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Genetic analysis of fertility-restorer genes in rice

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Abstract

Wild-abortive (WA), Honglian (HL) and Baro-II (BT) are three important cytoplasmic male sterility (CMS) types in rice. It is essential to investigate genetic mode and allelism of fertility restorer (*Rf*) genes and the relationship between *Rf* and CMS. Fertility of the all test-cross F_1 plants shows that the restorer-maintainer relationship is similar for HL-CMS and BT-CMS, while that is variance for WA-CMS and HL-CMS (or BT-CMS), respectively. Genetic analysis of *Rf* genes indicates that HL- or BT-CMS are controlled by single dominant *Rf* gene and WA-CMS is controlled by one or two pairs of dominant *Rf* genes, which reflects the characters of the gametophytic and sporophytic restoration CMS type. It is concluded that there are at least three *Rf* loci in different accessions with *Rf* genes for each CMS type.

Additional key words: allelism test, cytoplasmic male sterility, *Oryza sativa* L.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal plants. In the latest forty years, the discovery of cytoplasmic male sterility (CMS) not only facilitates hybrid seed production, but also provides an excellent system for the study of nucleus-cytoplasm interaction. Since the discovery of Wild-abortive (WA) CMS line of rice, there were over sixty CMS lines including Dissi, Gambodia, Indonesia rice, Dian I, Honglian (HL), Baro-II (BT) and Maxie were developed from crossing between interspecies, intersubspecies and even intervarieties of *Oryza* species with AA genome (Zhu *et al*. 2000, Li and Yuan 2000). The cytological, physiological and genetic characteristics of these CMS systems have been extensively studied, due to their important economic value in agriculture. They were usually categorized into WA-CMS, HL-CMS and BT-CMS based on the evidence from genetic and cytological studies (Rao 1988, Li and Yuan 2000).

CMS can be restored by nuclear restorer gene (*Rf*). Multiple *Rf* loci are certainly determined by the multiple CMS systems in the natural populations within a plant species and one or more than one major restoring loci confer complete fertility restoration in a majority of CMS systems, such as in *Brassica napus*, *Zea mays* and *Plantago coronopus* (Gabay-Laughnan and Laughnan 1994, Bellaoui *et al*. 1999, Van Damme *et al*. 2004). In rice, a variable number of fertility restorer genes can restore complete fertility of certain a CMS line in various restorer lines. One or two dominant restorer alleles (*Rf3* and/or *Rf4*) are usually suggested be responsible for the fertility of WA-CMS (Tan *et al*. 1998). BT-CMS is also regulated by one or two restorer alleles, *Rf1a* and/or *Rf1b* (Komori *et al*. 2003, Akagi *et al*. 2004, Wang *et a*l. 2006). HL-CMS is always restored by single dominant gene, *Rf5* or *Rf6*, in the different restorer accession (Liu *et al*. 2004).

According to the characteristics, we speculated that there were the various fertility restorer genes and genetic mode for the different CMS types. To understand better the genetic mode of the fertility restorer genes and the *Rf* alleles in wild and cultivated rice for these three CMS, we summarized the more information about those through genetic analysis and allelism test.

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Abbreviations: BT - Baro-II; CMS - cytoplasmic male sterility; HL - Honglian; *Rf* - fertility restorer; WA - Wild-abortive.

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Materials and methods

Plants: A total of 73 accessions of *Oryza* species with AA-genomes, including 52 cultivated rice (*indica* and *japonica*) and 21 wild rice were used in this study (Table 1). A typical wild-abortive CMS line of Zhenshan 97A (ZsA) (*indica*) and the corresponding maintainer Zhenshan 97B (ZsB) (*indica*), a typical Honglian CMS line of Yuetai A (YtA) (*indica*) and the corresponding maintainer Yuetai B (YtB) (*indica*) and a typical BT-CMS line of Baoyuan A (ByA) (*indica*) and the corresponding maintainer Baoyuan B (ByB) (*indica*) were used as female parents with all those accessions. These plant materials and their derived progenies were all planted in the experimental field within Wuhan University campus in the summer and Hainan Island, Hainan, China in the winter during the 2004 - 2006 year. All photoperiod-sensitive accessions and their derived progenies were given a 10-h short photoperiod (08:00–18:00 h) after they were grown for about 2 months at Wuhan.

Population construction for genetic analysis: All the accessions with the fertility restore ability were crossed as the male parent with emasculated YtB and ByB. Then, YtA and ByA were test-crossed as female parents with every fertile hybrid F_1. To WA-CMS, we constructed the F_2 generation segregation populations by ZsA as the female parents with all accessions with fertility restore ability. The fertility segregation of the BC_1F_1 or F_2 population was evaluated for genetic analysis of the fertility restorer genes.

Allelism test for fertility restorer genes (*Rf*): Some accessions with the fertility restore ability were crossed as the male parent with Teqing and Minghui63 as bridge parents for WA-CMS, with 9311 and Milyang23 as bridge parents for HL-CMS and with 9149 and Milyang23 as bridge parents for BT-CMS. To compare the relationship of restorer genes, seven fertile F_1 plants for WA-CMS, twenty fertile F_1 plants for HL-CMS and five fertile F_1 plants for BT-CMS were selected and test-crossed as male parents with their respective CMS line ZsA, YtA and ByA. If the sterile plants are not observed in the population derived from the test-cross of A//Rf/Rf', the restoring loci between two different restorer lines (*Rf* and *Rf'*) are thought to be allelic. Otherwise, the two restoring loci are considered non-allelic.

Fertility scoring for all F_1 hybrids tested: Pollen fertility and seed-setting rate were used as the main criteria for the evaluation of fertile and sterile plants. Mature anthers were harvested, and the pollen was stained with 1 % I_2-KI solution. The numbers of dark blue (stainable) and clear pollen grains (unstainable) in each individual were counted under an optical microscope, and the numbers of the seed set on a spikelet were counted. Fertility evaluation was conducted as described by Li *et al*. (2005). Natural seed-setting rates of F_1 hybrid were used as the main criteria for the evaluation of compatibility. At least three different plants must be tested for every hybrid combination.

Statistical analysis: The χ^2 was used to determine whether the ratio between the number of fertile and sterile plants expected for the inheritance mode. The ratio between the number of fertile and sterile plants fit the 3:1 ratio expected for the inheritance mode of a single dominant gene or fit the 15:1 and 9:7 ratio expected for the inheritance mode of two pairs of dominant genes in the F_2 populations. The ratio between the number of fertile and sterile plants fit the 1:1 ratio expected ratio expected for the inheritance mode of a single dominant gene or fit the 3:1 ratio expected for the inheritance mode of two pairs of dominant genes in the backcross populations BC_1F_1. *P* values were determined from χ^2 tables (Rao 1998).

Results

Fertility of F_1 hybrids for WA-CMS, HL-CMS and BT-CMS: Outcrossing is a widely used method to enlarge the genetic background in rice breeding programs, but the unpredictable reproductive-isolation between species usually leads to the male sterility. In this research, the accessions used as male parents belong to seven different rice species and two subspecies, the sterility of some testcross may be caused by reproductive barriers. In order to understand if the sterility of the testcrosses came from the possible incompatibility of outcrossing, ZsB, YtB and ByB, the maintainers corresponding to ZsA, YtA and ByA, holding the same nuclear genetic background with their corresponding CMS line besides the cytoplasm, were emasculated and crossed respectively as female parents with the other accessions. Nature seed-setting rate of F_1 hybrids were tested for all accessions, of which no and low fertile test-crossed F_1 hybrids (Table 2). These data eliminate reproductive barriers likelihood and indicate that there are compatible between all accessions and ZsB, YtB and ByB and the sterility of the testcrosses have nothing to do with reproductive barriers.

Fertility of WA-type, HL-type and BT-type F_1 hybrids were carefully evaluated, the F_1 hybrids with both over 10 % stainable pollen and 20 % seed-setting rate were regard as fertile. 65 out of 73 HL-type and BT-type F_1 plants were fertile and eight F_1 plants were sterile respectively. 55 WA-type F_1 hybrids plants were fertile and 18 F_1 plants were sterile (Table 1). The distribution frequency of *Rf* for HL- and BT-CMS (89.0 %) was obviously higher than that for WA-CMS (75.3 %). For these three CMS types, the restorer-maintainer relationship of HL-CMS is consistent nearly with that of

Table 1. The *Oryza* genus accessions used in this study and the fertility of their F_1 hybrids tested for WA-CMS, HL-CMS and BT-CMS lines. Means of three replicates ± SD.

Series name	Accessions	Species	Source country	WA-CMS [%]		HL-CMS [%]		BT-CMS [%]	
				fertile pollen	seed setting	fertile pollen	seed setting	fertile pollen	seed setting
W1	101255	*O. barthii*	Cameroon	0	0	0	0	0	0
W2	101791	*O. glaberrima*	Senegal	0	0	0	0	0	0
W3	101978	*O. nivara*	India	0	0	40.5±1.3	11.7±1.2	30.0±1.4	54.0±1.6
W4	102641	*O. glaberrima*	Liberia	0	0	0	0	0	0
W5	104085	*O. meridionalis*	Australia	0	0	0	0	0	0
W6	104540	*O. glaberrima*	Nigeria	0	0	1.0	0	5.0±1.4	9.4±2.3
W7	105668	*O. glumaepatula*	Brazil	0	0	0	0	0	0
W8	106194	*O. barthii*	Guinea	0	0	0	0	0	0
W9	103836	*O. nivara*	Bangladesh	8.6±1.2	5.5±1.1	71.1±5.3	23.1±2.4	54.3±2.1	34.2±1.8
W10	106102	*O. nivara*	India	10.0±2.1	23.0±2.3	46.7±3.2	54.0±3.6	33.3±1.9	35.3±1.7
W11	103821	*O. nivara*	China	27.5±2.6	32.0±1.3	30.5±1.7	43.3±2.3	43.3±3.2	45.0±4.1
W12	Dongxiang2	*O. rufipogon*	China	31.6±2.2	40.7±1.5	50.9±1.7	27.6±3.3	43.3±6.1	54.3±4.3
W13	105824	*O. nivara*	Thailand	43.3±2.3	65.0±4.3	70.0±4.4	53.5±2.3	92.7±6.7	78.0±4.1
W14	105887	*O. rufipogon*	Bangladesh	51.0±2.3	70.0±3.2	50.3±1.4	49.2±2.1	50.3±3.5	49.2±4.3
W15	101855	*O. glaberrima*	Burkina Faso	52.7±3.3	10.0±1.3	33.7±2.4	22.7±3.4	35.5±2.3	28.3±1.3
W16	106309	*O. nivara*	Cambodia	68.0±2.7	77.1±3.6	84.8±3.4	75.6±4.6	56.7±3.2	73.2±2.7
W17	106158	*O. rufipogon*	Laos	71.4±2.3	52.6±2.1	48.3±2.5	20.5±5.4	53.2±4.3	40.5±4.4
W18	106083	*O. rufipogon*	India	77.6±5.6	46.0±3.2	67.4±4.6	62.7±4.7	43.6±3.2	75.4±1.9
W19	101971	*O. nivara*	India	79.4±2.2	23.7±3.4	78.3±4.5	24.1±2.3	58.7±4.1	34.6±1.7
W20	104705	*O. nivara*	India	86.6±3.3	37.4±1.7	73.6±2.9	66.1±1.4	63.4±1.7	73.2±6.7
W21	106344	*O. nivara*	Myanmar	88.3±5.7	86.0±5.3	0	0	0	0
C1	64786	*O. sativa* ssp. indica	Bangladesh	25.0±3.4	29.2±4.4	65.0±4.1	93.7±2.5	43.0±4.3	65.0±5.6
C2	66535	*O. sativa* ssp. indica	Indonesia	30.0±2.7	43.0±3.8	40.0±3.1	59.3±2.5	34.0±1.6	45.0±3.5
C3	78260	*O. sativa* ssp. indica	Thailand	31.7±3.1	38.9±2.3	45.5±3.9	63.1±3.1	80.5±2.6	78.0±3.5
C4	76343	*O. sativa* ssp. indica	Pakistan	41.4±3.1	73.0±2.3	5.0±5.2	35.0±3.0	20.0±3.9	25.0±2.1
Zhenzhuai	Zhenzhuai	*O. sativa* ssp. indica	China	0	0	66.7±2.3	74.9±3.3	81.7±2.4	54.0±4.4
II-32B	II-32B	*O. sativa* ssp. indica	China	0	0	31.7±6.3	58.7±3.4	56.7±4.4	73.7±4.7
D62B	D62B	*O. sativa* ssp. indica	China	0	0	33.3±3.2	55.7±2.2	50.0±3.3	43.0±5.4
Jin23B	Jin23B	*O. sativa* ssp. indica	China	0	0	45.5±2.1	62.5±1.2	43.3±3.3	54.0±3.1
C418	C418	*O. sativa* ssp. indica	China	5.0±1.0	10.0±2.3	25.0±3.4	25.8±5.2	6.7±1.6	24.0±1.5
9311	9311	*O. sativa* ssp. indica	China	5.0±3.0	10.0±2.3	43.3±3.6	81.5±4.2	53.3±3.3	65.0±4.1
Ezao11	Ezao11	*O. sativa* ssp. indica	China	5.0±1.9	9.7±0.7	35.0±1.7	54.0±2.3	54.0±3.8	88.9±4.7
Guangluai4	Guangluai4	*O. sativa* ssp. indica	China	6.7±1.1	23.0±0.9	71.7±2.1	70.8±3.6	43.3±4.7	83.9±5.5
Shengtai1	Shengtai1	*O. sativa* ssp. indica	China	7.3±1.3	4.0±2.3	41.7±2.1	84.9±3.1	73.3±2.6	82.8±4.5
Ezao6	Ezao6	*O. sativa* ssp. indica	China	12.0±2.1	13.8±2.0	36.7±3.1	59.9±3.2	50.0±2.7	81.7±3.4
CDR22	CDR22	*O. sativa* ssp. indica	China	20.0±1.1	25.6±1.8	53.3±5.7	19.2±4.9	53.3±3.2	32.7±10.1
9149	9149	*O. sativa* ssp. indica	China	20.0±3.5	40.2±3.7	73.3±4.1	35.6±4.2	86.7±2.8	72.6±2.8
PC311	PC311	*O. sativa* ssp. japonica	China	20.0±3.3	31.0±2.4	40.0±5.6	49.3±3.8	18.3±1.9	72.9±2.4
Dular	Dular	*O. sativa* ssp. indica	India	31.7±2.7	43.0±3.8	11.7±1.9	25.4±2.6	23.3±3.3	32.0±2.3
908	908	*O. sativa* ssp. indica	China	32.0±1.6	42.3±2.3	43.3±4.3	75.7±1.9	53.0±2.0	76.5±2.1
Cpso17	Cpso17	*O. sativa* ssp. japonica	America	32.0±3.3	45.0±2.3	61.7±2.5	60.5±3.5	52.0±2.1	75.0±1.9
Aijiaonante	Aijiaonante	*O. sativa* ssp. indica	China	33.3±2.3	12.3±3.4	72.5±3.2	72.6±2.1	53.3±2.2	65.0±3.3
955	955	*O. sativa* ssp. indica	China	40.0±3.5	54.0±4.6	50.0±4.5	62.5±3.2	43.3±2.5	32.0±2.6
Mianhui725	Mianhui725	*O. sativa* ssp. indica	China	43.3±3.0	65.3±3.7	46.7±8.5	76.3±8.5	53.3±5.4	54.0±3.5
Milyang23	Milyang23	*O. sativa* ssp. indica	China	45.0±3.1	75.0±1.0	55.0±3.1	84.9±3.4	48.5±4.3	72.5±3.2
Kanghui63	Kanghui63	*O. sativa* ssp. indica	China	50.0±2.4	60.5±3.4	43.3±5.4	85.4±2.7	71.7±5.6	84.1±8.2
Wanhui38	Wanhui38	*O. sativa* ssp. indica	China	50.0±2.3	88.3±4.3	50.0±2.1	55.1±3.2	50.0±2.5	66.2±3.7
Taichung nat.	Taichung nat.	*O. sativa* ssp. indica	China-Taiwan	56.7±5.1	16.1±2.3	6.7±2.6	23.5±3.4	8.3±2.0	26.7±3.2
Ce64	Ce64	*O. sativa* ssp. indica	China	60.0±2.0	44.6±3.1	30.0±2.4	9.2±3.2	40.0±2.1	54.0±2.5
Duohui1	Duohui1	*O. sativa* ssp. indica	China	60.0±2.3	59.2±3.2	53.3±2.1	28.8±3.1	53.3±3.4	20.5±2.7
IR8	IR8	*O. sativa* ssp. indica	Philippines	60.0±1.9	65.2±2.5	56.7±3.4	32.0±2.5	50.0±4.7	73.7±3.6
J413	J413	*O. sativa* ssp. indica	China	68.3±1.7	33.3±3.4	18.3±2.1	81.9±3.2	46.7±2.7	93.8±4.5
6078	6078	*O. sativa* ssp. indica	China	71.7±2.9	80.0±4.6	54.5±2.6	81.0±3.2	53.3±4.3	84.0±3.3
Shenghui743	Shenghui743	*O. sativa* ssp. indica	China	75.0±6.7	33.9±5.4	71.7±4.3	68.8±2.1	61.7±2.7	15.2±1.7
IR527	IR527	*O. sativa* ssp. indica	Philippines	75.0±3.3	18.7±2.3	50.0±5.3	36.4±4.5	56.7±3.3	13.1±2.5
9628	9628	*O. sativa* ssp. indica	China	75.0±4.5	39.1±1.9	50.0±1.7	78.9±5.7	65.0±4.5	82.7±5.7

cont.

Ce49	Ce49	*O. sativa* ssp. indica	China	76.0±4.2	91.6±3.7	40.0±4.2	91.9±4.2	36.7±6.8	93.5±5.3
Fuhui838	Fuhui838	*O. sativa* ssp. indica	China	76.7±2.4	78.9±2.9	63.3±2.3	90.1±1.8	36.7±3.2	86.6±6.7
DX1	DX1	*O. sativa* ssp. indica	China	76.7±2.1	81.9±7.3	36.7±3.2	80.2±2.7	53.3±1.6	89.1±3.3
288	288	*O. sativa* ssp. indica	China	76.7±3.2	89.6±3.9	40.0±2.7	67.0±2.2	30.0±6.7	60.0±3.3
Minghui63	Minghui63	*O. sativa* ssp. indica	China	77.5±2.8	87.0±4.8	43.3±3.6	74.8±3.2	51.7±3.3	85.7±4.5
Mbp98	MBP98	*O. sativa* ssp. indica	China	78.3±6.8	86.0±6.3	50.0±6.7	82.5±3.3	78.3±2.2	54.0±3.4
Minghui86	Minghui86	*O. sativa* ssp. indica	China	78.3±4.5	71.7±2.7	56.7±3.3	72.3±2.1	66.7±4.1	76.0±2.2
Manghui	Manghui	*O. sativa* ssp. indica	China	78.3±4.9	83.5±4.9	60.0±2.9	81.9±3.6	36.7±3.3	77.6±3.3
Teqing	Teqing	*O. sativa* ssp. indica	China	81.7±6.7	64.5±5.3	53.3±4.2	75.0±4.8	50.0±2.7	56.0±6.7
Shenghui11	Shenghui11	*O. sativa* ssp. indica	China	81.7±7.8	54.5±3.7	43.0±3.5	58.2±6.5	51.7±4.3	61.1±4.5
IR38	IR38	*O. sativa* ssp. indica	Philippines	85.0±9.1	50.8±5.3	51.7±3.3	80.9±3.8	71.7±2.8	69.1±1.7
71068	71068	*O. sativa* ssp. indica	China	90.0±4.6	67.2±5.2	6.7±5.2	12.5±5.1	53.3±3.4	60.3±3.2
Shuhui725	Shuhui725	*O. sativa* ssp. indica	China	90.0±6.5	70.9±2.3	53.3±2.7	80.6±1.9	71.7±1.7	72.4±1.7
Pecos	Pecos	*O. sativa* ssp. japonica	America	90.0±4.8	58.3±3.1	50.0±3.3	64.8±1.1	65.0±3.3	67.1±3.3
IR36	IR36	*O. sativa* ssp. indica	Philippines	90.0±6.4	70.3±2.7	65.0±4.6	78.3±3.4	63.3±4.9	87.4±2.1
924	924	*O. sativa* ssp. indica	China	91.7±5.7	62.7±6.2	15.0±2.7	73.7±3.2	36.7±2.9	73.0±3.4
Mianhui734	Mianhui734	*O. sativa* ssp. indica	China	91.7±7.1	83.1±3.4	50.0±2.2	81.9±1.9	43.0±2.0	67.0±3.1

Table 2. Natural seed-setting rate of F_1 hybrids for some accessions and low fertile tested F_1 hybrids with ZsB, YtB and ByB as female parents. Means of three replicates ± SD.

Series	WA-CMS [%]	HL-CMS [%]	BT-CMS [%]
W1	50.3 ± 2.1	54.3 ± 1.3	62.3 ± 1.3
W2	56.3 ± 1.3	43.8 ± 2.1	54.6 ± 1.6
W3	77.8 ± 1.6	82.9 ± 1.9	81.9 ± 2.3
W4	57.2 ± 2.0	45.3 ± 1.0	65.3 ± 1.9
W5	76.0 ± 3.1	55.3 ± 1.7	59.2 ± 1.5
W6	55.3 ± 1.1	43.2 ± 1.9	56.2 ± 1.7
W7	63.6 ± 0.8	77.5 ± 1.3	74.3 ± 2.2
W8	42.3 ± 3.4	50.2 ± 1.7	59.1 ± 2.1
W9	75.5 ± 5.1	66.5 ± 2.6	69.3 ± 1.9
W10	65.4 ± 1.3	58.3 ± 2.1	58.3 ± 0.7
W21	85.4 ± 2.1	83.0 ± 1.7	82.3 ± 1.3
Zhenzhuai	88.3 ± 1.1	92.1 ± 3.1	90.2 ± 2.0
II-32B	90.2 ± 1.4	93.2 ± 2.4	91.2 ± 2.1
D62B	96.1 ± 1.5	94.1 ± 1.3	93.3 ± 1.7
Jin23B	89.3 ± 1.7	92.5 ± 1.1	91.5 ± 3.3
C418	76.8 ± 1.9	86.1 ± 3.2	86.5 ± 1.3
9311	88.7 ± 2.1	89.2 ± 1.3	79.2 ± 1.5
Ezao11	79.8 ± 2.3	87.3 ± 0.9	89.1 ± 2.1
Guangluai4	78.9 ± 2.4	82.1 ± 1.3	80.1 ± 3.2
Shengtai1	87.3 ± 1.4	78.9 ± 1.1	77.9 ± 1.7
Ezao6	89.2 ± 1.5	76.8 ± 1.0	73.8 ± 2.4

BT-CMS, and different from that of WA-CMS. Further analysis showed that the *Rf* genes collected mainly to in two wild rice, *i.e. O. nivara* and *O. rufipogon* and cultivated rice, *i.e. O. sativa*. The accessions without fertility restorer ability for the three CMS distributed to the wild rice, *i.e. O. barthii, O. meridionalis* and *O. glumaepatula*. The ratio of the accessions with *Rf* in the cultivated rice (100 %) was higher than that in wild rice (66.7 %).

Genetic analysis of fertility restorer genes: To understand the genetic patterns of the fertility restorer genes for the different CMS type and the different restoring ability in the same CMS type, the backcross populations BC_1F_1 were carried out for HL- and BT-CMS, which belonged to two deputies for the gametophytic restoration CMS type. Correspondingly, the F_2 generation populations were carried out for WA-CMS, a deputy for the sporophytic restoration CMS type. According to the different pollen fertility and spikelet fertility, such as ranging 20 to 60 % and over 60 %, we constructed six F_2 generation populations for WA-CMS and six backcross populations for HL- and BT-CMS type, respectively. The size of all the populations was about 150 plants. Fertility analysis showed that the ratios between fertile and sterile plants were equal to 3:1 in three F_2 segregation populations, fit to the action mode of one pair of *Rf* gene, while the ratios between fertile and sterile plants nearly fit to 15:1 in two F_2 segregation populations and were true of 9:7 in the residual for WA-CMS, indicating the genetic pattern of two pairs of genes (Table 3). In the same way, fertility analysis showed that the ratios between the fertile and sterile plants were almost all equal to 1:1, which suggested the genetic mode of only one pair of *Rf* gene for HL- and BT-CMS (Tables 4, 5).

Allelism test of fertility restorer gene for three CMS types: To investigate the relationship among the fertility restorer genes and loci for WA-CMS, Minghui 63, a cultivar with *Rf*, was used as bridge parents and carried out a series of populations with the accessions having higher fertility to test F_1 hybrids. These results showed that the

Table 3. Fertility segregation in the F_2 populations for WA-CMS line, ZsA. χ^2 ($df = 1, P = 0.05$) = 3.84.

F_2 populations	Fertile:sterile	Expected ratio	χ^2 value	*Rf* gene number
ZsA/C1	114:49	3:1	2.227	1
ZsA/Milyang23	117:36	3:1	0.176	1
ZsA/W14	78:33	3:1	1.324	1
ZsA/W17	87:10	15:1	2.728	2
ZsA/Minghui63	144:13	15:1	1.104	2
ZsA/W19	89:84	9:7	1.623	2

Table 4. Fertility segregation in the backcross BC_1F_1 populations for HL-CMS line, YtA. χ^2 (df = 1, P = 0.05) = 3.84.

Backcross populations	Fertile: sterile	Expected ratio	χ^2 value	*Rf* gene number
YtA//YtB/9311	83:76	1:1	0.308	1
YtA//YtB/Milyang23	79:70	1:1	0.544	1
YtA//YtB/W12	54:63	1:1	0.692	1
YtA//YtB/Minghui63	87:77	1:1	0.610	1
YtA//YtB/W19	75:57	1:1	2.455	1
YtA//YtB/9149	89:68	1:1	2.809	1

Table 5. Fertility segregation in the backcross BC_1F_1 populations for BT-CMS line, ByA. χ^2 (df = 1, P = 0.05) = 3.84.

Backcross populations	Fertile: sterile	Expected ratio	χ^2 value	*Rf* gene number
ByA//ByB/9311	79:73	1:1	0.237	1
ByA//ByB/Milyang23	77:70	1:1	0.333	1
ByA//ByB/W12	67:57	1:1	0.806	1
ByA//ByB/Minghui63	94:79	1:1	1.301	1
ByA//ByB/W19	79:69	1:1	0.676	1
ByA//ByB/9149	87:69	1:1	2.077	1

Table 6. Allelism analysis of the fertility-restorer genes for WA-CMS.

Test crosses	Sterile plants	Popul. size	Recomb. frequency [%]	Allelism
ZsA//Minghui63/IR38	16	94	12.77	non-all.
ZsA//Minghui63/Teqing	10	136	14.71	non-all.
ZsA//Minghui63/Shuhui725	8	96	16.67	non-all.
ZsA//Minghui63/Fuhui838	8	84	19.05	non-all.
ZsA//Minghui63/IR36	8	90	17.78	non-all.
ZsA//Minghui63/Milyang23	9	90	20.00	non-all.
ZsA//Teqing/Milyang23	8	105	15.24	non-all.

restoring loci among Milyang23, Teqing, Shuhui725, Fuhui838, IR36 and IR38 and Minghui63 were non-allelic. In addition, fertility analysis showed that the sterile plants were observed in the population derived from test-cross of ZsA//Teqing/Milyang23, indicating that the restoring loci between Teqing and Milyang23 were also non-allelic (Table 6). So it was concluded that there were at least three restoring loci for WA-CMS.

In the same way, ten accessions were selected and hybridized with 9311 and Milyang23, two cultivated lines, as bridge parents for HL-CMS. Fertility analysis showed that the sterile plants were observed in the test-cross populations derived from the combination between 9311 and CDR22, IR8, Cpslo17, W12 and W16, and that between Milyang23 and CDR22, IR8, Cpslo17, W12, MBP98, Shuhui725, Minghui63, Fuhui838 and Ce49. It suggested that the *Rf* genes or loci of these combinations were non-allelic. In addition, there were not sterile plants in the backcross populations derived from the combination between 9311 and MBP98, Shuhui725, Minghui63, Fuhui838 and Ce49 and that between Milyang23 and W16. It indicated that the *Rf* genes or loci of these combinations were allelic (Table 7). So it was also concluded that there were at least three restoring loci for HL-CMS.

Table 7. Allelism analysis of the fertility-restorer genes for HL-CMS.

Test crosses	Sterile plants	Popul. size	Recomb. frequency [%]	Allelism
YtA//9311/CDR22	2	106	3.77	non-all.
YtA//9311/IR8	7	119	11.76	non-all.
YtA//9311/Cpslo17	6	96	12.50	non-all.
YtA//9311/MBP98	0	95	0	allelic
YtA//9311/Shuhui725	0	87	0	allelic
YtA//9311/Minghui63	0	86	0	allelic
YtA//9311/Fuhui838	0	127	0	allelic
YtA//9311/Ce49	0	101	0	allelic
YtA//9311/W12	12	89	26.97	non-all.
YtA//9311/W16	7	79	17.72	non-all.
YtA//Milyang23/CDR22	8	132	12.12	non-all.
YtA//Milyang23/IR8	5	65	15.38	non-all.
YtA//Milyang23/Cpslo17	5	150	6.67	non-all.
YtA//Milyang233/MBP98	5	120	8.33	non-all.
YtA//Milyang23/Shuhui725	2	132	3.03	non-all.
YtA//Milyang23/Minghui63	4	121	6.61	non-all.
YtA//Milyang23/Fuhui838	13	138	18.84	non-all.
YtA//Milyang23/Ce49	14	155	18.06	non-all.
YtA//Milyang23/W12	8	91	17.58	non-all.
YtA//Milyang23/W16	0	103	0	allelic

Table 8. Allelism analysis of the fertility-restorer genes for BT-CMS.

Test crosses	Sterile plants	Popul. size	Recomb. frequency [%]	Allelism
ByA//9149/D62B	8	128	12.5	non-all.
ByA//9149/Jin23B	6	132	9.09	non-all.
ByA//9149/Ezao6	12	168	14.26	non-all.
ByA//9149/ Milyang23	8	170	9.41	non-all.
ByA//Milyang23/Ezao6	4	160	5.00	non-all.

For BT-CMS, five combinations, ByA//9149/D62B, ByA//9149/Jin23B, ByA//9149/Ezao6, ByA//9149/Milyang23 and ByA//Milyang23/Ezao6, were carried out (Table 8). The sterile plants were found in all the above combinations, suggesting that the *Rf* genes or loci between the accessions were non-allelic, and that among Milyang23, 9149 and Ezao6 were non-allelic. So there were at least three restoring loci for BT-CMS system.

Discussion

So far, more than sixty CMS lines usually categorized into WA-, HL- and BT-CMS were developed. WA-CMS aborted at uninucleate stage with irregular microspores, belongs to a sporophytic restoration CMS type. HL-CMS system aborted at dinucleate stage with negative stainability in 1 % I_2-KI solution and BT-CMS aborted at trinucleate stage with stainability in 1 % I_2-KI solution, which are all ranked to gametophytic restoration CMS type.

Some accessions with *Rf* gene can restore the three CMS types synchronously, while some accessions with *Rf* gene can restore only HL-CMS (BT-CMS) and maintain WA-CMS, *i.e.* 9311, w9, or can restore only WA-CMS and maintain HL-CMS (BT-CMS), *i.e.* w21. These results showed the following conclusion: *1*) there is a certain dependent evolution between CMS and *Rf* in rice (this result is consistent with the documented results of Touzet *et al*. 2004 and Li *et al*. 2005); *2*) the sterile line can be developed ceaselessly from crossing between interspecies, intersubspecies and even intervarieties of *Oryza* species, such as MotiA, PadminiA, IR66707A and IR69700A (Hoan *et al*. 1998, Dalmacio *et al*. 1995, Pradhan *et al*. 2000); *3*) HL-CMS are identical to BT-CMS, expect the tiny different of fertility power. However, the relationship of fertility-maintenance between HL-CMS (BT-CMS) and WA-CMS are different, and the frequency of *Rf* for HL-CMS and BT-CMS are higher than that for WA-CMS. It indicates that it is different relationship of restorer-maintainer between sporophytic restoration CMS type and gametophytic restoration CMS type and it is similar to that within the same restoration CMS type (sporophytic type or gametophytic type). *4*) Based on allelic analysis *Rf* loci derived from the different cultivated and wild accessions distributed in Asia, Oceania, Latin America and Africa, there are at least three *Rf* loci for WA-CMS, HL-CMS, which are consistent with results of Li *et al*. (2005), and BT-CMS is also restored by at least three different *Rf* loci. It indicated that the multiple *Rf* loci are certainly determined by the multiple CMS systems in the natural populations.

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