

Genetic characterisation of a novel male sterile two-type line system 19F08AB in *Brassica napus* L.

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Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Table S1. The primers used in the present study

Primer	Sequence (5'-3')	Purpose
P11	GAAACGGGAAGTGACAAT	Orf138
P12	GCATTATTTTCTCGGTCCAT	
P21	AGCTGTCTGGAGGGAATC	Orf222
P22	GCGGTCTCACGCACTAATC	
P31	AGCTGTCTGGAGGGAATC	Orf224
P32	ACGACATCAAGGAGGAAC	
Ms5a-1F	GTAATAATCCATATTCGCGTGG	<i>Mf</i>
Ms5a-1R	ATACGAGACAGGCATGAGGGTG	
5c-12F	GTTGCCTGTCTGTAAACAATC	<i>ms</i>
ac-2R	AATCTGGCCTAAACTTCTTTGG	
Ms5a-1F	GTAATAATCCATATTCGCGTGG	<i>Ms</i>
Ms5b-1R	CAACGTCTGTTGTACCAGTGTAACC	
RFP-F	GGGATGCGATCCTGATATTG	<i>Rfp</i>
RFP-R1	GAGAGAGGCTACAGAACAAAC	
rfp-9F	GGGATGCGATCCTGATATCGTG	<i>rfp</i>
rfp-8R	GTCCAATTCACTTGTATAG	

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Table S2. Comparison of floral organs of fertile and sterile plants of 19F08AB in *Brassica napus* L. (in cm)

Samples	Diameter of corolla	Length of pedicel	Length of petal	Width of petal	Length of four stronger stamens	Length of two weaker stamens	Length of four stronger anthers	Length of two weaker anthers
19F08B	2.29 ± 0.14 ^a	1.81 ± 0.27 ^a	1.55 ± 0.15 ^a	0.91 ± 0.13 ^a	1.05 ± 0.05 ^a	0.88 ± 0.04 ^a	0.29 ± 0.04 ^a	0.30 ± 0.04 ^a
19F08A	1.93 ± 0.14 ^b	1.94 ± 0.45 ^a	1.29 ± 0.07 ^b	0.82 ± 0.06 ^b	0.49 ± 0.05 ^b	0.41 ± 0.04 ^b	0.21 ± 0.02 ^b	0.21 ± 0.01 ^b

Data are expressed by mean ± SD ($n = 20$); different lowercase letters indicate significant difference at $P < 0.05$ level

Table S3. Comparison of main agronomic traits of fertile and sterile plants of 19F08AB in *Brassica napus* L.

Samples	Plant height (cm)	Branch height (cm)	Branch No.	Length of the main inflorescence (cm)	Total siliques per plant (No.)	Seed numbers per silique	Pod length (cm)
19F08B	181.10 ± 7.89 ^a	54.00 ± 13.89 ^B	7.50 ± 2.32 ^a	73.70 ± 23.30 ^a	520.50 ± 174.68 ^A	33.40 ± 2.99 ^a	7.66 ± 0.55 ^a
19F08A	186.50 ± 9.01 ^a	75.60 ± 13.90 ^A	8.40 ± 0.84 ^a	68.90 ± 9.90 ^a	329.30 ± 45.76 ^B	32.10 ± 2.02 ^a	6.48 ± 0.29 ^a

Data are expressed by mean ± SD ($n = 10$) for all traits except for total siliques per plant and seed numbers per silique, where $n = 20$; different capital or lowercase letters indicate significant differences at $P < 0.01$ or $P < 0.05$ levels

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Table S4. Fertility performance of the progenies between 19F08AB and other male sterile lines in *Brassica napus* L.

No.	Code	Genetic background	Fertile plants	GMS plants	Expected ratio (F:GMS)	χ^2_c value
1	1A85-2 × 1A26-2F	19F08A × YY10B	44	39	1:1	0.19
2	2A95-7S × 2A17-2F	19F08A × YY10B	18	14	1:1	0.28
3	9F08S × 9A43-1S	19F08A × H50S	18	27	1:1	1.42
4	1A92-5S × 1A108-9	19F08A × H50S	16	27	1:1	2.33
5	1A93-2 × 1A108-9	19F08A × H50S	19	24	1:1	0.37
6	1A96-3 × 1A120-7	19F08A × H50S	33	32	1:1	0.00
7	2A77-1S × 2A127	19F08A × H50S	50	40	1:1	0.90
8	2A77-2S × 2A127	19F08A × H50S	30	36	1:1	0.38
9	9F08-2S × 9A64-4S	19F08A × 373S	8	14	1:1	1.14
10	1A93-4S × 1A201-1	19F08A × 373S	26	14	1:1	3.03
11	1A94-3S × 1A201-2	19F08A × 373S	25	31	1:1	0.45
12	1A94-2S × 1A202-1	19F08A × 373S	24	30	1:1	0.46
13	2A77-3S × 2A330	19F08A × 373S	15	18	1:1	0.12
14	2A77-4S × 2A330	19F08A × 373S	12	15	1:1	0.15
15	2A30-4S × 2A95-1F	9012A × 19F08B	25	0	1:0	–
16	2A30-5S × 2A95-2F	9012A × 19F08B	11	0	1:0	–
17	2A95-3S × 2A34-1	19F08A × TAM	15	16	1:1	0
18	2A95-14S × 2A35-1	19F08A × TAM	16	9	1:1	1.44
19	2A95-13S × 2A35-2	19F08A × TAM	25	24	1:1	0
20	2A95-14S × 2A35-3	19F08A × TAM	7	9	1:1	0.06
21	1A80-2 × 1A23-2	19F08A × ZS05R	33	0	1:0	–
22	1A81-1 × 1A23-2	19F08A × ZS05R	33	0	1:0	–
23	1A82-2 × 1A23-2	19F08A × ZS05R	31	0	1:0	–
24	2A95-4S × 2A14-1	19F08A × ZS05R	37	0	1:0	–
25	2A95-5S × 2A14-2	19F08A × ZS05R	17	0	1:0	–
26	2A95-8S × 2A16-1F	19F08A × WW1291	30	0	1:0	–
27	2A95-9S × 2A16-2	19F08A × WW1291	8	0	1:0	–
28	2A95-18S × 2C70-2	19F08A × 2011B2	16	15	1:1	0.00
29	9F08-2S × 9C303-2	19F08A × S11R	23	15	1:1	1.29
30	2A95-15S × 2C396-2	19F08A × S11R	27	17	1:1	1.84
31	2A95-7S × 2C396-1	19F08A × S11R	28	16	1:1	2.75
32	1A85-3 × 1C272-1	19F08A × T16	17	10	1:1	1.33
33	2A99-7S × 2C380-2	19F08A × T16	27	23	1:1	0.18
34	2A95-16S × 2C404-1	19F08A × Q10C	24	22	1:1	0.02
35	2A95-15S × 2C404-2	19F08A × Q10C	26	15	1:1	2.44
36	9F08S × 9C324-5	19F08A × CZL-20A	14	16	1:1	0.03
37	2A95-16S × 2C413-1	19F08A × CZL-20A	25	22	1:1	0.09
38	2A95-17S × 2C413-2	19F08A × CZL-20A	11	11	1:1	0.05
39	9F08-2S × 9C31F-1	19F08A × ZS09	21	18	1:1	0.10
40	1A80-3 × 1C31-1	19F08A × ZS09	17	11	1:1	0.89
41	1A83-1 × 1C31-1	19F08A × ZS09	15	9	1:1	1.04
42	1A83-2 × 1C31-1	19F08A × ZS09	15	18	1:1	0.12
43	1A85-5 × 1C27-1	19F08A × ZS09	17	12	1:1	0.55
44	1A84-7 × 1C267-6	19F08A × SH11	17	7	1:1	3.38
45	1A85-4 × 1C267-6	19F08A × SH11	18	21	1:1	0.10
46	1A85-5 × 1C267-6	19F08A × SH11	14	16	1:1	0.03
47	1A85-6 × 1C267-6	19F08A × SH11	4	6	1:1	0.10
48	1A85-7 × 1C267-6	19F08A × SH11	17	8	1:1	2.56
49	1A85-8 × 1C267-6	19F08A × SH11	6	5	1:1	0.00

F – fertile plants; GMS – genic male sterile plants; $\chi^2_{0.05,1} = 3.84$; $\chi^2_{0.01,1} = 6.63$; – not applied

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Table S5. Comparison of floral organs of fertility, genic male sterility (GMS), cytoplasmic male sterility (CMS) and double MS (CMS + GMS) plants of 2A65 in *Brassica napus* L. (in cm)

Samples	Diameter of corolla	Length of pedicel	Length of petal	Width of petal	Length of four stronger stamens	Length of two weaker stamens	Length of four stronger anthers	Length of two weaker anthers
2A65-F	2.18 ± 0.06 ^a	2.51 ± 0.29 ^a	1.69 ± 0.05 ^a	1.13 ± 0.03 ^a	1.14 ± 0.03 ^a	0.92 ± 0.02 ^a	0.32 ± 0.03 ^a	0.28 ± 0.03 ^a
2A65-GMS	1.76 ± 0.07 ^b	2.04 ± 0.15 ^{ab}	1.54 ± 0.04 ^b	0.91 ± 0.03 ^b	0.55 ± 0.03 ^c	0.45 ± 0.01 ^b	0.25 ± 0.02 ^b	0.24 ± 0.01 ^{ab}
2A65-CMS	1.73 ± 0.06 ^b	1.89 ± 0.19 ^b	1.23 ± 0.04 ^c	0.70 ± 0.04 ^c	0.68 ± 0.04 ^b	0.46 ± 0.05 ^b	0.21 ± 0.01 ^b	0.22 ± 0.01 ^b
2A65-double MS	1.01 ± 0.09 ^c	1.86 ± 0.04 ^b	1.01 ± 0.04 ^d	0.47 ± 0.03 ^d	0.50 ± 0.03 ^c	0.40 ± 0.03 ^b	0.24 ± 0.01 ^b	0.22 ± 0.01 ^b

Data are expressed by mean ± SD ($n = 20$); different lowercase letters indicate significant difference at $P < 0.05$ level

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Table S6. Genotyping results of BC₁ population (2A69) derived from the cross between 19F08A and ZS09 (19F08A/ZS09//ZS09) by *Rfp/rfp* gene-specific molecular markers

No.	Accessions	Genotype	<i>Rf</i>	<i>rf</i>
1	ZS11	<i>rfrf</i>	0	1
2	3C243	<i>Rfrf</i>	1	1
3	SH11	<i>RfRf</i>	1	0
4	2A69-1F	<i>Rfrf</i>	1	1
5	2A69-2F	<i>Rfrf</i>	1	1
6	2A69-3F	<i>Rfrf</i>	1	1
7	2A69-5F	<i>Rfrf</i>	1	1
8	2A69-6F	<i>Rfrf</i>	1	1
9	2A69-7F	<i>Rfrf</i>	1	1
10	2A69-1GMS	<i>Rfrf</i>	1	1
11	2A69-2GMS	<i>Rfrf</i>	1	1
12	2A69-3GMS	<i>Rfrf</i>	1	1
13	2A69-4GMS	<i>Rfrf</i>	1	1
14	2A69-1MS	<i>rfrf</i>	0	1
15	2A69-2MS	<i>rfrf</i>	0	1
16	2A69-3CMS	<i>rfrf</i>	0	1
17	2A69-4CMS	<i>rfrf</i>	0	1
18	2A69-5CMS	<i>rfrf</i>	0	1
19	2A69-6MS	<i>rfrf</i>	0	1
20	2A69-7CMS	<i>rfrf</i>	0	1
21	2A69-8MS	<i>rfrf</i>	0	1
22	2A69-9MS	<i>rfrf</i>	0	1
23	2A69-10MS	<i>rfrf</i>	0	1
24	2A69-11MS	<i>rfrf</i>	0	1
25	2A69-12MS	<i>rfrf</i>	0	1
26	2A69-13MS	<i>rfrf</i>	0	1
27	2A69-14CMS	<i>rfrf</i>	0	1
28	2A69-15MS	<i>rfrf</i>	0	1
29	2A69-16MS	<i>rfrf</i>	0	1

Three accessions (No.1~3) are used as references; GMS – genic male sterile plants; CMS – cytoplasmic male sterile plants; MS – cytoplasmic and genic male sterility (CMS + GMS) plants

Table S7. Genotyping results of BC₁ population (2A69) derived from the cross between 19F08A and ZS09 (19F08A/ZS09//ZS09) by the *Ms/ms/Mf* gene-specific molecular markers

No.	Accessions	Genotype	<i>Ms</i>	<i>Mf</i>	<i>ms</i>
1	2A07-1F	<i>MfMs</i>	1	1	0
2	2A07-1S	<i>MsMs</i>	1	0	0
3	3A03-1F	<i>msms</i>	0	0	1
4	3A03-1S	<i>Msms</i>	1	0	1
5	2A16	<i>MfMf</i>	0	1	0
6	2A69-1F	<i>msms</i>	0	0	1
7	2A69-2F	<i>msms</i>	0	0	1
8	2A69-3F	<i>msms</i>	0	0	1
9	2A69-5F	<i>msms</i>	0	0	1
10	2A69-6F	<i>msms</i>	0	0	1
11	2A69-7F	<i>msms</i>	0	0	1
12	2A69-1GMS	<i>Msms</i>	1	0	1
13	2A69-2GMS	<i>Msms</i>	1	0	1
14	2A69-3GMS	<i>Msms</i>	1	0	1
15	2A69-4GMS	<i>Msms</i>	1	0	1
16	2A69-1MS	<i>Msms</i>	1	0	1
17	2A69-2MS	<i>Msms</i>	1	0	1
18	2A69-3CMS	<i>msms</i>	0	0	1
19	2A69-4CMS	<i>msms</i>	0	0	1
20	2A69-5CMS	<i>msms</i>	0	0	1
21	2A69-6MS	<i>Msms</i>	1	0	1
22	2A69-7CMS	<i>msms</i>	0	0	1
23	2A69-8MS	<i>Msms</i>	1	0	1
24	2A69-9MS	<i>Msms</i>	1	0	1
25	2A69-10MS	<i>Msms</i>	1	0	1
26	2A69-11MS	<i>Msms</i>	1	0	1
27	2A69-12MS	<i>Msms</i>	1	0	1
28	2A69-13MS	<i>Msms</i>	1	0	1
29	2A69-14CMS	<i>msms</i>	0	0	1
30	2A69-15MS	<i>Msms</i>	1	0	1
31	2A69-16MS	<i>Msms</i>	1	0	1
32	2A69-17MS	<i>Msms</i>	1	0	1

Five accessions (No.1~5) are used as references; GMS – genic male sterile plants; CMS – cytoplasmic male sterile plants; MS – cytoplasmic and genic male sterility (CMS + GMS) plants

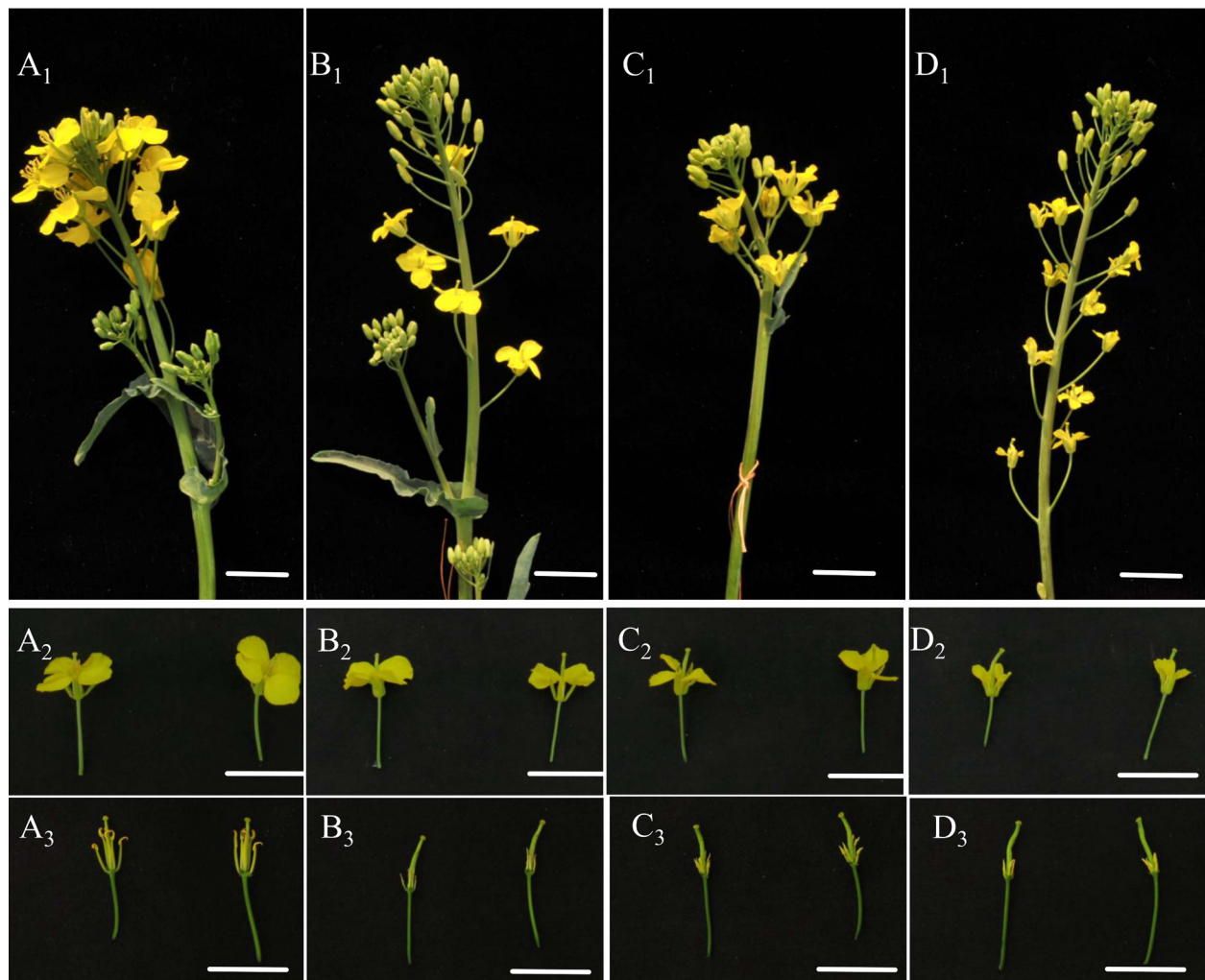


Figure S1. Inflorescence and flowers of fertile (A₁–A₃), GMS (B₁–B₃), CMS (C₁–C₃) and double MS (GMS + CMS) (D₁–D₃) samples in 2A65, an F₂ population derived from the cross between 19F08A and ZS05R
Scale bar = 2 cm; MS – male sterility; GMS – genic male sterility; CMS – cytoplasmic male sterility

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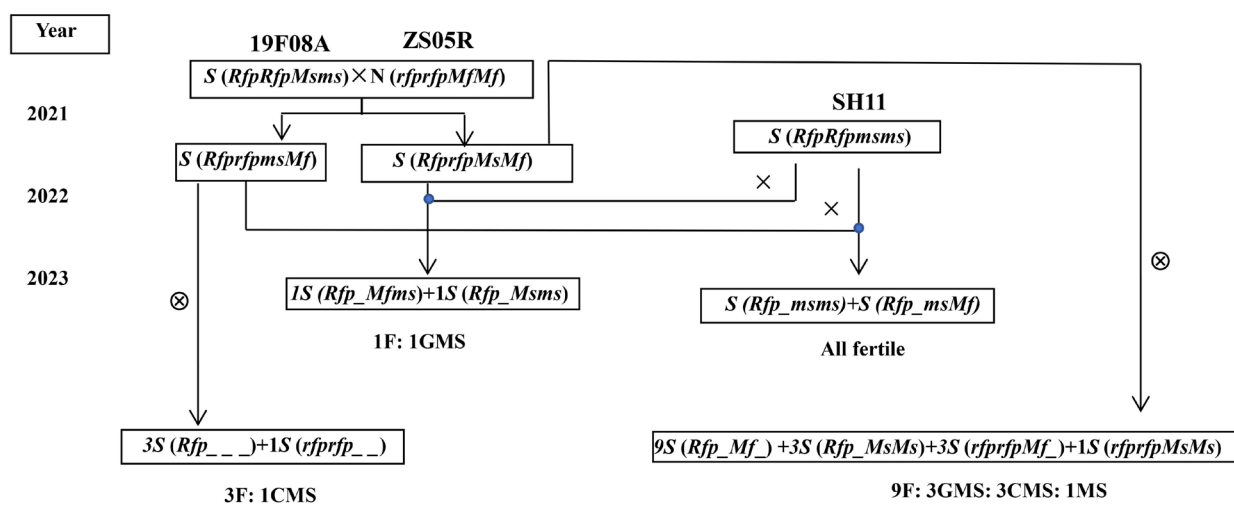


Figure S2. Genetic investigation of 19F08A and its relationship with Shaan-GMS in *Brassica napus*

F – fertile plants; GMS – genic male sterile plants; CMS – cytoplasmic male sterile plants; MS – cytoplasmic and genic male sterility (CMS + GMS) plants; *S* – *pol* CMS; *N* – normal cytoplasm; *Rfp* – restorer allele for *pol* CMS; *rfp* – maintainer allele for *pol* CMS; *Ms* – dominant male sterile gene for Shaan-GMS; *ms* – male fertile gene for Shaan-GMS; *Mf* – male restorer gene for Shaan-GMS

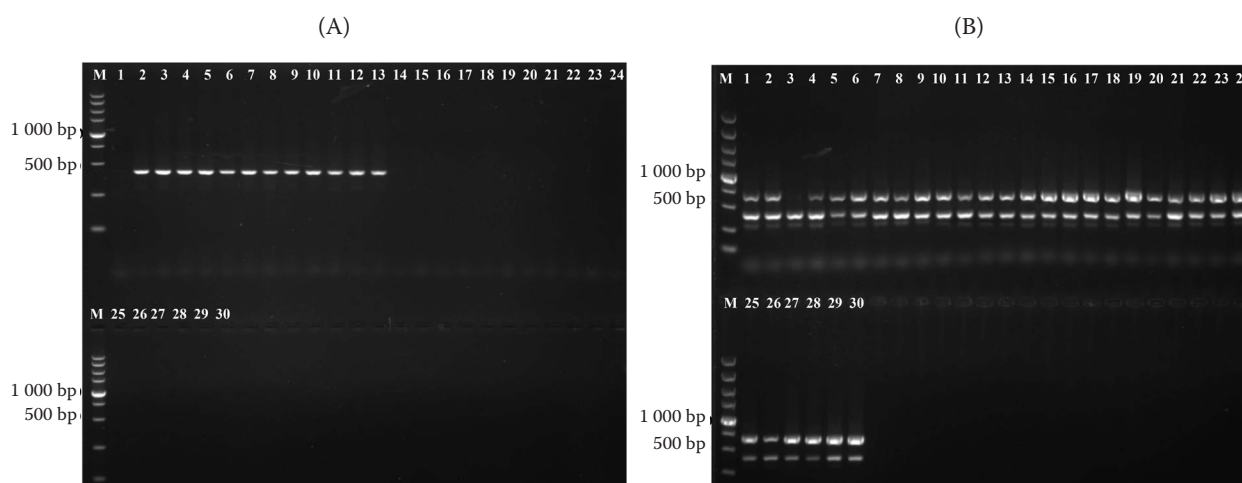


Figure S3. Genotyping of BC₁ population (2A69) derived from the cross between 19F08A and ZS09 (19F08A/ZS09//ZS09) by the *Rfp/rfp* gene-specific molecular markers: PCR amplification results by primers RFP-F/RFP-R1 (A), by primers rfp-9F/(rfp-8R+RFP-R1) (B)

Lane 1 – ZS11 (*rfprfp*); lane 2 – 3C243 (*Rfprfp*); lane 3 – SH11 (*RfRf*); lanes 4~30 – genic male sterile (GMS), cytoplasmic male sterile (CMS), and CMS + GMS plants in 2A69 population (Table S6)

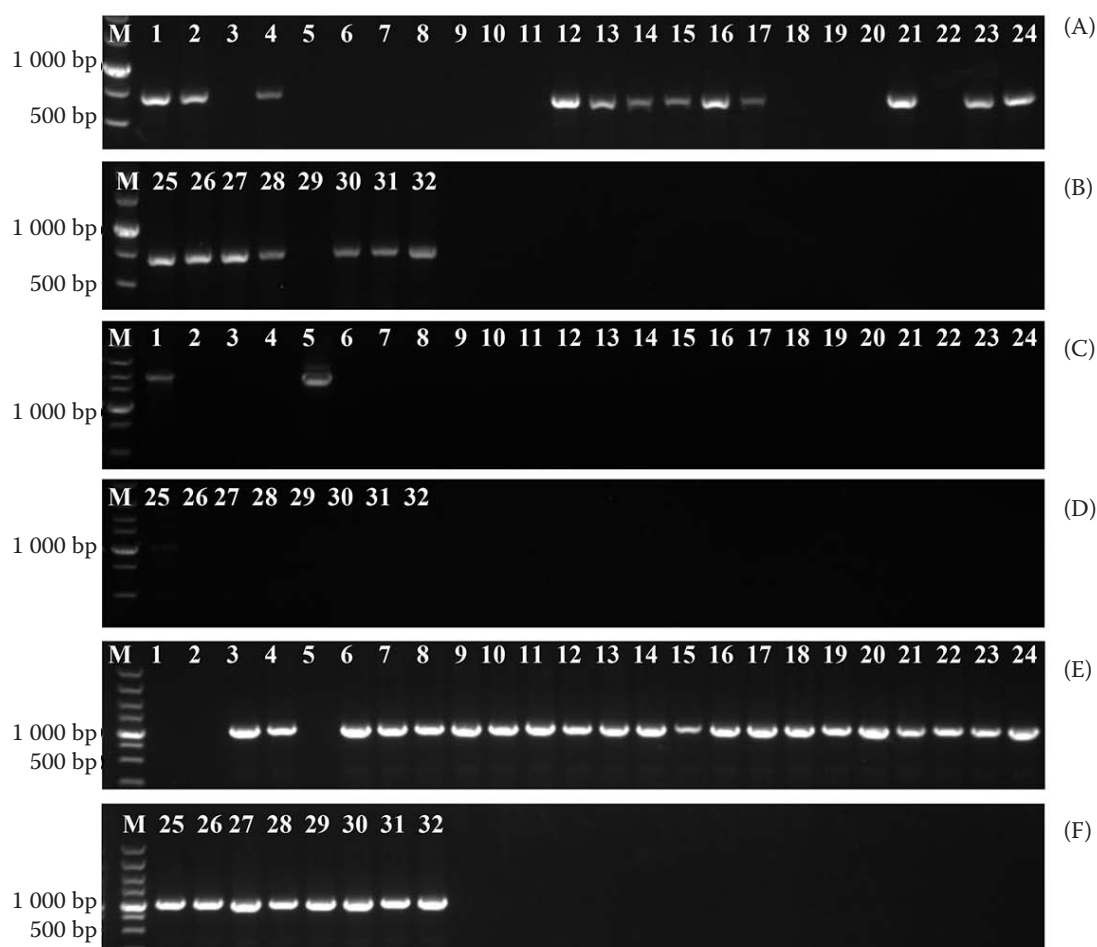


Figure S4. Genotyping of BC₁ population (2A69) derived from the cross between 19F08A and ZS09 (19F08A/ZS09//ZS09) by the *Ms/ms/Mf* gene-specific molecular markers: PCR amplification results by primers Ms5a-1F and Ms5b-1R for detecting the *Ms* allele (A, B); P by primers 670-2F and ac-2R for detecting the *Mf* allele (C, D); by primers 5c-12F and ac-2R for detecting the *ms* allele (E, F)

M – marker DL 5000; lane 1 – 2A07-1F (*MfMs*); lane 2 – 2A07-1S (*MsMs*); lane 3 – 3A03-1F (*msms*); lane 4 – 3A03-1S (*Msms*); lane 5 – 2A16 (*MfMf*); lanes 6~32 – genic male sterile (GMS), cytoplasmic male sterile (CMS), and CMS + GMS plants in 2A69 population (Table S7)