 to 50% over inbred cultivars due to heterosis, a hybrid vigor (Barclay 2010; Huang et al. 2020; Ma and Yuan 2015; Tang et al. 2017b). WA-CMS
plants are most widely used as female parents in hybrid rice breeding, which accounts for approximately 90% of the three-line hybrids produced in China and 100% of the hybrids developed outside China (Huang et al. 2014; Sattari et al. 2007). Other CMS types used for japonica-type hybrid rice breeding include BT-CMS and HL-CMS (Huang et al. 2014, for a review).

The CW-CMS/Rf17 system is quite different from other CMS/Rf systems, as described above. It also has a unique feature that induces CMS in various elite indica cultivars, for which CMS lines could not be produced using other CMS systems such as BT-CMS or WA-CMS, because indica rice cultivars, in general, carry Rf genes for BT-CMS and WA-CMS. We have produced CW-CMS lines and Rf17-containing restorer lines of the elite indica cultivars, IR24 (a strong restorer of WA-CMS), IR64 (a popular mega-variety), NSIC Rc 160 (a high-quality eating cultivar), NSIC Rc 240 (a high-yielding cultivar) in the Philippines, Cihergang (a high-yielding cultivar) in Indonesia, BRRI dhan 29 (a high-yielding cultivar) in Bangladesh, Local Basmati and Pusa Basmati (aromatic cultivars) in India, and NERICA-L-19 (a high-yielding cultivar with an IR 64 genetic background) in Africa. These CW-CMS lines did not set any seeds, whereas fertility was fully restored by the introduction of Rf17 (Toriyama and Kazama 2016; Toriyama et al. 2019). These results demonstrate that the CW-CMS/Rf17 system will be useful for the production of CMS lines of various indica cultivars and for hybrid rice breeding.

In Japan, a couple of hybrid rice cultivars, namely, Mitsuhikari 2002, Mitsuhikari 2005 and Togo 1 to Togo 4, have been developed using BT-CMS by Mitsui Chemicals Agro (https://mitsui-agro.com/product/tabid/114/Default.aspx) and Research Institute of Rice Production & Technology Co., Ltd. (http://www.rrpt.co.jp/hp/). Hybrid rice is not very popular in Japan; the volume of hybrid rice on the market was 5,572 tons in 2020, accounting for 0.1% of the total rice production in Japan (https://www.maff.go.jp/j/seisan/syoryu/kensa/kome/). Across the world, however, the hybrid rice plantation area in 2014 was 15.5 million ha in China, accounting for 50% of the rice plantation area, and 6.36 million ha outside China, accounting for 6% on average (Ma and Yuan 2015; Zheng et al. 2020). Although the cultivation of two-line hybrids based on photoperiod-or thermo-sensitive genic male sterile lines is gradually increasing in China, accounting for up to one-third of the total hybrid rice growing area (Huang et al. 2014; Tang et al. 2017b), a CMS/Rf system is still a promising system for hybrid rice production.

Acknowledgements
This study was partly supported by KAKENHI 20K21300 and 21H02161. I thank Dr. Tomohiko Kazama for critical reading of this manuscript.

References
Akagi H, Nakamura A, Yokozeki-Misono Y, Inagaki A, Takahashi H, Mori K, Fujimura T (2004) Positional cloning of the rice Rf-1 gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. Theor Appl Genet 108: 1449–1457

Akagi H, Sakamoto M, Shinjiyo C, Shimada H, Fujimura T (1994) A unique sequence located downstream from the rice mitochondrial atp6 may cause male sterility. Curr Genet 25: 52–58

Barclay A (2010) Hybridizing the world. Rice Today 9: 32–35

Bohra A, Jha UC, Adhimoolam P, Bisht D, Singh NP (2016) Cytoplasmic male sterility (CMS) in hybrid breeding in field crops. Plant Cell Rep 35: 967–993

Bentolila S, Alfonso AA, Hanson MR (2002) A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-stereile plants. Proc Natl Acad Sci USA 99: 10887–10892

Brown G, Formanova N, Jin H, Wargachuk R, Dendy C, Patil P, Laforest M, Cheung WY, Landry BS (2003) The radial Rfo restorer gene of Ongura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. Plant J 35: 262–272

Chen G, Zou Y, Hu J, Dign Y (2018) Genome-wide analysis of the rice PPR gene family and their expression profiles under different stress treatments. BMC Genomics 19: 720

Chen LT, Liu YG (2014) Male sterility and fertility restoration in crops. Annu Rev Plant Biol 65: 579–606

Cheng S, Gutmann B, Zhong X, Ye Y, Fisher MF, Bai F, Castleden I, Song Y, Song B, Huang J, et al. (2016) Redefining the structural motifs that determine RNA binding and RNA editing by pentatricopeptide repeat proteins in land plants. Plant J 85: 532–547

Desloire S, Gherbi H, Laloui W, Marhadour S, Clouet V, Cattolico L, Falentin C, Giancola S, Renard M, Budar F, et al. (2003) Identification of the fertility restoration locus, Rfo, in radish, as a member of the pentatricopeptide-repeat protein family. EMBO Rep 4: 588–594

Fujii S, Bond CS, Small I (2011) Selection patterns on restorer-like genes reveal a conflict between nuclear and mitochondrial genomes throughout angiosperm evolution. Proc Natl Acad Sci USA 108: 1723–1728

Fujii S, Kazama T, Ito Y, Kojima S, Toriyama K (2014) A candidate factor that interact with RF2, a restorer of fertility of Lead rice-type cytoplasmic male sterility in rice. Rice (N Y) 7: 21

Fujii S, Kazama T, Yamada M, Toriyama K (2010) Discovery of global genomic re-organization based on comparison of two newly sequenced rice mitochondrial genomes with cytoplasmic male sterility-related genes. BMC Genomics 11: 209

Fujii S, Toriyama K (2008) Genome barriers between nuclei and mitochondria exemplified by cytoplasmic male sterility. Plant Cell Physiol 49: 1484–1494

Fujii S, Toriyama K (2009) Suppressed expression of RETROGRADE-REGULATED MALE STERILITY restores pollen fertility in cytoplasmic male sterile rice plants. Proc Natl Acad Sci USA 106: 9513–9518

Hanson MR, Bentolila S (2004) Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell 16(Suppl): S154–S169

He W, Chen C, Amede YMN, Dong X, Xi K, Sun Y, Dang T, Jin T
(2020) Multicentric origin and diversification of \textit{atp6}-orf79-like structures reveal mitochondrial gene flows in \textit{Oryza rufipogon} and \textit{Oryza sativa}. \textit{Evol Appl} 13: 2284–2299

Hu J, Wang K, Huang W, Liu G, Gao Y, Wang J, Huang Q, Ji Y, Qin X, Wan L, et al. (2012) The rice pentatricopeptide repeat protein Rf5 restores fertility in Hong-Lian cytoplasmic male-sterile lines via a complex with the glycine-rich protein GRP162. \textit{Plant Cell} 24: 109–122

Huang JZ, Guo Z, Zhang HL, Shu QY (2014) Workable male sterility systems for hybrid rice: Genetics, biochemistry, molecular biology, and utilization. \textit{Rice (N Y)} 7: 13

Huang W, Hu J, Yu C, Huang Q, Wan L, Wang L, Qin X, Ji Y, Zhu R, Li S, et al. (2012) Two non-allelic nuclear genes restore fertility in a gametophytic pattern and enhance abiotic stress tolerance in the hybrid rice plant. \textit{Theor Appl Genet} 124: 799–807

Huang W, Yu C, Hu J, Wang L, Dan Z, Zhou W, He C, Zeng Y, Yao G, Qi J, et al. (2015) Pentatricopeptide-repeats family protein RF6 functions with hexokinase 6 to rescue rice cytoplasmic male sterility. \textit{Proc Natl Acad Sci USA} 112: 14984–14989

Igarashi K, Kazama T, Motomura K, Toriyama K (2013) Whole genomic sequencing of RT98 mitochondria derived from \textit{Oryza rufipogon} and northern blot analysis to uncover a cytoplasmic male sterility-associated gene. \textit{Plant Cell Physiol} 54: 237–243

Igarashi K, Kazama T, Toriyama K (2016) A gene encoding pentatricopeptide repeat protein partially restores fertility in RT98-type cytoplasmic male sterile rice. \textit{Plant Cell Physiol} 57: 2187–2193

Itabashi E, Iwata N, Fujii S, Kazama T, Toriyama K (2011) The fertility restorer gene, \textit{Rf2}, for Lead Rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. \textit{Plant J} 65: 359–367

Itabashi E, Kazama T, Toriyama K (2009) Characterization of cytoplasmic male sterility of rice with Lead Rice cytoplasm in comparison with that with Chinsurah Boro II cytoplasm. \textit{Plant Cell Rep} 28: 233–239

Iwabuchi M, Kyozuka J, Shimamoto K (1993) Processing followed by complete editing of an altered mitochondrial \textit{atp6} RNA restores fertility of cytoplasmic male sterile rice. \textit{EMBO J} 12: 1437–1446

Katara JL, Verma RL, Nayak D, Ngangkham U, Ray S, Subudhi H, Behera L, Samantaray S, Rao RN, Singh ON, et al. (2017) Frequency and fertility restoration efficiency of \textit{Rf3} and \textit{Rf4} genes in Indian rice. \textit{Plant Breed} 136: 74–82

Katsuo K, Mizushima U (1958) Studies on the cytoplasmic male sterility of hybrids obtained reciprocally between cultivated and wild varieties, \textit{Oryza sativa} \textit{L.}. \textit{Jpn J Breed} 12: 81–84 (in Japanese)

Kawahara Y, Bastide M, Hamilton JP, Kanamori H, McCombie WR, Ouyang S, Schwartz DC, Tanaka T, Wu J, Zhou S, et al. (2013) Improvement of the \textit{Oryza sativa} Nipponbare reference genome using next generation sequence and optical map data. \textit{Rice (N Y)} 6: 4

Kazama T, Itabashi E, Fujii S, Nakamura T, Toriyama K (2016) Mitochondrial ORF79 levels determine pollen abortion in cytoplasmic male sterile rice. \textit{Plant J} 85: 707–716

Kazama T, Nakamura T, Watanabe M, Sugita M, Toriyama K (2008) Suppression mechanism of mitochondrial ORF79 accumulation by Rf1 protein in BT-type cytoplasmic male sterile rice. \textit{Plant J} 55: 619–628

Kazama T, Okuno M, Watari Y, Yanase S, Koizuka C, Tsuruta Y, Sugaya H, Toyoda A, Itoh T, Tsutsumi N, et al. (2019) Curing cytoplasmic male sterility via TALEN-mediated mitochondrial genome editing. \textit{Nat Plants} 5: 722–730

Kazama T, Toriyama K (2003) A pentatricopeptide repeat-containing gene that promotes the processing of aberrant \textit{atp6} RNA of cytoplasmic male-sterile rice. \textit{FEBS Lett} 544: 99–102

Kazama T, Toriyama K (2014) A fertility restorer gene, \textit{Rf4}, widely used for hybrid rice breeding encodes a pentatricopeptide repeat protein. \textit{Rice (N Y)} 7: 28

Kim YJ, Zhang D (2018) Molecular control of male fertility for crop hybrid breeding. \textit{Trends Plant Sci} 23: 53–65

Kinoshita T (1997) Gene symbols and information on male sterility. \textit{Rice Genet Newslett} 14: 13–22

Kitamura E (1962) Studies on cytoplasmic sterility of hybrids in distinctly related varieties of rice, \textit{Oryza sativa} \textit{L.}: I. Fertility of the \textit{F1} hybrids between strains derived from certain Philippine\texttimes Japanese variety crosses and Japanese varieties. \textit{Ipn J Breed} 12: 81–84 (in Japanese)

Koizuka N, Imai R, Fujimoto H, Hayakawa T, Kimura Y, Kohno-Murase J, Sakai T, Kawasaki S, Imamura J (2003) Genetic characterization of a pentatricopeptide repeat protein gene, \textit{orf687}, that restores fertility in the cytoplasmic male-sterile Kosena radish. \textit{Plant J} 34: 407–415

Kojima H, Kazama T, Fuji S, Toriyama K (2010) Cytoplasmic male sterility-associated ORF79 is toxic to plant regeneration when expressed with mitochondrial targeting sequence of ATPase gamma subunit. \textit{Plant Biotechnol} 27: 111–114

Komori T, Ohta S, Murai N, Takakura Y, Kuraya Y, Suzuki S, Hiei Y, Imaseki H, Nitta N (2004) Map-based cloning of a fertility restorer gene, \textit{Rf-1}, in rice (\textit{Oryza sativa} \textit{L.}). \textit{Plant J} 37: 315–325

Li S, Tan Y, Wang K, Wan C, Zhu Y (2008) Gametophytically alloplasmic CMS line of rice (\textit{Oryza sativa} \textit{L.}) with variant \textit{orfH79} haplotype corresponding to specific fertility restorer. \textit{Theor Appl Genet} 117: 1389–1397

Li SQ, Yang DC, Zhu YG (2007) Characterization and use of male sterility in hybrid rice breeding. \textit{J Integr Plant Biol} 49: 791–804

Lin SC, Yuan LP (1980) Hybrid rice breeding in China. In: IRRI (eds), \textit{Innovative Approaches to Rice Breeding}. IRRI, Manila, Philippines, pp 35–51

Luo D, Xu H, Liu Z, Guo J, Li H, Chen L, Zhang Q, Bai M, Yao N, Wu H, et al. (2013) A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. \textit{Nat Genet} 45: 573–577

Ma GH, Yuan LP (2015) Hybrid rice achievements, development and prospects in China. \textit{J Integr Agric} 14: 197–205

Melonek J, Stone JD, Small I (2016) Evolutionary plasticity of restorer-of-fertility-like proteins in rice. \textit{Sci Rep} 6: 35152

Motomura K, Ishimine Y, Murayama S, Higa T, Goya A, Motoyose T (2001) Inheritance of cytoplasmic male sterility and restorer of fertility gene in a developed rice line, RT98C. \textit{Ipn J Trop Agr} 45: 202–208 (in Japanese)

Motomura K, Moromizato Z, Adaniya S (2003) Inheritance of cytoplasmic male sterility and restoration of fertility in rice line, RT102C, derived from \textit{Oryza rufipogon}. \textit{Ipn J Trop Agr} 47: 70–76

Okazaki M, Kazama T, Murata H, Motomura K, Toriyama K (2013) Whole mitochondrial genome sequencing and transcriptional analysis to uncover an RT102-type cytoplasmic male sterility-associated candidate gene derived from \textit{Oryza rufipogon}. \textit{Plant Cell Physiol} 54: 1560–1568

Omukai S, Arimura S, Toriyama K, Kazama T (2021) Disruption of mitochondrial open reading frame 352 partially restores pollen
development in cytoplasmic male sterile rice. *Plant Physiol* 187: 236-246

O'Toole N, Hattori M, Andres C, Iida K, Lurin C, Schmitz-Linneweber C, Sugita M, Small I (2008) On the expansion of the pentatricopeptide repeat gene family in plants. *Mol Biol Evol* 25: 1120–1128

Peng X, Wang K, Hu C, Zhu Y, Wang T, Yang J, Tong J, Li S, Zhu Y (2010) The mitochondrial gene *orfH79* plays a critical role in impairing both male gametophyte development and root growth in CMS-Honglian rice. *BMC Plant Biol* 10: 125

Qin X, Huang Q, Xiao H, Zhang Q, Ni C, Xu Y, Liu G, Yang D, Zhu Y, Hu J (2016) The rice DUF1620-containing and WD40-like repeat protein is required for the assembly of the restoration of fertility complex. *New Phytol* 210: 934–945

Rao YS (1988) Cytohistology of cytoplasmic male sterile lines in hybrid rice. In: Smith WH, Bostian LR, Cervantes EP (eds), *Hybrid Rice*. IRRI, Manila, Philippines, pp 115–128

Sakai H, Lee SS, Tanaka T, Numata H, Kim J, Kawahara Y, Wakimoto H, Yang CC, Iwamoto M, Abe T, et al. (2013) Rice Annotation Project Database (RAP-DB): An integrative and interactive database for rice genomics. *Plant Cell Physiol* 54: e6

Sattari M, Kathiresan A, Gregorio G, Hernandez JE, Nas TM, Virmani SS (2007) Development and use of two-gene marker-aided selection system for fertility restorer genes in rice. *Euphytica* 153: 35–42

Schmitz-Linneweber C, Small I (2008) Pentatricopeptide repeat proteins: A socket set for organelle gene expression. *Trends Plant Sci* 13: 663–670

Shinjyo C (1984) Cytoplasmic male sterility and fertility restoration in rice having genome A. In: Tsunoda S, Takahashi N (eds) *Database for rice genomics*. Plant Cell Physiol 45: 22–1: 57

Shinjyo C (1969) Cytoplasmic-genetic male sterility in cultivated rice, *Oryza sativa* L. *Ipn J Genet* 44: 149–156

Shinjyo C (1975) Genetical studies of cytoplasmic male sterility and fertility restoration in rice, *Oryza sativa* L. *Sci Bull Coll Agr Univ Ryukyu* 22: 1–57

Suketomo C, Kazama T, Toriyama K (2020) Fertility restoration of Chinese wild rice-type cytoplasmic male sterile rice by CRISPR/Cas9-mediated genome editing of nuclear-encoded *RETROGRADE-REGULATED MALE STERILITY*. *Plant Biotechnol* 37: 285–292

Suresh PB, Srikanth B, Kishore VH, Rao IS, Vemireddy LR, Dharika N, Sundaram RM, Ramesha MS, Rao KRSS, Virakamath BC, et al. (2012) Fine mapping of *Rf3* and *Rf4* fertility restorer loci of WA-CMS of rice (*Oryza sativa* L.) and validation of the developed marker system for identification of restorer lines. *Euphytica* 187: 421–435

Takatsuka A, Kazama T, Toriyama K (2021) Cytoplasmic male sterility-associated mitochondrial gene *orf312* derived from rice (*Oryza sativa* L.) cultivar Tadukan. *Rice (N Y)* 14: 46

Tang H, Luo D, Zhou D, Zhang Q, Tian D, Zheng X, Chen L, Liu YG (2014) The rice restorer *Rf4* for wild-aborive cytoplasmic male sterility encodes a mitochondrial-localized PPR protein that functions in reduction of WA352 transcripts. *Mol Plant* 7: 1497–1500

Tang H, Zheng X, Li C, Xie X, Chen Y, Chen L, Zhao X, Zheng H, Zhou J, Ye S, et al. (2017a) Multi-step formation, evolution and functionalization of new cytoplasmic male sterility genes in the plant mitochondrial genomes. *Cell Res* 27: 130–146

Tang L, Xu ZJ, Chen WF (2017b) Advances and prospects of super rice breeding in China. *J Integr Agric* 16: 984–991

Toriyama K, Kazama T (2016) Development of cytoplasmic male sterile lines and restorer lines of various elite Indica Group rice cultivars using CW-CMS/Rf17 system. *Rice (N Y)* 9: 22

Toriyama K, Kazama T, Sato T, Fukuta Y, Oka M (2019) Development of cytoplasmic male sterile lines and restorer lines of various elite Indica Group rice cultivars using CW-CMS/Rf17 system. *Rice (N Y)* 12: 73

Wang K, Gao F, Ji Y, Liu Y, Dan Z, Yang P, Zhu Y, Li S (2013) ORFH79 impairs mitochondrial function via interaction with a subunit of electron transport chain complex III in Honglian cytoplasmic male sterile rice. *New Phytol* 198: 408–418

Wang X, Guan Z, Gong Z, Yan J, Yang G, Liu YG, Yin P (2018) Crystal structure of WA352 provides insight into cytoplasmic male sterility in rice. *Biochem Biophys Res Commun* 501: 898–904

Wang Z, Zou Y, Li X, Zhang Q, Chen L, Wu H, Su D, Chen Y, Guo J, Luo D, et al. (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: 676–687

Watanabe Y (1971) Establishment of cytoplasmic and genetic male-sterile lines by means of *Indica-Japonica* cross. *Oryza Cuttack* (Suppl 2): 9–16

Xie H, Peng X, Qian M, Cai Y, Ding X, Chen Q, Cai Q, Zhu Y, Lan L, Cai Y (2018) The chimeric mitochondrial gene *orf182* causes non-pollen-type abortion in Dongxiang cytoplasmic male-sterile rice. *Plant J* 95: 715–726

Yabuno T (1977) Genetic studies on the interspecific cytoplasm substitution lines of an Asian perennial strain of *Oryza rufipogon* GRiFF. and *O. glaberrima* STEUD. *Euphytica* 26: 451–463

Yagi H, Hayashi S, Kobayashi K, Hirayama T, Nakamura T (2013) Elucidation of the RNA recognition code for pentatricopeptide repeat proteins involved in organelle RNA editing in plants. *PLoS One* 8: e57286

Yan J, Yao Y, Hong S, Yang Y, Shen C, Zhang Q, Zhang D, Zou T, Yin P (2019) Delineation of pentatricopeptide repeat codes for target RNA prediction. *Nucleic Acids Res* 47: 3728–3738

Yi P, Wang L, Sun QP, Zhu YG (2002) Discovery of mitochondrial chimeric gene associated with cytoplasmic male sterility of HL rice. *Chin Sci Bull* 47: 744–747

Zheng W, Ma Z, Zhao M, Xiao M, Zhao J, Wang C, Gao H, Bai Y, Wang H, Sui G (2020) Research and development strategies for hybrid *japonica* rice. *Rice (N Y)* 13: 36