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Maintaining and restoring cytoplasmic male sterility systems in pepper (*Capsicum annuum* L.)

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ABSTRACT. We studied the efficiency of maintaining and restoring cytoplasmic male sterility (CMS) systems in pepper (*Capsicum annuum* L.). An *Rf*-linked molecular marker was employed to analyze the interaction between 6 CMS lines (A), 5 maintainers (B), and 6 restorers (C). Sterility was maintained in the matings of lines 201A x 200B, 203A x 200B, 206A x 200B, 200A x 201B, 206A x 201B, 200A x 202B, 200A x 203B, 200A x 206B, and 201A x 206B. All 6 restorers restored the fertility of lines 200A, 202A, 203A, and 204A, except that 213C could not restore the fertility of lines 200A and 204A. However, the 6 restorers had diverse restoring abilities in individual CMS lines. The *Rf*-linked molecular marker was amplified by PCR in lines 207C, 208C, and 213C. This DNA marker was only found in the F1 hybrids M39, M14, M19, M25, M13, M20, and M22. We conclude that the restorers 208C and 207C can transmit the *Rf*

gene or the *Rf*-linked marker to F1 hybrids.

Key words: Pepper; Cross-combination; Fertility investigation; *Rf*-linked molecular marker

INTRODUCTION

Cytoplasmic genetic male sterility (CMS) is a method that is widely used for producing F1 hybrid seeds as well as the study of nucleo-cytoplasmic interactions (Budar et al., 2003; Hanson and Bentolila, 2004; Chase, 2007). The CMS three-line-hybridizing method is an important method for heterosis utilization of the crop. The genotype of the CMS line (A), maintainer (B), and restorer (C) is *S rfrf*, *N rfrf*, *S/N RFRF*, or *S RFrf*, respectively (Guo, 2001). CMS is the result of the coaction of the karyogenes and the cytogenes. The nuclear gene of the CMS line is controlled by the recessive gene, while the novel expression of the *orf* gene in the mitochondrial genome inhibits the plant from producing normal pollen (Hanson, 1991; Linke and Borner, 2005). The restoration of the fertility of the CMS line is controlled by the *Rf* gene (Schnable and Wise, 1998; Hanson and Bentolila, 2004; Horn, 2006).

Male sterility in pepper (*Capsicum annuum* L.) was first reported by Peterson (1958). The inheritance of the pepper is quite complex, as there are 2 types of male sterility in pepper; genic male sterility is controlled by the nuclear gene and CMS is controlled by both the karyogene and the cytogene. Meanwhile, CMS is not only inherited stably, but also is affected by the environment. Both Peterson (1958) and Shifriss (1997) reported that the expression of the male-sterility gene could be affected by environmental temperature (Shifriss and Guri, 1979). Partial sterility of pollen, which may be affected by the environment or controlled by other specific genes, was first reported by Zhang et al. (2000). Thus, changes in temperature may lead to alterations in fertility ranging from complete sterility to partial fertility, which may also lead to CMS lines self-crossing or being crossed as the male parent (Gergely et al., 2006). Therefore, the application of the CMS line is based on the understanding of the genetic characteristics of the CMS line and the relationship between maintaining and restoring.

Regarding the developing of molecular markers, partial markers were found and applied to fertility selection in pepper. For example, Peterson (1958) first reported that there were multiple alleles of the restoring gene (*Rf*) in pepper. Kim et al. (2001) and Kim and Kim (2005) found that there were differences in the mtDNA region between the *coxII* and *atp6-2* gene of the maintainer and CMS line, and then identified 2 sequence-characterized amplified region (SCAR) markers related to CMS. Zhang et al. (2000) selected two *Rf*-linked random amplified polymorphism DNA markers, OP131400 (0.37 cM) and OW18800 (8.12 cM), which were converted to CRF-SCAR [OPT-02/570 (5 cM)] by Lee et al. (2004) and Gulyas et al. (2006). The exploitation of this molecular marker can speed up the process of CMS selection and application of CMS in pepper, which can improve the breeding effect.

We selected and bred several types of CMS lines as well as their related maintainers and restorers by the combination of interspecific crossing and the induced mutation technique (Gong et al., 2008); however, the relationship between maintaining and restoring is unknown. In this study, we aimed to reveal the relationship between the CMS line, the related maintainer, and the restorer by field crossing, surveys, and indoor evaluation. Meanwhile, we analyzed the differences of the restoring gene among different materials using the CRF-SCAR marker (Gulyas et al., 2006). These

data will provide the theoretical foundation for the application of the CMS system.

MATERIAL AND METHODS

Plant materials

The experiment employed 5 CMS lines (A; 200A, 201A, 202A, 203A, and 206A), 5 near-isogenic maintainers (B; 200B, 201B, 202B, 203B, and 206B), an additional CMS line (204A), and 6 restorers (C; 207C, 208C, 209C, 211C, 213C, and 214C) of the pepper (*C. annuum* L.), which were provided by the pepper research team at Northwest A&F University (Table 1). Each pair of A and B differed in cytoplasm types; these were used for performing crosses and analyzing the relationship between maintaining and restoring.

Table 1. Cytoplasmic male sterility (CMS) lines used in the crossing test.

Lines	Genotype	Phenotype	Fruit type	Line function
200A	*S*(*msms*)	Sterile	H	CMS, A line
201A	*S*(*msms*)	Sterile	H	CMS, A line
202A	*S*(*msms*)	Sterile	H	CMS, A line
203A	*S*(*msms*)	Sterile	H	CMS, A line
204A	*S*(*msms*)	Sterile	H	CMS, A line
206A	*S*(*msms*)	Sterile	S	CMS, A line
200B	*N*(*msms*)	Fertile	H	CMS, maintainer, B line
201B	*N*(*msms*)	Fertile	H	CMS, maintainer, B line
202B	*N*(*msms*)	Fertile	H	CMS, maintainer, B line
203B	*N*(*msms*)	Fertile	H	CMS, maintainer, B line
206B	*N*(*msms*)	Fertile	S	CMS, maintainer, B line
207C	*N*(*RfRf*)	Fertile	H	CMS, restorer, C line
208C	*N*(*RfRf*)	Fertile	H	CMS, restorer, C line
209C	*N*(*RfRf*)	Fertile	S	CMS, restorer, C line
211C	*N*(*RfRf*)	Fertile	S	CMS, restorer, C line
213C	*N*(*RfRf*)	Fertile	S	CMS, restorer, C line
214C	*S*(*RfRf*)	Fertile	S	CMS, restorer, C line

H = hot pepper; S = sweet pepper.

Crossing test

The 5 maintainers (200B, 201B, 202B, 203B, and 206B) were crossed separately to 5 CMS lines (200A, 201A, 202A, 203A, and 206A; A x B), and 6 restorers (207C, 208C, 209C, 211C, 213C, and 214C) were crossed separately to 4 CMS lines (200A, 202A, 203A, and 204A; Table 2). All F1 hybrids (M is prefixed for the code of each cross-combination) of 40 crossing combinations were harvested in September 2010. The following April, all F1 hybrid seeds were grown in a greenhouse, and the fertility of the F1 hybrids was identified from the early stage of anthesis.

Pollen assay

Field statistical experiment

In the blooming stage of anthesis, 5 flowers were randomly selected to evaluate the F1 hybrid fertility according to the method of Gulyas et al. (2006).

Table 2. Crossing combinations of cytoplasmic male sterility system in pepper.

Hybrid combination	Code	Hybrid combination	Code	Hybrid combination	Code	Hybrid combination	Code
206A x 200B	M1	202A x 207C	M14	201A x 206B	M28	203A x 207C	M39
206A x 201B	M2	206A x 203B	M15	201A x 203B	M29	203A x 211C	M40
202A x 201B	M3	202A x 214C	M16	201A x 202B	M30	203A x 206B	M42
200A x 211C	M5	200A x 201B	M18	204A x 214C	M31	203A x 201B	M43
201A x 200B	M7	200A x 207C	M19	200A x 209C	M33	200A x 203B	M44
203A x 202B	M8	204A x 208C	M20	203A x 213C	M34	200A x 206B	M45
203A x 200B	M9	204A x 209C	M21	200A x 214C	M35	206A x 202B	M46
202A x 206B	M10	200A x 208C	M22	203A x 209C	M36	200A x 202B	M41
202A x 209C	M11	204A x 211C	M23	204A x 213C	M37	200A x 213C	M24
202A x 208C	M13	203A x 214C	M26	203A x 208C	M38	204A x 207C	M25

Analysis of pollen quantity

The pollen quantity was measured according to the method described by Wang et al. (2008). One hundred anthers from 30 large flowers of each combination were put into vials to desiccate the anther. When the pollen was fully released, 5 mL 1% sodium hexametaphosphate was added, and the vial was shaken to resuspend the pollen. After resuspending the pollen, a drop of the suspension was added to a counting chamber to evaluate the quantity of pollen. Each experiment was repeated 6 times and the values obtained for each experiment were averaged. The pollen quantity of each anther (N) was equal to the medium value of the 6 values, which were calculated in accordance with the following formula:

N = (total number of pollen grains in 400 grids x 10,000 x 5 x 3) / 100

Analysis of pollen germination

After drying in the shade, pollen grains isolated from flowers were germinated *in vitro* in culture medium (0.01% boric acid + 10% sucrose + 1% agar) at 25°C in the dark (Ye, 2010). The percentage of pollen germination was measured at 3, 6, 18, and 21 h. For each experiment, 6 fields were randomly observed by light microscope. The pollen germination rate was calculated in accordance with the following formula:

Germination rate (%) = number of germinated pollen grains / total pollen grains x 100

Fruit setting and seed analysis

Ten flowers were randomly marked in each plant to measure the fruit-setting rate and the number of seeds in each fruit; finally, the median value was obtained.

Rf-linked molecular marker analysis

Total DNA was extracted from the fresh leaves of each plant (Walbot and Warren, 1988). The primers were designed according to the method of Gulyas et al. (2006). The marker was prepared using the following PCR protocol: an initial denaturation at 95°C for 5 min, 40 cycles of amplification, each consisting of 95°C for 45 s, annealing at 62°C for 45 s, 72°C for 90 s, and a final extension at 72°C for 10 min (Lee et al., 2008).

RESULTS

Maintaining ability of 5 maintainers

The pollen quantity and the self-crossed fruit-setting rate of F1 hybrids from each cross-combination (A x B) were observed in the field to grossly predict the maintaining ability of the maintainers in each CMS line. The self-crossed fruit-setting rate of M45, M7, M28, M9, M1, and M2 were nil and these 6 F1 hybrids did not have normal pollen or traces of pollen, which indicated that the 6 F1 hybrids were sterile and that the sterility could be maintained in the mating of 200A x 206B, 201A x 206B, 201A x 200B, 203A x 200B, 206A x 200B, and 206A x 201B. However, it was observed that M3, M18, M30, and M41 did not have pollen or traces of pollen, with both their self-crossed fruit-setting rate less than 20%. These findings demonstrated that the 4 F1 hybrids were partially fertile, thus the fertility of the 4 was relatively low (Table 3).

Table 3. Pollen quantity and self-crossed fruit-setting rate in F1 hybrid of cytoplasmic male sterility lines and maintainers in pepper.

Code	Number			Self-crossed fruit-setting rate (means ± SE)	Fertility	Code	Number			Self-crossed fruit-setting rate (means ± SE)	Fertility
	++	+-	-				++	+-	-		
M1	0	0	36	0.00 ± 0.00	S	M28	0	0	36	0.00 ± 0.00	S
M2	0	0	38	0.00 ± 0.00	S	M29	0	32	0	32.00 ± 11.36	F
M3	0	0	30	13.00 ± 5.75	PF	M30	0	0	35	5.00 ± 0.27	PF
M7	0	0	21	0.00 ± 0.00	S	M42	0	0	32	26.00 ± 10.75	F
M8	0	32	12	26.00 ± 8.75	F	M43	38	0	0	27.00 ± 9.49	F
M9	0	0	24	0.00 ± 0.00	S	M44[a]	0	37	0	43.00 ± 9.49	F
M10	22	0	0	46.00 ± 13.50	F	M45	0	0	39	0.00 ± 0.00	S
M15	38	0	0	14.00 ± 5.67	F	M46	38	0	0	14.00 ± 7.00	F
M18[a]	0	0	34	14.00 ± 5.67	PF	M41[a]	0	0	39	7.00 ± 0.75	PF

(++) = pollen quantity basically equal to that of the male parent; (+-) = pollen quantity basically equal to 50% of the male parent; (-) = traces of pollen grains or no pollen; SE = standard error; F = fertile; S = sterile; PF = partially fertile; [a]no seed.

The indoor tests of pollen quantity, pollen germination of each anther, and the number of seeds in each fruit revealed that F1 hybrids M18, M41, M44, M45, M7, M28, M9, M1, and M2 could not seed, thereby suggesting the sterility of the plants. These findings indicated that sterility could be maintained in the mating of 200A x 201B, 200A x 202B, 200A x 203B, 200A x 206B, 201A x 200B, 201A x 206B, 206A x 200B, 203A x 200B, and 206A x 201B. Other F1 hybrids of the cross-combinations (A x B) were fertile, thus indicating no maintaining ability of the male parent (Table 4).

The field survey and indoor test showed that different maintainers had different maintaining ability to the same CMS line, although the same maintainer had different maintaining ability to different CMS lines, such as the maintaining ability of 200B to 201A, 203A, and 206A, just as that of 201B to 206A and 206B to 200A and 201A. However, sterility could not be maintained in the mating of 203A x 201B, 203A x 202B, 206A x 202B, 201A x 203B, 206A x 203B, 202A x 206B, and 203A x 206B. Of note, M3, M18, M30, and M41 were partially fertile, as M3 and M30 had traces of pollen grains and lower than 20% self-crossed fruit-setting rates (Figure 1), although they had a few seeds in the fruit; the pollen was also viable. These results showed that 201B and 202B, the male parents of M3 and M30, could not maintain the sterility of 202A and 201A, respectively. In addition, M18 and M41 had a small quantity of pollen

and their fruit-setting rates were lower than 20% in the field; however, their fruits had no seeds while the pollen quantity, pollen germination of each anther, and the number of seeds in each fruit were both practically nil according to the indoor test. These results suggested that M18 and M41 were sterile, thus 201B and 202B, the male parents of M18 and M41, respectively, could maintain the sterility of 200A. Moreover, by field survey, M44 had pollen to some extent and could yield fruit by self-pollination, while the number of its seeds was nil despite the viable pollen. Therefore, M44 was sterile, thus indicating that 203B could maintain the sterility of 200A.

Table 4. Pollen quantity, germination rate, and the number of the seed in F1 hybrid of cytoplasmic male sterility lines and maintainers in pepper.

Code	Pollen quantity (means ± SE)	Germination rate (%)	Seed No.	Fertility	Code	Pollen quantity (means ± SE)	Germination rate (%)	Seed No.	Fertility
M18	0 ± 0	0.00 ± 0.00	0.00 ± 0.00	S	M10	41921 ± 1639	52.50 ± 3.54	43.40 ± 7.61	F
M41	0 ± 0	0.00 ± 0.00	00.00 ± 0.00	S	M9	3848 ± 612	0.00 ± 0.00	0.00 ± 0.00	S
M44	11152 ± 1581	44.81 ± 5.01	0.00 ± 0.00	S	M43	12500 ± 1936	57.14 ± 15.54	44.50 ± 6.64	F
M45	3466 ± 503	0.00 ± 0.00	0.00 ± 0.00	S	M8	13269 ± 2172	-	58.40 ± 7.11	F
M7	2900 ± 200	0.00 ± 0.00	0.00 ± 0.00	S	M42	2803 ± 488	-	54.78 ± 7.34	F
M30	3464 ± 681	51.67 ± 6.86	18.00 ± 5.93	F	M1	0 ± 0	0.00 ± 0.00	0.00 ± 0.00	S
M29	9358 ± 413	66.60 ± 25.51	38.00 ± 7.02	F	M2	22238 ± 2129	0.00 ± 0.00	0.00 ± 0.00	S
M28	916 ± 72	0.00 ± 0.00	0.00 ± 0.00	S	M46	19166 ± 2714	49.39 ± 11.76	54.90 ± 9.64	F
M3	2955 ± 787	50.98 ± 10.14	37.60 ± 6.70	F	M15	36924 ± 4306	43.33 ± 2.36	78.80 ± 11.82	F

SE = standard error; F = fertile; S = sterile; (-) = deficit.

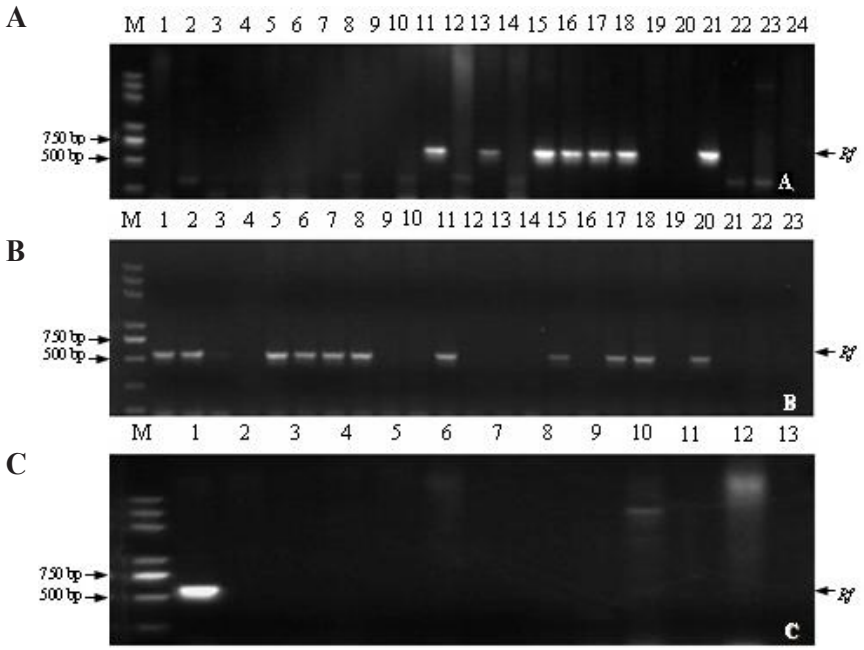

Figure 1. PCR amplification of *Rf*-linked CRF-SCAR DNA marker in the parents and their F1 hybrids. *Lane M* = *Trans* 2K plus DNA ladder marker. **A.** *Lanes 1* to *24* = M10, M1, M2, M3, M4, M5, M6, M7, M8, M9, M11, M12, M13, 206B, M15, M16, 207C, 208C, 209C, 211C, 213C, 214C, 200A, and 200B. **B.** *Lanes 1* to *23* = M26, M33, M38, M18, M19, M20, M21, M22, M23, M24, M25, M28, M29, M30, M31, M34, M35, M36, M37, M39, M40, M41, and M42. **C.** *Lanes 1* to *13* = M14, M43, M44, M45, M46, 201A, 201B, 202A, 202B, 203A, 203B, 204A, and 206A.

Restoring ability of 6 restorers

The pollen quantity and spontaneous fruit-setting rate of F1 hybrids in each combination (A x C) tested in the field showed that M37 and M24 did not have viable pollen or traces of pollen, with no self-crossed fruit-setting. These findings indicated that 213C could not restore the fertility of 200A and 204A (Table 5). M21 and M31 were partially fertile, with lower than 20% self-crossed fruit-setting rates, 11.00 ± 5.68 and 10.00 ± 6.67%, respectively, indicating that 209C and 214C had relatively low restoring ability to 204A. Other F1 hybrids of the cross-combinations (A x C) were fertile.

Table 5. Pollen quantity and self-crossed fruit-setting rate in F1 hybrid of cytoplasmic male sterility lines and restorers in pepper.

Code	Number			Self-crossed fruit-setting rate (%, means ± SE)	Fertility	Code	Number			Self-crossed fruit-setting rate (%, means ± SE)	Fertility
	++	+-	-				++	+-	-		
M5	38	0	0	23.00 ± 6.75	F	M31	38	0	0	10.00 ± 6.67	PF
M11	40	0	0	48.00 ± 10.33	F	M33	33	0	0	48.00 ± 13.17	F
M13	38	0	0	38.00 ± 15.49	F	M34	35	0	0	49.00 ± 11.97	F
M14	34	0	0	34.00 ± 12.65	F	M35	37	0	0	44.00 ± 9.67	F
M16	42	0	0	42.00 ± 12.29	F	M36	37	0	0	37.00 ± 9.49	F
M19	34	0	0	50.00 ± 11.55	F	M37	0	0	35	0.00 ± 0.00	S
M20	0	32	0	55.00 ± 8.50	F	M38	37	0	0	33.00 ± 9.49	F
M21	40	0	0	11.00 ± 5.68	PF	M39	0	0	30	23.00 ± 12.52	F
M22	38	0	0	47.00 ± 9.49	F	M40	33	0	0	61.00 ± 11.92	F
M23	32	0	0	31.00 ± 7.38	F	M24	0	0	44	0.00 ± 0.00	S
M26	32	0	0	25.00 ± 7.07	F	M25	35	0	0	25.00 ± 10.80	F

(++) = pollen quantity basically equal to that of the male parent; (+-) = pollen quantity basically equal to 50% of the male parent; (-) = traces of pollen grains or no pollen; SE = standard error; F = fertile; S = sterile; PF = partially fertile.

The seeds per fruit of M24 and M37 were found to be practically nil (Table 6), indicating sterility, as 213C could not restore the fertility of 200A and 204A. The number of seeds per fruit of M23 was lower, approximately 11.20 ± 4.67, indicating that 211C had decreased restoring ability to 204A. Furthermore, the pollen quantity of M39 and M31 was lower than that of the male parent (restorer), 4,609 ± 309 and 1,675 ± 168, respectively. Therefore, the restoring abilities of 207C to 203A and 214C to 204A are decreased.

Table 6. Pollen quantity, germination rate, and number of the seeds in F1 hybrid of cytoplasmic male sterility lines and restorers in pepper.

Code	Pollen quantity (means ± SE)	Germination rate (%)	Seed No.	Fertility	Code	Pollen quantity (means ± SE)	Germination rate (%)	Seed No.	Fertility
M19	45,699 ± 3514	39.36 ± 1.25	51.80 ± 8.55	F	M38	36,950 ± 1977	41.19 ± 9.21	57.30 ± 4.85	F
M22	29,768 ± 1586	89.52 ± 5.39	36.30 ± 5.17	F	M36	23,108 ± 4190	52.63 ± 16.76	136.90 ± 26.92	F
M33	52,576 ± 2092	40.68 ± 8.53	53.70 ± 7.09	F	M40	53,265 ± 1774	45.56 ± 5.10	78.80 ± 5.18	F
M5	65,494 ± 4742	41.14 ± 6.09	45.30 ± 5.27	F	M34	42,295 ± 2639	59.89 ± 6.32	44.4 ± 4.01	F
M24	-	50.11 ± 28.52	0.00 ± 0.00	S	M26	24,404 ± 2572	26.06 ± 5.13	92.4 ± 8.75	F
M35	51,598 ± 1840	81.53 ± 9.21	69.80 ± 6.21	F	M25	50,568 ± 3841	44.29 ± 2.02	31.1 ± 8.72	F
M14	34,816 ± 3679	54.89 ± 16.73	38.20 ± 2.97	F	M20	9,700 ± 786	48.85 ± 21.25	59.60 ± 5.25	F
M13	-	46.42 ± 19.76	67.7 ± 6.95	F	M21	21,487 ± 1420	59.46 ± 16.37	54.20 ± 8.12	F
M11	36,950 ± 3163	-	60.30 ± 5.27	F	M23	37,833 ± 2468	36.57 ± 16.63	11.20 ± 4.67	F
M16	44,340 ± 2306	82.14 ± 13.25	77.80 ± 5.83	F	M37	3,628 ± 608	47.78 ± 13.47	0.00 ± 0.00	S
M39	4,609 ± 309	21.25 ± 12.37	48.7 ± 6.22	F	M31	1,675 ± 168	41.11 ± 1.92	39.80 ± 8.85	F

SE = standard error; F = fertile; S = sterile; (-) = deficit.

The findings described above showed that all of the restorers tested in our experiments could restore the fertility of all of the CMS lines tested, with the exception that 213C could not restore the fertility of 200A and 204A. However, the restoring ability of the different restorers differed; this finding was primarily exemplified in the pollen quantity, pollen germination, fruit-setting rates, and seeds per fruit of the F1 progeny in the cross-combination (the same restorer to different CMS lines). It is worth noting that the self-crossed fruit-setting rate of M21 was only 11.00 ± 5.68%, while the pollen quantity, pollen germination, and seeds per fruit indicated that M21 was fertile; therefore, 209C could restore the fertility of 204A, while the low fruit-setting rate might be due to the lower vitality of the pistil. The self-crossed fruit-setting rates of M39 with decreased pollen quantity and germination, M31 with decreased pollen quantity, and M23 with fewer seeds per fruit were 23.00 ± 12.52, 10.00 ± 6.67, and 31.00 ± 7.38%, respectively. These results indicate that 211C and 214C may have worse restoring ability to 204A, as did 207C to 203A.

Analysis of the *Rf*-linked DNA marker

The CMS lines, maintainers, and their F1 individuals were polymorphic for the *Rf*-linked DNA marker, CRF-SCAR. The CRF-SCAR marker was reported to be linked to the major *Rf* locus at the distance of 5 cM. The presence of the CRF-SCAR PCR product was associated with the presence of the *Rf* allele. The results of the present study showed that the CRF-SCAR PCR product was only present in M15, which indicated that M15 (206A x 203B) has the *Rf* gene; however, we could not amplify any objective PCR product in 206A and 203B, which may have resulted from gene rearrangement caused by the crossing of parents, and further study is warranted to investigate the specific cause.

In the 6 restorers tested, the *Rf* gene was polymorphic for the presence of the CRF-SCAR PCR product, and only occurred in 207C, 208C, and 213C. Further studies are required to determine the mechanisms involved in controlling the restoring ability of restorers.

The CRF-SCAR PCR product was also found in M39 (203A x 207C), M14 (202A x 207C), M19 (200A x 207C), M25 (204A x 207C), M13 (202A x 208C), M20 (204A x 208C), and M22 (200A x 208C), the F1 hybrids of CMS lines and restorers, and also the male parents 207C and 208C. These results may indicate that 207C and 208C can transmit the *Rf*-linked marker or the *Rf* gene to F1 hybrids.

Although there was no CRF-SCAR PCR product in 209C and 214C, the CRF-SCAR marker was present in the F1 hybrids M33 (200A x 209C), M11 (202A x 209C), M21 (204A x 209C), M36 (203A x 209C), M35 (200A x 214C), M16 (202A x 214C), M26 (203A x 214C), and M31 (204A x 214C). These results might be due to the nucleo-cytoplasmic interactions of CMS lines and restorers in pepper.

In M5, M40, and M23 as well as their male parents such as 211C, no PCR product was found, although 211C could restore the fertility of 200A, 203A, and 204A. These findings may be related to the genetic diversity of the *Rf* gene. Taken together, these data demonstrated that genetic differences of the *Rf* gene exist in different materials (Figure 2).

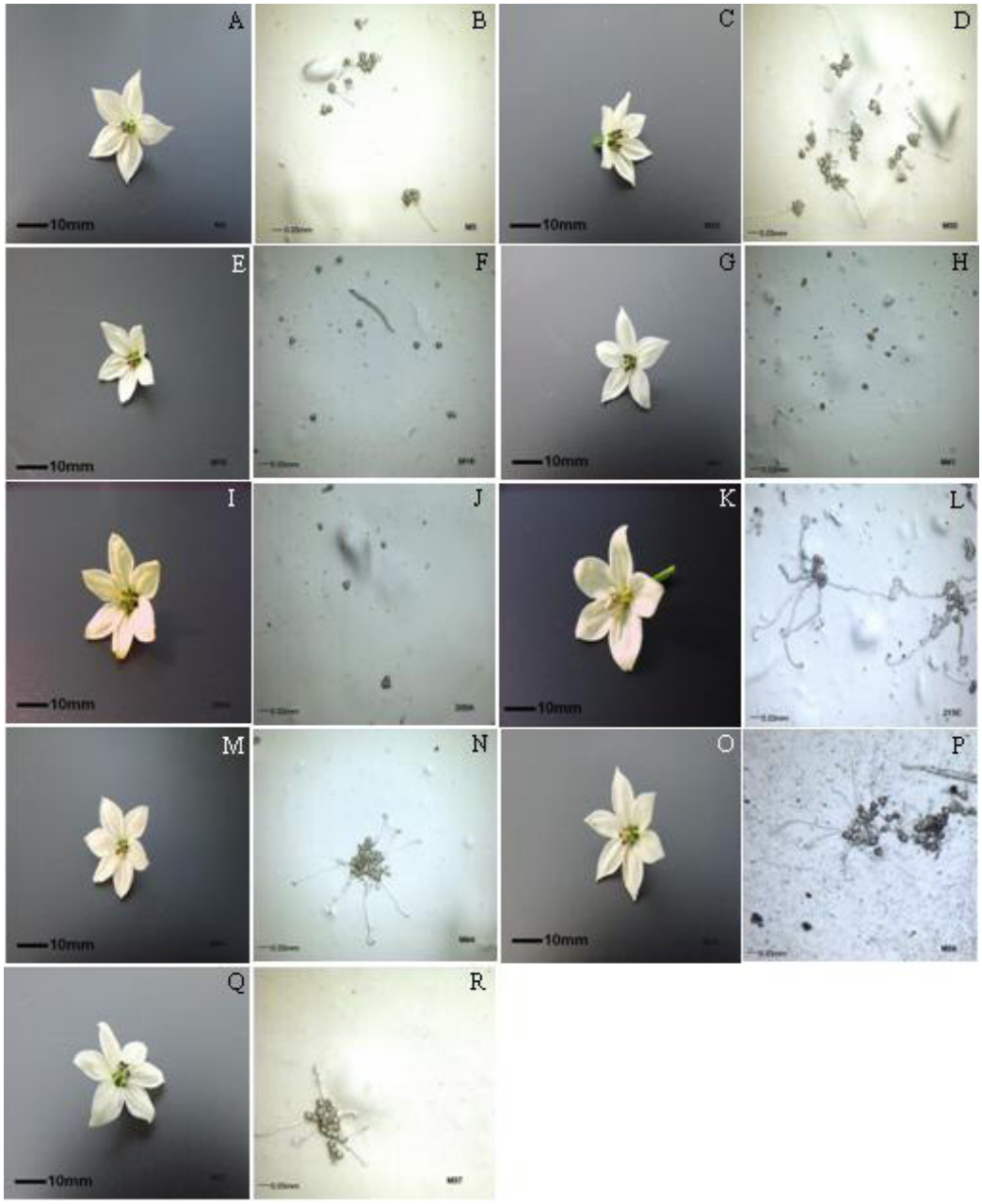

Figure 2. Pollen germination and the morphological characters of the flower in the parents and their F1 hybrids. **A. C. E. G. I. K. M. O.** and **Q.** Morphological characters of the flower of M3, M30, M18, M41, 200A, 213C, M44, M24, and M37, respectively, bar = 10 mm. **B. D. F. H. J. L. N. P.** and **R.** Pollen germination of M3, M30, M18, M41, 200A, 213C, M44, M24, and M37, respectively, bar = 0.03 mm.

DISCUSSION

It is thought that the differences of maintaining ability are caused by the different sources of the cytoplasm and nucleolus (Wang et al., 2009). Our studies of the relationship of CMS lines, maintainers, and restorers revealed that a same maintainer had different maintaining ability to the CMS lines with different sources of cytoplasm; for example, 201B could maintain the sterility of 201A and 206A and partially maintain the sterility of 200A, while it could not maintain the sterility of 202A and 203A. In addition, different maintainers had different maintaining ability to the same CMS line; for instance, 200B might completely maintain the sterility of 201A, 203A, and 206A, while 202B did not have any maintaining ability to 201A, 203A, and 206A. These results were similar to those of rice (Wang et al., 2009). The crossing test between CMS lines and restorers in pepper demonstrated that different restorers had different restoring ability to the same CMS line; for instance, 207C, 208C, and 209C could restore the adequately fertility of 204A, while 211C and 214C had worse restoring ability to 204A. We inferred that it was the variation in the *Rf* genes of the restorers that might lead to these differences. Furthermore, the same restorer had different restoring ability to the CMS lines with different sources of cytoplasm; for example, 214C had better restoring ability to 200A, but had worse restoring ability to 204A. This might be explained by the different cytoplasm leading to the differences of the restoring ability among the CMS lines with the same nucleolus (Tang et al., 2005).

The pairs of *Rf* genes are different among the different restorers of radish (Zhang et al., 2002), with some radishes having 1 pair and others having 2 pairs. Our experiment revealed that different maintainers and restorers might carry different *Rf* genes and different pairs of alleles (Table 7). The complexity of the differentiation of the cytoplasm and nucleolus with different sources in addition to the difference of the regions may likely result in the diversity of nuclear genes.

Table 7. Genotypes and function of cytoplasmic male sterility three-lines in pepper.

Line	Original genotype	Previous line function	Complete cytoplasm and nuclear genotypes	New line function
200A	$S\ ms_0ms_0$	A line	$S\ ms_0ms_0\ ms_1ms_1ms_3ms_3\ ms_6ms_6$	A line of ms_0
201A	$S\ ms_1ms_1$	A line	$S\ ms_0ms_0\ ms_1ms_1\ Ms_2Ms_2\ Ms_3Ms_3\ ms_6ms_6$	A line of ms_1
202A	$S\ ms_2ms_2$	A line	$S\ ms_0ms_0\ Ms_1Ms_1\ ms_2ms_2\ Ms_3Ms_3\ Ms_6Ms_6$	A line of ms_2
203A	$S\ ms_3ms_3$	A line	$S\ ms_0ms_0\ Ms_1Ms_1\ ms_3ms_3\ Ms_6Ms_6$	A line of ms_3
204A	$S\ ms_4ms_4$	A line	-	A line of ms_4
206A	$S\ ms_6ms_6$	A line	$S\ ms_0ms_0\ ms_1ms_1\ Ms_2Ms_2\ Ms_3Ms_3\ ms_6ms_6$	A line of ms_6
200B	$N\ ms_0ms_0$	B line	$N\ ms_0ms_0\ ms_1ms_1ms_3ms_3\ ms_6ms_6$	B line of m_0, ms_1, ms_3, and ms_6
201B	$N\ ms_1ms_1$	B line	$N\ ms_0ms_0\ ms_1ms_1\ Ms_2Ms_2\ Ms_3Ms_3\ ms_6ms_6$	B line of ms_0, ms_1, and ms_6 C line of ms_2, ms_3
202B	$N\ ms_2ms_2$	B line	$N\ ms_0ms_0\ Ms_1Ms_1\ ms_2ms_2\ Ms_3Ms_3\ Ms_6Ms_6$	B line of ms_0, ms_2 C line of ms_1, ms_3, and ms_6
203B	$N\ ms_3ms_3$	B line	$N\ ms_0ms_0\ Ms_1Ms_1\ ms_3ms_3\ Ms_6Ms_6$	B line of ms_0, ms_3 C line of ms_1, ms_6
206B	$N\ ms_6ms_6$	B line	$N\ ms_0ms_0\ ms_1ms_1\ Ms_2Ms_2\ Ms_3Ms_3\ ms_6ms_6$	B line of ms_0, ms_1, and ms_6 C line of ms_2, m_3
207C	$N\ Rf_7Rf_7$	C line	$N\ Rf_7Rf_7\ Ms_0Ms_0\ Ms_2Ms_2\ Ms_3Ms_3\ Ms_4Ms_4$	C line of ms_0, ms_2, ms_3, and ms_4
208C	$N\ Rf_8Rf_8$	C line	$N\ Rf_8Rf_8\ Ms_0Ms_0\ Ms_2Ms_2\ Ms_3Ms_3\ Ms_4Ms_4$	C line of ms_0, ms_2, ms_3, and ms_4
209C	$N\ Rf_9Rf_9$	C line	$N\ Rf_9Rf_9\ Ms_0Ms_0\ Ms_2Ms_2\ Ms_3Ms_3\ Ms_4Ms_4$	C line of ms_0, ms_2, ms_3, and ms_4
211C	$N\ Rf_{11}Rf_{11}$	C line	$N\ Rf_{11}Rf_{11}Ms_0Ms_0\ Ms_3Ms_3\ Ms_4Ms_4$	C line of ms_0, ms_3, and ms_4
213C	$N\ Rf_{13}Rf_{13}$	C line	$N\ Rf_{13}Rf_{13}\ ms_0ms_0\ Ms_3Ms_3\ ms_4ms_4$	C line of ms_3, B line of ms_3, and ms_4
214C	$S\ Rf_{14}Rf_{14}$	C line	$S\ Rf_{14}Rf_{14}Ms_0Ms_0\ Ms_2Ms_2\ Ms_3Ms_3\ Ms_4Ms_4$	C line of ms_0, ms_2, ms_3, and ms_4

In practical breeding, the adaptive capacity of a same type of CMS line in one crop is limited. Hence, it is necessary to select and breed several types of CMS lines and their related restorers for improving the resistance and stability of the yield of F1 hybrids (Liu et al., 2011). The genotypes of the 5 CMS lines in our study were different, and difference also existed in the maintainers and restorers (Table 7). These findings provide the foundation for further study and use of these rare plant materials.

CONCLUSIONS

The present study of the relationship between maintaining and restoring among different CMS systems revealed the maintaining ability of the maintainers to the CMS lines and also the restoring ability of the restorers to the CMS lines. In addition, the detection of the *Rf*-linked marker among all of the plant materials showed the inheritance of the *Rf* gene between the male parent, 207C, 208C, and the F1 hybrid. As there was a consistent relationship of maintaining in some CMS lines with different cytoplasm, it is possible to breed many sterile lines with the same nucleus to exploit this cytoplasmic diversity; additionally, the different restoring ability of the restorers may indicate that there are other unknown *Rf* genes in pepper. In future studies, some other new *Rf* genes may be found by analyzing the F2 segregations, which could enrich the genetic diversity of the restorers. These studies will promote the resistance of pepper to abiotic stresses encountered in practical production.

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