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Relationship between CMS-specific mitochondrial structures and pollen abortive phenotype in rice CMS lines

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Abstract Cytoplasmic male sterile (CMS) lines varied at pollen abortive phenotypes (PAP) have been used effectively for production of hybrid seeds in rice. To investigate the relationship between PAP of CMS lines and CMS-specific mitochondrial genes or structures in rice, CMS lines varied at PAP developed on 18 cytoplasmic sources were examined with seven PCR primers specific to three CMS-specific mitochondrial regions. The 18 CMS lines were classified into stained abortive (SA) and typical abortive (TA) types according to their PAP. Two CMS cytoplasms-specific PCR fragments, by which genotypes between the CMS lines and their maintainers were distinguished, were identified with primer LD29 or LD30. Furthermore, it was discovered that phenotypes of SA-CMS and TA-CMS were related to CMS-specific mitochondrial genes or structures, *LD-orf310*, *atp6-orf79*-like, and *orf288*-like structures. The results suggested that CMS phenotypes were mainly controlled by cytoplasmic genes, and it could be speculated that the SA-CMS phenotype was related to *LD-orf310* and *atp6-orf79*-like structure, while TA-CMS phenotype was related to *WA*352.

Keywords Cytoplasmic male sterility (CMS) · CMS-specific mitochondrial structure · Stained pollen abortion · Typical pollen abortion · Rice

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Introduction

Cytoplasmic male sterility (CMS), a phenotype of failure to produce functional pollens, is a popular phenomenon found in more than 150 flowering species (Young and Hanson 1987; Schnable and Wise 1998), and it plays a key role in commercial production of hybrid seeds in self-pollinating crops, such as rice (Ngangkham et al. 2010). Besides its great contribution in hybrid rice production, CMS in rice is also important in the study of nuclei–mitochondria interactions (Fujii and Toriyama 2008; Chen and Liu 2014), since more than 60 CMS sources have been identified in rice (reviewed by Li et al. 1982; Virmani 1994; Kinoshita 1997).

Of the CMS sources identified in rice, some have been used to develop CMS lines, and CMS lines on nine CMS sources, namely, Boro type (BT), Dissi (D), Dwarf-wild abortive (DA), Dian-type (DT), Gambiaka (G), Honglian (HL), Indonesia Paddy (ID), 83-K52 (K), and Wild abortive (WA), have

been popularly used for hybrid seed production. CMS lines are not only varied at cytoplasm sources but also pollen abortive phenotypes (PAP). According to their PAP, the CMS lines are classified into three types: stained abortive (SA), which produces very high ratios of abortive pollen grains in a round shape with starch partially accumulated and stainable by I_2–KI; round abortive (RA), which produces very high ratios of pollen grains in a round shape, but without accumulation of the starch and unstainable by I_2–KI; and the typical abortive (TA) type, which produces very high ratios of pollen grains in irregular shapes, without accumulation of the starch and unstainable by I_2–KI (reviewed by Yuan 2002; Li et al. 2007).

CMS phenotypes in plants are always related to mitochondrial dysfunction resulted by abnormal rearrangement, modifications, and chimerism of mitochondrial DNA (mtDNA) (Dewey et al. 1986; Levings 1990; Kadowaki et al. 1990; Hanson 1991; Kaleikau et al. 1992; Iwabuchi et al. 1993; Akagi et al. 1994, 1995; Hernould et al. 1998; Yi et al. 2002; Hanson and Bentolila 2004; Kazama et al. 2008; Das et al. 2010; Chen and Liu 2014). In WA-CMS, which is the most important CMS in hybrid rice production (Yuan 2002), a novel structure *WA 352* was detected in mitochondrial, which was also detected in other popularly used sporophytic CMSs, such as D, DA, G, ID, and K (Luo et al. 2013). While *orf79* structure was detected in BT- and DT-CMS (Wang et al. 2006; Kazama et al. 2008; Luan et al. 2013), and *orfH79* was detected in HL-CMS (Yi et al. 2002; Peng et al. 2010).

Although it is well known that the CMS phenotype is conferred by CMS-specific mitochondrial genes/structures, the relationship between the mitochondrial genes/structures and the PAP of CMS lines in rice is still unclear.

Materials and methods

Plant materials

Rice materials include 18 CMS lines and their corresponding maintainers (nuclei donors), both of them are isonuclear-alloplasmic lines. Of the 18 CMS, the first nine in Table 1 are used for commercial production of *indica* or *japonica* hybrid rice, and the other nine are developed at RRI-YAU, Kunming, Yunnan, China (Table 1).

Evaluation of pollen fertility

During the rice plant flowering, nine nearly blooming spikelets were collected from three plants of each CMS line in the morning, and kept in 75 % ethanol. Pollen grains from three spikelets of each CMS lines were stained with 1 % I_2–KI solution and then examined under a microscope. Pollen grains were classified into stained-, round-, and typical-abortive (SA, RA, and TA) types according to their PAP as described by Li et al. (1982).

DNA preparation and PCR assay

Total genomic DNA was extracted from freshly harvested young etiolated seedlings with CTAB method (Rogers and Bendich 1985). Seven PCR primers specific to CMS-related genes or structures were synthesized by Beijing Genomics Institution (BGI) according to the sequences from the references (Table 2). Target DNA fragments were amplified with a total volume of 25 μl containing 20 pmol primers, 2 μl of 10 mM dNTP mixture (2.5 mM each), 1U of *Taq* DNA polymerase (Takara, Dalian, China), 5 ng of total DNA, and 1× PCR buffer (1.5 mM Mg^{2+}). The PCR was performed on a thermocycler (Applied Biosystems, Carlsbad, CA, USA) under the following thermo conditions: initial denaturation at 94 °C for 4 min, followed by 20–30 cycles at 94 °C for 30 s, 55 °C for 30 s, then 72 °C for 40 s-3 min (details in Table 2), with a final extension for 10 min at 72 °C. The PCR products were resolved by electrophoresis through a 2.0 % agarose gel and stained by ethidium bromide.

Sequence of the PCR products

PCR products were purified by using SanPrep column DNA gel extraction kit (Sangon, Shanghai, China), cloned by p-EASY T1 vector (Transgene, Beijing, China), then sequenced with bi-directional method in five replications by Sunbio Biotech (Bejjing Sunbiotech Co., Ltd., China). The sequences of PCR products were analyzed on NCBI Blast (http://www.ncbi.nlm.nih.gov/BLAST/).

Table 1 The rice CMS lines and maintainers

No.	CMS line	CMS type	Maintainer	Cytoplasm donor	Refs.
1	BT-J139 A	BT	J139	Chinsurah Boro II	Shinjyo (1969, 1975)
2	D-I25 A	D	I25	Dissi (D52/37)	Li (1997)
3	DA-H42 A	DA	H42	A dwarf wild abortive rice plant	Wu (1986)
4	DT-H42 A	DT	H42	High altitude a *O.* ssp. *indica* cultivar	Li et al. (1980)
5	G-I25 A	G	I25	Gambiaka	Li (1997)
6	HL-DY A	HL	DY	A red-awned wild rice plant	Rao (1988)
7	ID-H42 A	ID	H42	Indonesian paddy rice no. 6	Zhang (1980, 1983)
8	K-L207 A	K	L207	83-K52	Zheng et al. (1995)
9	WA-H42 A	WA	H42	A wild abortive rice plant	Lin and Yuan (1980); Yuan (2002)
10	D7-CJ23 A	D7	CJ23	D7	Wang et al. (2009)
11	D502-H23 A	D502	H23	Diantun502	Wang et al. (2009)
12	IR58-H42 A	IR58	H42	IR58025	Wang et al. (2009)
13	LJ454-Qu 2 A	LJ454	Qu 2	Liaojing454	RRI-YAU 2011 (Unpublished)
14	MH63-H34 A	MH63	H34	Minghui63	Wang et al. (2009)
15	MY23-DY A	MY23	DY	Milyang23	Wang et al. (2009)
16	MY77-YJY 14 A	MY77	YJY14	Milyang77	Tan et al. 2011 (Unpublished)
17	N29-DY A	N29	DY	Nan29	Wang et al. (2009)
18	N34-CJ23 A	N34	CJ23	Nan34	Wang et al. (2009)

Results

Variations of pollen abortive phenotype characteristics in the CMS lines

Three kinds of pollen grains, TA, RA, or SA, were observed in most of the CMS lines, but the ratios of TA or SA pollen grains were much higher than those of RA ones in all the CMS lines. The CMS lines with D, DA, G, ID, K, and WA cytoplasm, and other three CMS lines developed on D502, IR58, and MY23 cytoplasm, had much higher ratios of TA pollen grains. Therefore, these CMS lines were termed TA-CMS lines. While the CMS lines on BT, DT cytoplasm, and the other six CMS lines developed on D7, LJ454, MH63, MY77, N29, and N34, cytoplasm had much higher ratios of SA pollen grains, and were termed SA-CMS lines. The HL-CMS line, HL-DY A, which possessed *O. sativa* ssp. *japonica* nuclear background donated by a *japonica* cultivar DY, had a much higher ratios of SA pollen grains, but very low ratios of RA and TA pollen grains (Table 3).

Relationship between pollen abortive phenotypes and PCR products on mtDNA

All the CMS lines varied at PAPs showed the genotype difference from their maintainers on fragments amplified by LD29 and LD30. The CMS lines possessed a 905-bp or a 2555-bp fragment amplified by LD29, named as LD29-SA and LD29-TA, respectively, and a 788-bp fragment by LD30, named as LD30-A, but their maintainers did not possess any fragment with these two primers (Fig. 1). The genotype differences between CMS lines and their maintainers were also revealed by the other five primers with the similar results, in which PCR fragments were obtained only in SA- or TA-CMS lines, but not in their maintainers (data not shown).

Genotype difference on mtDNA was detected not only between CMS lines and their maintainers but also between SA- and TA-CMS lines. With primers on CMS-specific genes or structures, the SA- and TA-CMS can be distinguished clearly by PCR products amplified in all of the SA-CMS lines, but not in a TA-CMS line, or in contrast, in all of the TA-CMS lines,

Table 2 Information of PCR primers and thermo conditions

No.	Primer	Sequence (5′–3′)	Cycles	Elongation	Refs.
1	LD29	TTCCCATCAGTGCTGCTACT(F) CGTGCTTTCTTGAAAGGAGG(R)	20	3 min	Luan et al. (2013)
2	LD30	GAGTAAGGCAATTTGGCTTG(F) AGACCTGTCATCAGTAGTTC(R)	30	40 s	Luan et al. (2013)
3	orWA352	GTTGATGGGTATGGATAGAG(F) CGCAGGGCCTCGGTATATCTA(R)	30	1 min 30 s	Luo et al. (2013)
4	Fragment I	ATTTTUCTCGAGUCTATGGCAAATCTGGTC(F) GTATGTCTAGACCACCACTGTCC(R)	20	40 s	Wang et al. (2006)
5	Fragment III	GGCCGGTCATAGTTCAGT(F) GTATGTCTAGACCACCACTGTCC(R)	30	40 s	Wang et al. (2006)
6	B-atp6	TCTCCCTTTCTAGGAGCAGAG(F) TAACGCAATACACTTCCGCG(R)	30	1 min 30 s	Wang et al. (2006)
7	atp6-orf79	TCTCCCTTTCTAGGAGCAGAGC(F) AGGGGTGGGATATTTGCCTGGTCCACC(R)	30	2 min	Luan et al. (2013)

but not in a SA-CMS line. For instance, SA- and TA-CMS lines showed different genotypes on PCR products amplified by LD29. All the SA-CMS lines possessed LD29-SA fragment, but no TA-CMS possessed this fragment. In contrast, all the TA-CMS lines possessed LD29-TA fragment, but no SA-CMS line possessed this fragment (Fig. 1a; Table 4). Genotype difference between SA- and TA-CMS lines was observed not only on PCR fragment size difference, as revealed by LD29, but also on presence/absence of the PCR fragment revealed by the other five primers (Table 4). For example, all the TA-CMS possessed a 1432-bp fragment, with orWA352 primer, but no fragment was amplified in SA-CMS (Fig. 2a). In contrast, all the SA-CMS possessed a 1592-bp fragment amplified with primers atp6-orf79, but no fragment was obtained in TA-CMS with the primer (Fig. 2b).

Analysis of the PCR fragments

The sequences of all the eight PCR fragments from the CMS lines were aligned to the sequences on three mtDNA regions, *atp6-orf79*-like, *orf288*-like, and an intergenic region before *ccmFn*, with 99 or 100 % of identity (Fig. 3). Four fragments, FI-SA, FIII-SA, Ao79-SA, and Ba6-SA, were aligned on *atp6-orf79*-like region discovered in BT-CMS and LD-CMS, and these four fragments shared the same sequences with each other at upstream or downstream regions. Fragment FI-SA covered downstream of *atp6-orf79*-like and whole *orf79*, which overlapped with a downstream sequence of fragment FIII-SA and a sequence near the downstream of fragment Ao79-SA. Fragment FIII-SA overlapped a sequence in the downstream region of *atp6-orf79*-like structure, which included a downstream of *atp6* and whole *orf79*, plus a 207-bp intergenic sequence between *atp6* and *orf79*. Except for being aligned to the *atp6-orf79*-like structure, FIII-SA covered a downstream sequence of fragment Ba6-SA with a 289-bp upstream sequence, and overlapped a sequence near downstream sequence of fragment Ao79-SA with whole sequence. Fragment Ba6-SA overlapped with an upstream sequence of *atp6-orf79*-like structure, which covered the entire *atp6* and an upstream sequence of fragment Ao79-SA, while fragment Ao79-SA, the largest fragment amplified in the region, covered

Table 3 Pollen abortive phenotypes of the CMS lines

No.	CMS lines	CMS type	Pollen abortive phenotype	Ratios of pollen abortive type (%)		
				SA	RA	TA
1	BT-J139 A	BT	SA	97.82	1.34	0.84
2	D-I25 A	D	TA	0	40.34	59.66
3	DA-H42 A	DA	TA	1.42	4.18	94.4
4	DT-H42 A	DT	SA	99.03	0.42	0.55
5	G-I25 A	G	TA	4.11	12.92	82.97
6	HL-DY A	HL	SA	91.42	3.34	5.24
7	ID-H42 A	ID	TA	2.15	7.61	90.24
8	K-L207 A	K	TA	13.12	4.44	82.44
9	WA-H42 A	WA	TA	9.72	3.63	86.66
10	D7-CJ23 A	D7	SA	97.73	0.34	1.93
11	D502-H23 A	D502	TA	3.54	13.39	83.07
12	IR58-H42 A	IR58	TA	0.31	6.17	93.51
13	LJ454-Qu 2 A	LJ454	SA	95.27	0.9	3.83
14	MH63-H34 A	MH63	SA	94.93	3.65	1.43
15	MY23-DY A	MY23	TA	0.63	17.94	81.43
16	MY77-YJY 14 A	MY77	SA	97.56	1.01	1.43
17	N29-DY A	N29	SA	95.22	0.98	3.8
18	N34-CJ23 A	N34	SA	98.61	0	1.39

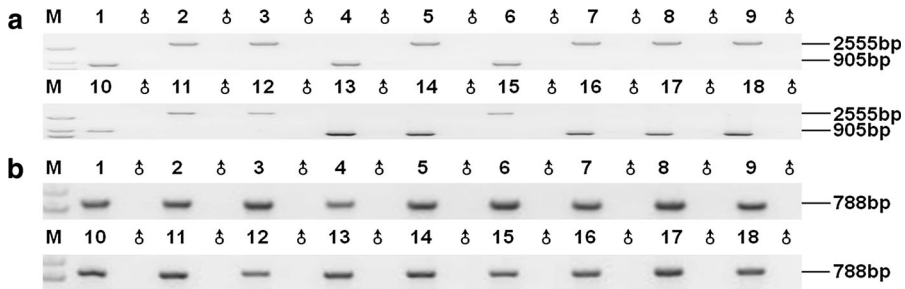

Fig. 1 Genotype difference between the CMS lines and their maintainers on fragments amplified with primer LD29 (**a**) and LD30 (**b**). Lanes 1–18 are the CMS lines in the same order as listed in Table 3, and the male symbol ♂ represents the corresponding maintainers

whole sequences of *atp6-orf79*-like structure and the other three PCR fragments amplified in this region (Fig. 3a).

Three fragments, LD29-SA, LD29-TA, and W352-TA, were aligned in *orf288*-like region. The downstream sequence of fragment LD29-SA covered a 393-bp upstream sequence of *LD-orf310*, and shared the same sequences with LD29-TA at upstream and downstream with a 499-bp and a 26-bp sequences, respectively (Fig. 3a). At the *orf288*-like region identified in WA-CMS, fragment LD29-TA covered an upstream sequence of *WA352*, and whole sequences of *rpl5-rps14*, plus two intergenic sequences and a single nucleotide insertion between *rpl5* and *rps14*, in which one was a 517-bp sequence at downstream of an intergenic sequence between *atp1* and *rpl5*, and another one was a 665-bp sequence between *rps14* and *WA352* (Fig. 3b). Some difference from LD29-TA, fragment WA352-TA, which overlapped with downstream of LD29-TA fragment with a 810-bp upstream sequence, covered whole *WA352*, plus two intergenic sequences, a 282-bp sequence between *rps14* and *WA352* and a 91-bp sequence between *WA352* and *atp6*. Unlike the above seven fragments, which were located at the

Table 4 Genotype difference between SA- and TA-CMS lines on PCR fragments

No.	CMS lines	CMS type	PAP	PCR fragments							
				LD29-SA	LD29-TA	LD30-A	W352-TA	Ao79-SA	Ba6-SA	FI-SA	FIII-SA
1	BT-J139 A	BT	SA	+	−	+	−	+	+	+	+
2	D-I25 A	D	TA	−	+	+	+	−	−	−	−
3	DA-H42 A	DA	TA	−	+	+	+	−	−	−	−
4	DT-H42 A	DT	SA	+	−	+	−	+	+	+	+
5	G-I25 A	G	TA	−	+	+	+	−	−	−	−
6	HL-DY A	HL	SA	+	−	+	−	+	+	+	+
7	ID-H42 A	ID	TA	−	+	+	+	−	−	−	−
8	K-L207 A	K	TA	−	+	+	+	−	−	−	−
9	WA-H42 A	WA	TA	−	+	+	+	−	−	−	−
10	D7-CJ23 A	D7	SA	+	−	+	−	+	+	+	+
11	D502-H23 A	D502	TA	−	+	+	+	−	−	−	−
12	IR58-H42 A	IR58	TA	−	+	+	+	−	−	−	−
13	LJ454-Qu2 A	LJ454	SA	+	−	+	−	+	+	+	+
14	MH63-H34 A	MH63	SA	+	−	+	−	+	+	+	+
15	MY23-DY A	MY23	TA	−	+	+	+	−	−	−	−
16	MY77-YJY14 A	MY77	SA	+	−	+	−	+	+	+	+
17	N29-DY A	N29	SA	+	−	+	−	+	+	+	+
18	N34-CJ23 A	N34	SA	+	−	+	−	+	+	+	+

Fragments LD29-SA and LD29-TA were amplified by primer LD29, other fragments LD30-A, W352-TA, Ao79-SA, Ba6-SA, FI-SA, and FIII-SA were amplified by primers LD30, orWA352, atp6-orf79, B-atp6, fragment I, and fragment III, respectively

PAP pollen abortive phenotype of the CMS lines

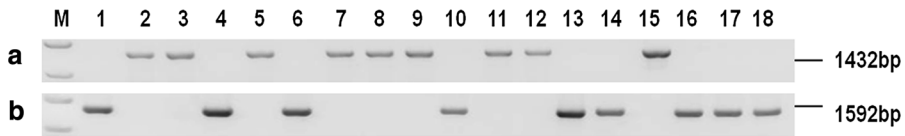

Fig. 2 PCR fragment presence/absence on TA- and SA-CMS. **a** Fragments amplified with primer orWA352. **b** Fragments amplified with primer atp6-orf79. Lanes 1–18 are the CMS lines as listed in Table 3

regions harboring CMS-specific genes or structures, fragment LD30-A was aligned to an intergenic region before *ccmFn* (Fig. 3a, b).

Discussion

Relationship between PAP and genetic background of CMS lines in rice

It has been well documented that the CMS lines possessing the same cytoplasm would produce pollen grains with similar PAP, regardless of the nuclear background, except for contamination by related restorer gene(s). For example, the CMS lines possessing WA cytoplasm produced very high ratios of TA pollen grains despite the nuclear background donated by either *O. sativa* ssp. *indica* or *O. sativa* ssp. *japonica* lines (Tang et al. 2005). Such phenomenon indicated that PAPs of CMS lines in rice were mainly controlled by CMS-specific genes or structures in cytoplasm. However, two PAP types, RA and SA, were observed in HL-CMS lines. The HL-CMS lines developed by cultivars with *indica* nuclei were classified as RA type, since they produced very high ratios of RA pollen grains (Yuan

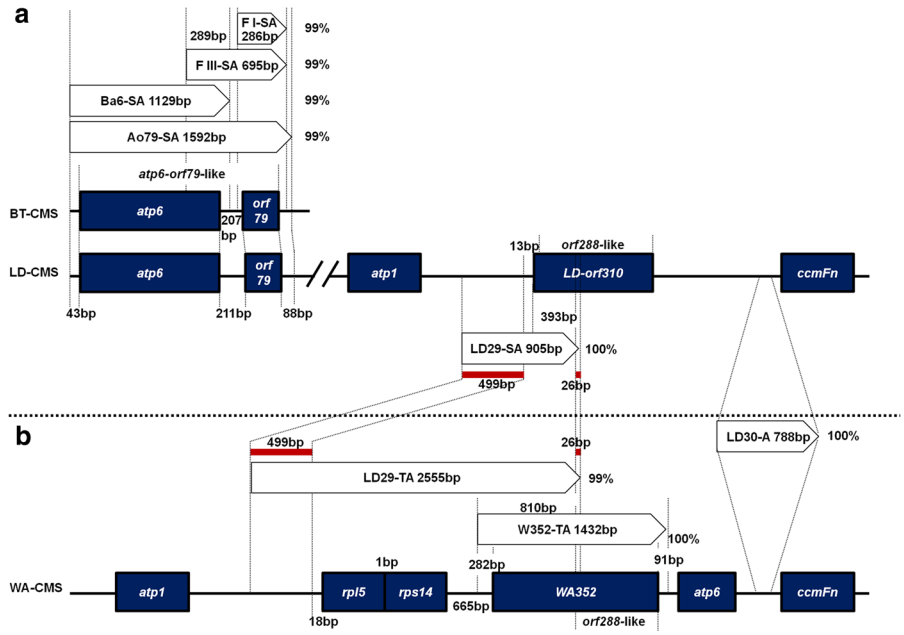

Fig. 3 PCR fragments aligned to mitochondrial genome of BT-CMS/LD-CMS (**a**) and WA-CMS (**b**) in rice. BT-CMS structure in **a** is based on D14339.1 (Akagi et al. 1994), and the LD-CMS structure is based on AB254027.1 (Itabashi et al. 2009), AP011077.1 (Fujii et al. 2010) in NCBI, in which the intergenic sequence between *atp6* and *orf79* is 207 bp in BT-CMS, while the intergenic sequence is 211 bp in LD-CMS. **b** Based on JF281154 (Bentolila and Stefanov 2012) and JX131325.1 (Luo et al. 2013) in NCBI. The *rectangular arrows* represent PCR fragments amplified in the current study. *Boxes filled with dark blue color* represent genes identified in mitochondrial genomes. The *red bars* represent consistent sequences between LD29-SA and LD29-TA fragments with 100 % identity. *Percentages at right side of the rectangular arrows* represent similarity between the PCR fragments and target sequences. The sizes of genes or PCR fragments are given in *Arabic numerals* with bp, and the *numbers* with bp between two *vertical lines* are the sizes of sequences

2002; reviewed by Li et al. 2007). However, when the HL-CMS lines possessing nuclei were donated by *japonica* cultivars, the CMS lines would produce very high ratios of SA pollen grains, therefore they would be classified as SA type. For example, HL-DY A, a CMS line possessing a *japonica* nucleus on HL cytoplasm, produced a very high ratio of SA pollen grains, and was classified as SA type in the current study (Table 2). HL-CMS lines with an SA phenotype were also reported in other study in the case of *indica* nuclei of HL-CMS lines replaced by the *japonica* nuclei (Tang et al. 2005). In rice CMS lines, such PAP change resulted by *O*. ssp. *indica* nuclei replaced by *O*. ssp. *japonica* ones was just observed in HL-CMS, but not in the TA-CMS lines, such as those possessing G, ID, and WA cytoplasm in the current study and in other research. The change difference of PAP type in HL-CMS lines indicated that genetic interaction between the CMS-specific mitochondrial genes or structures and nuclear background not only caused pollen abortion (Luo et al. 2013) but also determined PAP types of CMS lines, even though the abortion of pollen grain was mainly controlled by CMS-specific genes or structures in mitochondria. Furthermore, although environmental factors could have some effects on ratios of pollen abortive types, no cases of environmental factors resulted in a PAP type change were reported.

Different CMS-specific mtDNA structures corresponding to different CMS-PAP types

It is well known that CMS phenotypes in rice were mainly related to two mtDNA structures, *atp6-orf79*-like and *orf288*-like structures. *Atp6-orf79*-like structures, containing *atp6* and *orf79*-like genes, were related to CMS phenotypes of BT-, HL-, and LD-CMS lines (Wang et al. 2006; Itabashi et al. 2009; Peng et al. 2010), and *orf79*-like structure was considered to be a key structure in the most effective CMS-related

mitochondrial genes in BT-CMS (Iwabuchi et al. 1993; Wang et al. 2006; Fujii et al. 2010), while the *orf288*-like structures caused CMS phenotypes of CW-, LD-, and WA-CMS lines, in which *CW-orf307*, *LD-orf310*, and *WA352* were identified, respectively (Fujii et al. 2010; Luo et al. 2013). Our results revealed that all the SA-CMS lines had the same genotype on the PCR fragments amplified on *atp6-orf79*-like region, while none of the TA-CMS lines possessed those fragments. Similarly, another fragment LD29-SA, amplified on the *orf288*-like structure identified in LD-CMS (Fujii et al. 2010), was only detected in SA-CMS, but not in TA-CMS lines. It was reported that a fragment on *atp6-orf79*-like structure was observed only in gametophytic CMS lines possessing BT-, DT-, and HL-CMS, which had SA phenotype in the current study, but not in sporophytic CMS lines, such as those possessed WA-CMS, which had TA phenotype in the current and other study (Luan et al. 2013). In contrast, two fragments LD29-TA and W352-TA, amplified on *LD-orf310* and *orf288*-like structures, which were identified in LD-CMS and WA-CMS, respectively (Fujii et al. 2010; Luo et al. 2013), were only detected in TA CMS lines, but not in SA-CMS lines.

Since fragments on *atp6-orf79*-like or *LD-orf310* structures were detected only in SA-CMS lines in this study or in HL-CMS lines in other studies (Wang et al. 2006; Peng et al. 2010; Luan et al. 2013), which showed RA-phenotype when they possessed *indica* nuclei background (Yuan 2002), it could be speculated that *atp6-orf79*-like structure and *LD-orf310* identified in LD-CMS were mainly related to the CMS possessing SA or RA phenotype. The W352-TA and LD29-TA fragments, which contained an *orf288*-like structure or a part of the structure, which was identified in *WA352* and *LD-orf310* (Fujii et al. 2010; Luo et al. 2013) were only detected in TA-CMS, but not in SA-CMS. This suggested that *WA352* would be a major gene conferred TA phenotype in rice CMS. However, more studies are needed to clarify the relationship between these mtDNA regions and PAP phenotypes in rice CMS.

Interestingly, fragment LD30-A, which was located in a function-unknown region at front of *ccmFn* (Fig. 3), was detected only in the 18 CMS lines, but not in their maintainers, which did not possess CMS cytoplasm. This result might imply that fragment LD30-A could be also related to CMS-phenotypes, but more evidence is required.

Conclusions

CMS phenotypes in rice were mainly related to mitochondrial *WA352*, *LD-orf310*, and *atp6-orf79*-like structures. Besides these three structures, a function-unknown region before *ccmFn* could be related with CMS-phenotype in rice. SA-CMS phenotype could be mainly related to *LD-orf310* and *atp6-or f79*-like structure, and TA-CMS could be mainly related to *WA352*, which contained an *orf288*-like structure.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The authors note that this research is performed and reported in accordance with ethical standards of the scientific conduct. We confirm to have the authority to publish this work and that the manuscript has not been published before and is not under consideration for publication elsewhere.

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