

Map-based cloning of the nuclear restorer gene *Rfp* for pol CMS in rapeseed

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Heterosis



Left: Zhenshan97;

Right: Minghui63

Center: Zhenshan97 x Minghui63 hybrid



Left: B73;

Right: Mo17

Center: B73 x Mo17 hybrid

Rapeseed planting acreage and yield in China

Table 1 Rapeseed planting acreage and yield in different years in China

年份 Years	平均年种植面积 Yearly acreage/ ($\times 10^6 \text{ hm}^2$)	年平均产量 Yearly average yield/ ($\text{kg} \cdot \text{hm}^{-2}$)	平均总产 Yearly total yield/ ($\times 10^6 \text{ t}$)
1950~ 1959	1.936	463.5	0.885 5
1960~ 1969	1.731	478.9	0.833 2
1970~ 1979	2.127	652.0	1.386 5
1980~ 1989	4.246	1 153.7	4.899 0
1990~ 1999	6.249	1 331.5	8.321 0
2000	7.495	1 518.5	11.381 0
2001	7.095	1 596.8	11.331 0
2002	7.143	1 477.5	10.552 2
2003	7.221	1 581.0	11.420 0
2004	7.500	1 757.6	13.182 0
2005	7.240	1 802.5	13.050 0
2006	6.740	1 854.6	12.500 0

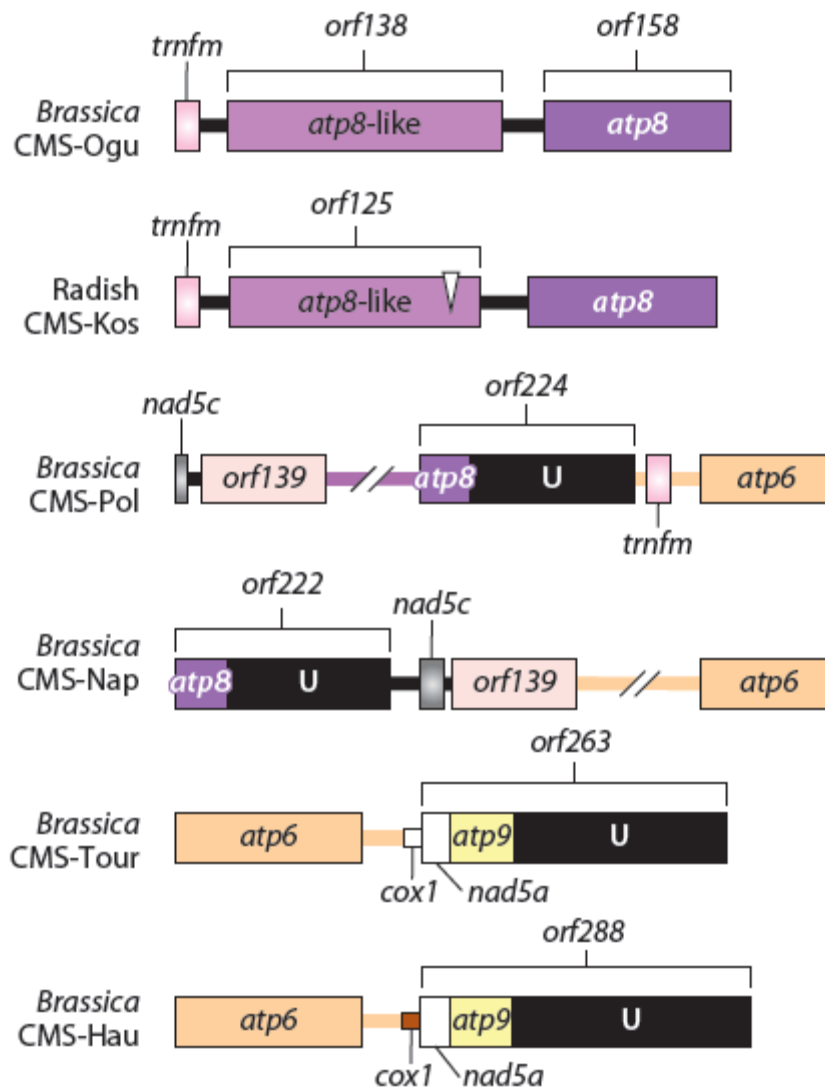
shen et al , 2007

Pollination control system for *B. napus*

There are four systems for hybrid seed production in *Brassica napus* L.

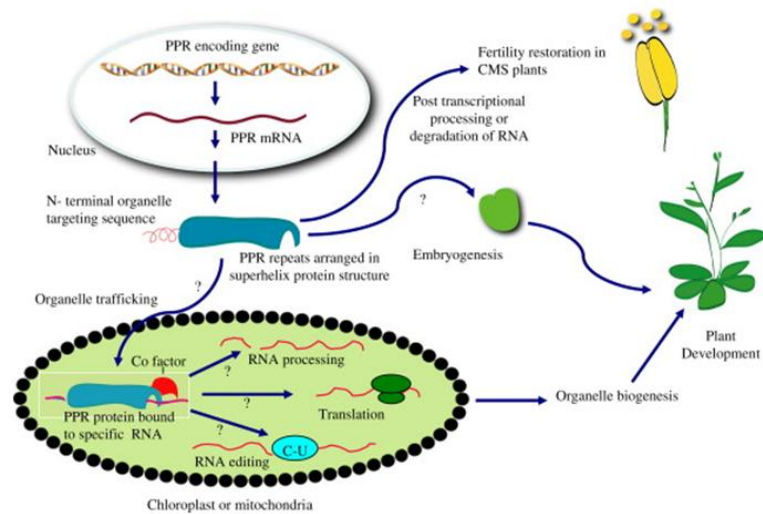
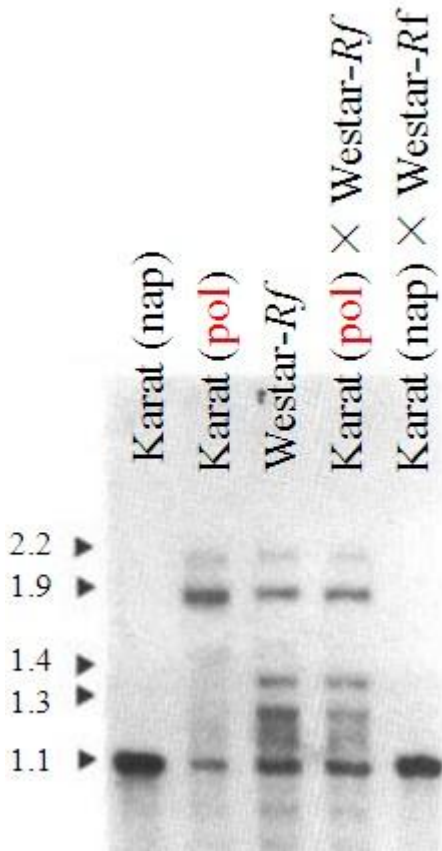
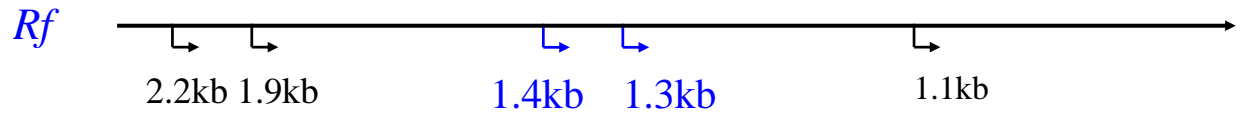
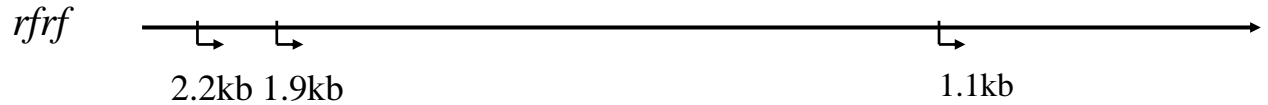
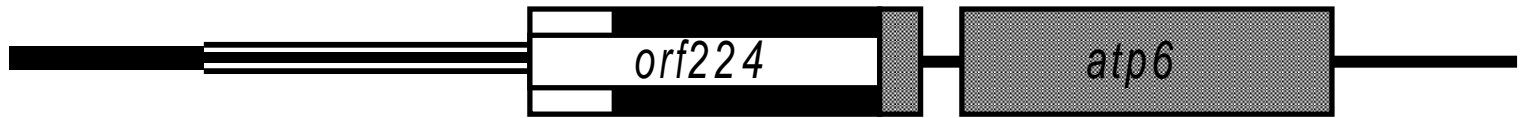
1. CMS (ogu CMS, Ogura, 1968; pol CMS, Fu, 1981)
2. GMS (RGMS, DGMS)
3. SI
4. CHA

CMS genes



Chen and Liu (2013)

orf224 gene



Singh and Brown, 1991

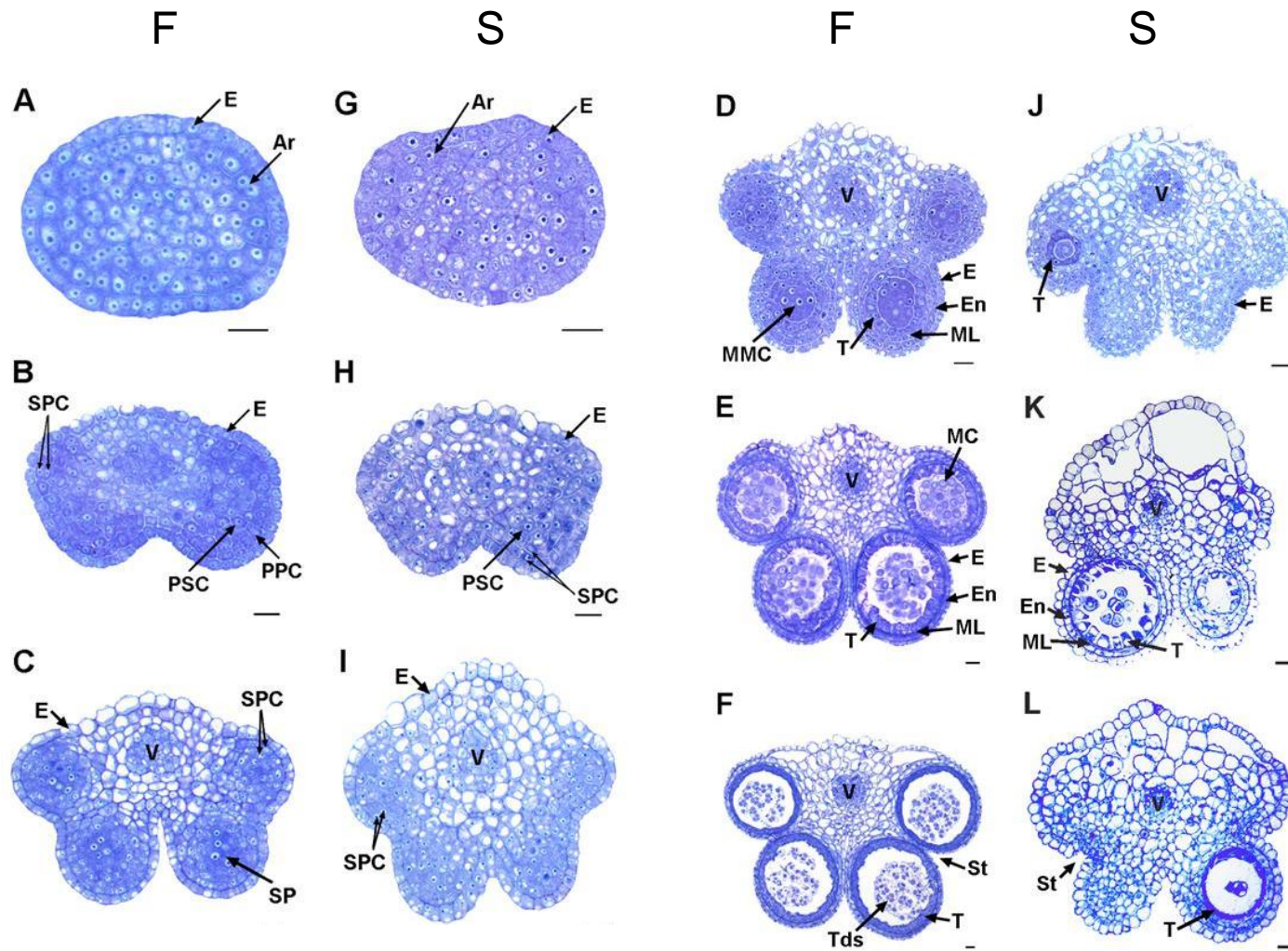
saha, et.al., 2007

Plant material



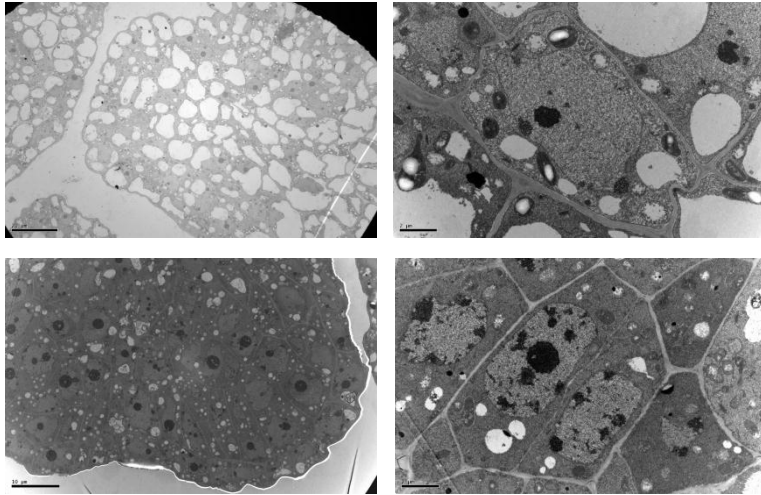
Mapping population: NIL population *S(rfprfp)* and *S(Rfprfp)*

The observations on semi-thin sections

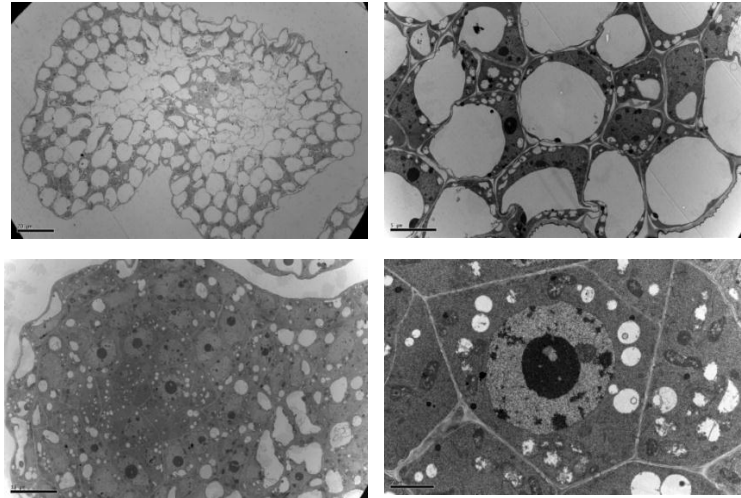


TEM

S3



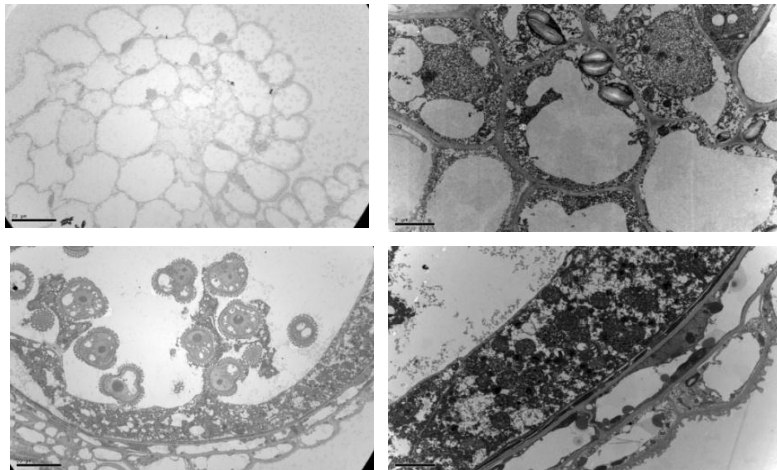
S4



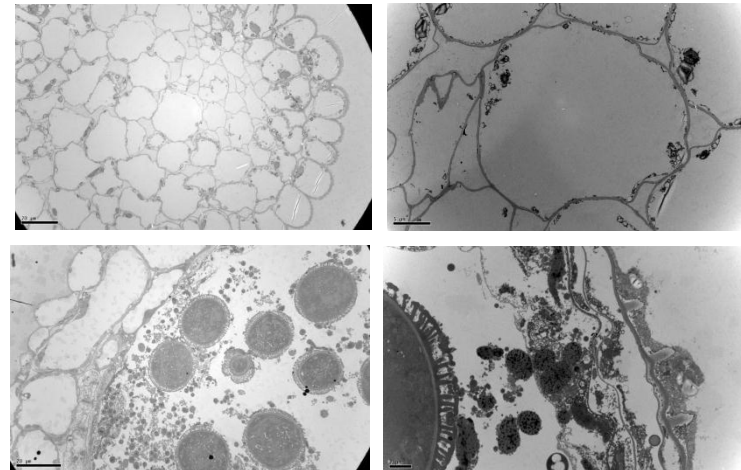
S

F

S10



S11



S

F

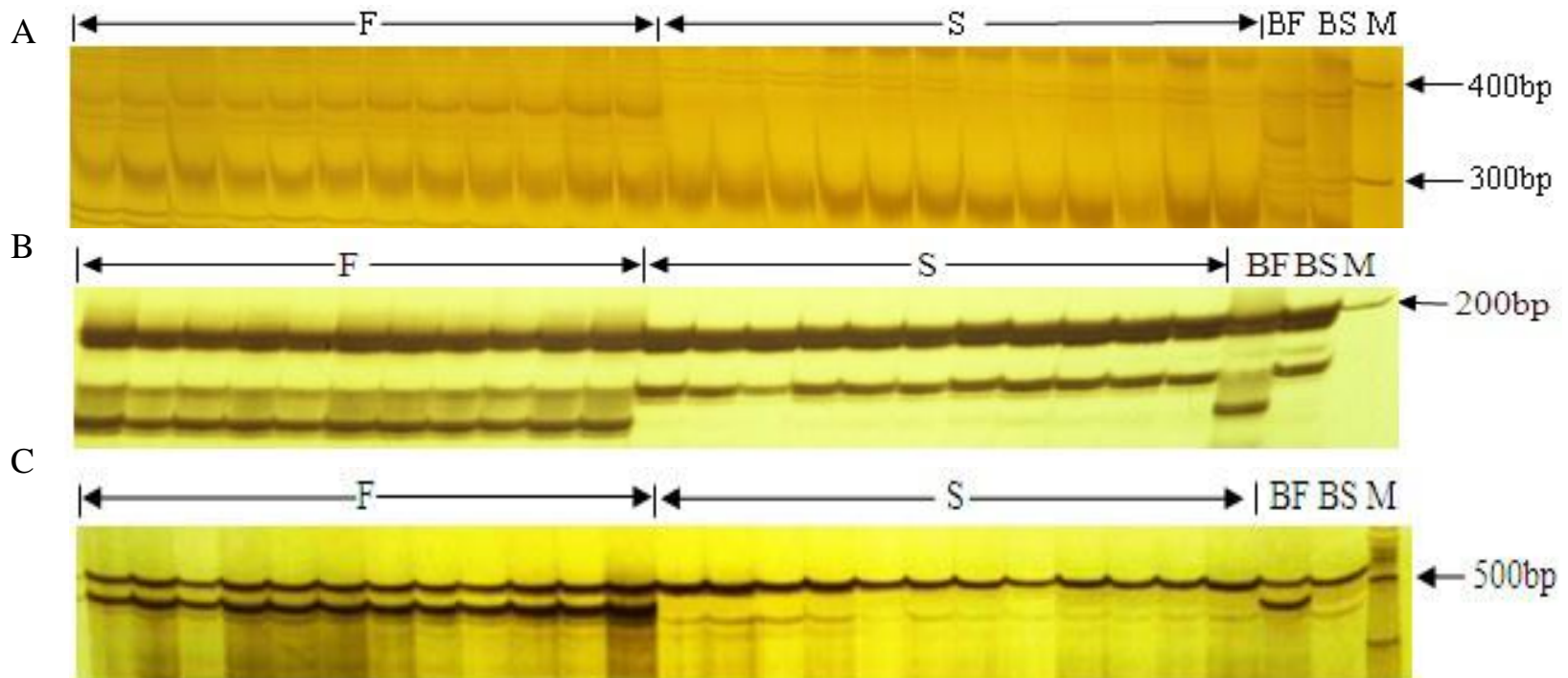
Genetic analysis of the *Rfp* gene

Table 3.1 Fertility segregation in two NIL populations

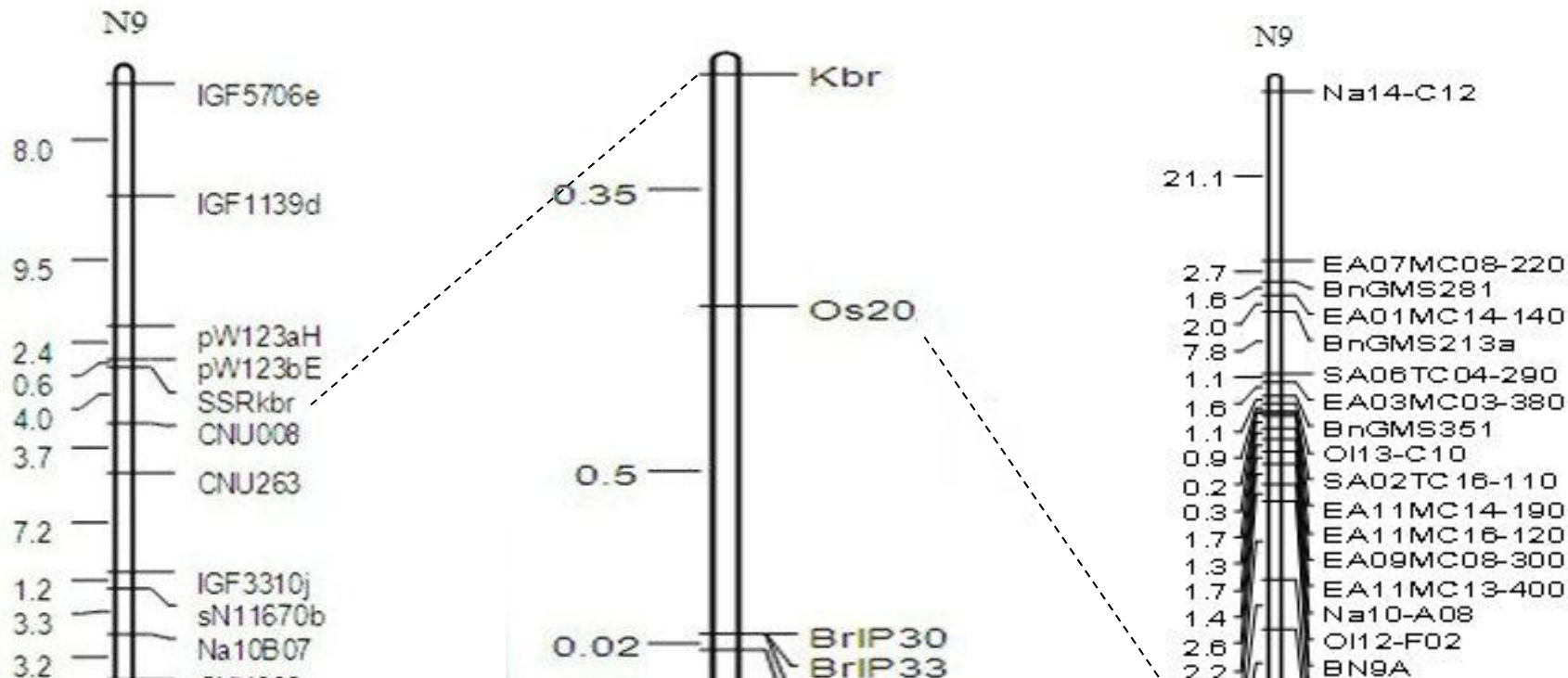
群体	单株总数	可育株数	不育株数	预期比例	χ^2
Pop.	Total number	Fertile plants	Sterile plants	Expected ratio	value
NIL ₁	1367	717	650	1:1	3.19
NIL ₂	3662	1827	1835	1:1	0.01

$$\chi^2_{0.05,1}=3.84$$

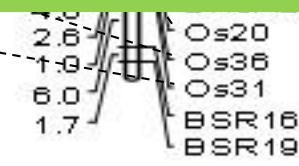
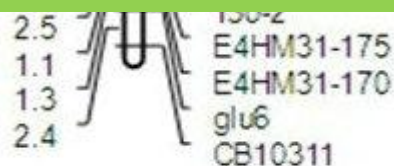
Molecular marker linkaged with *Rfp* gene



Linkage group



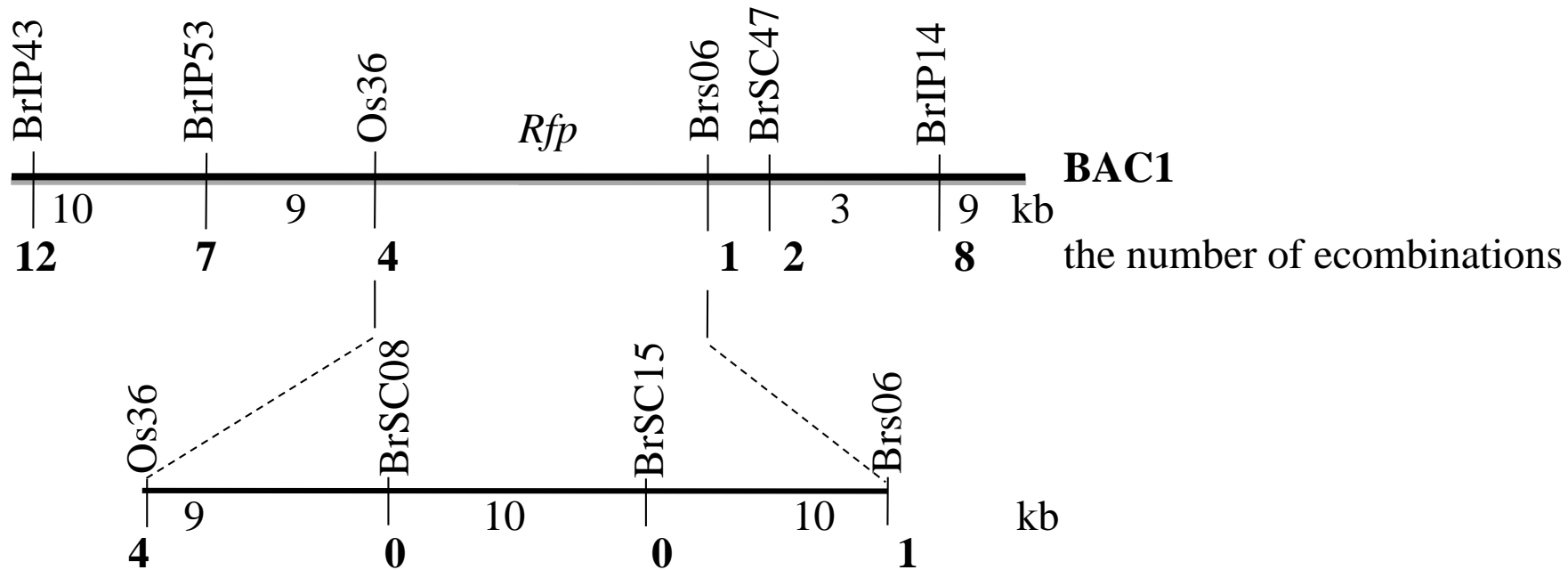
The *Rfp* gene was mapped on linkage group N9 of *B. napus*.



Fine mapping of the *Rfp* gene

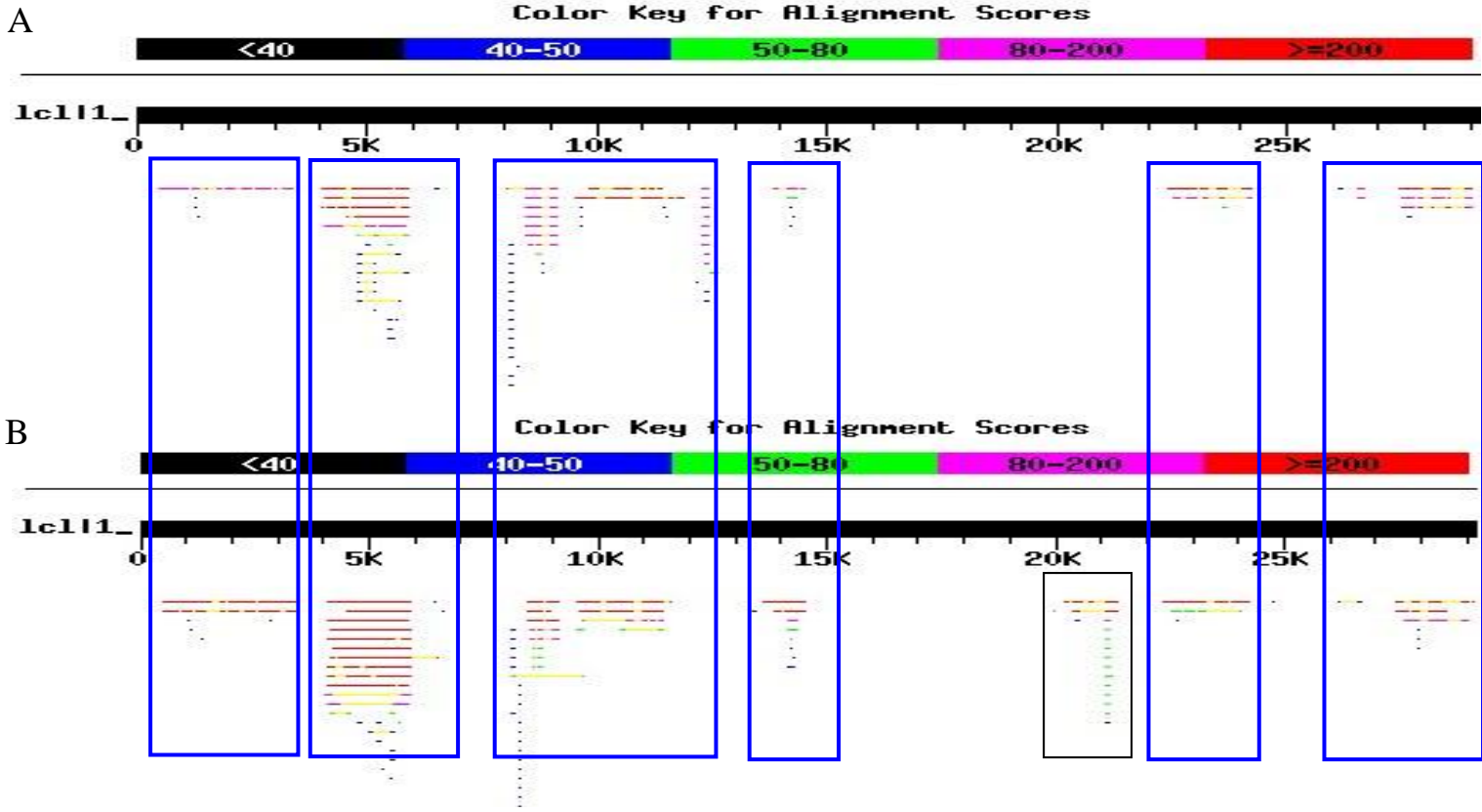
Individuals	表型 Phenotype (F/S) ^a	标记 Marker ^b								
		BrfP43	BrfP53	Os36	Os31	BrSC08	BrSC15	Brs06	BrSC47	BrfP14
MD13	F	A	H	H	H	H	H	H	H	H
MD77	F	A	H	H	H	H	H	H	H	H
MX19	S	H	A	A	A	A	A	A	A	A
MX60	F	A	H	H	H	H	H	H	H	H
OG28	S	H	A	A	A	A	A	A	A	A
MF69	S	H	H	A	A	A	A	A	A	A
MY04	F	A	A	H	H	H	H	H	H	H
OB60	S	H	H	A	A	A	A	A	A	A
ME76	S	H	H	H	A	A	A	A	A	A
MW17	S	H	H	H	A	A	A	A	A	A
MW63	F	A	A	A	H	H	H	H	H	H
OI47	F	A	A	A	H	H	H	H	H	H
MH01	F	H	H	H	H	H	H	H	H	A
ML56	S	A	A	A	A	A	A	A	A	H
MM67	S	A	A	A	A	A	A	A	A	H
MY49	S	A	A	A	A	A	A	A	A	H
NX92	F	H	H	H	H	H	H	H	H	A
NZ31	S	A	A	A	A	A	A	A	A	H
OE70	S	A	A	A	A	A	A	A	H	H
OF69	S	A	A	A	A	A	A	H	H	H

The physical map of *Rfp* gene



The physical distance between Os36 and Brs06 is 29.2kb.

Blast analysis



Candidate gene

The annotation of the candidate gene

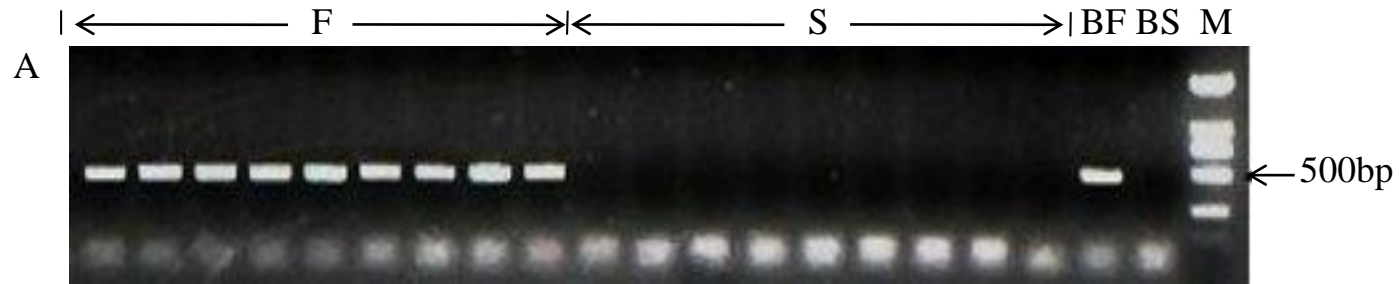
Number	Homologous <i>Arabidopsis</i> gene and E value	Gene annotation
ORF1	AT1G13030, 1e-34	Sphere organelles protein-related
ORF2	AT1G12300, 4e-121	PPR containing protein
ORF3	AT1G 13020, 1e-65	Eukaryotic translation initiation factor
ORF4	AT1G 12980, 3e-45	Enhancer of shoot regeneration 1
ORF5	AT3G 62330, 0.31	Zinc knuckle (CCHC-type) family protein
ORF6	AT1G 12970, 5e-170	Leucine-rich repeat family protein
ORF7	AT1G 12950, 2e-73	MATE efflux family protein

Comparative sequence analysis of the *PPR* gene

	223	224	228	268	301	382	384	406	423	479	542	648
<i>Rf</i>	A	N	P	T	F	N	K	W	D	A	S	S
<i>rf</i>	D	K	A	N	I	M	N	V	V	G	D	L

There are 45 SNPs between the *Rfp* and *rfp*, of them, 33 were synonymous mutations, and 12 were missense mutations.

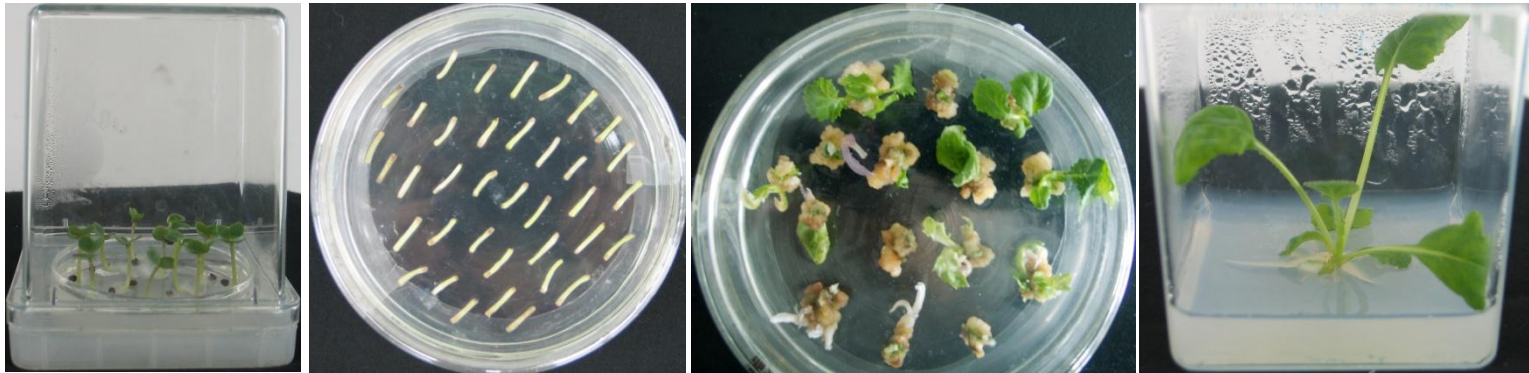
The SCAR marker converted from SNP



A NIL population

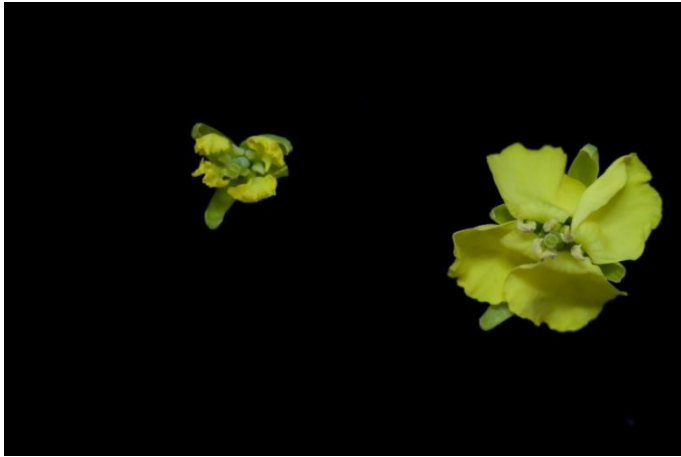
B Breeding materials R: restorer line, A: male sterile line

The complementation test of the *Rfp* gene



- ❖ To test for the ability of the *PPR* gene to complement male sterility in the pol CMS line, we introduced the candidate gene into the pol CMS line by *Agrobacterium tumefaciens*-mediated transformation.
- ❖ A total of 15 transgenic plants were recovered.

The *Rfp* gene could recover fertility of the T0 plants



A

B



A

B



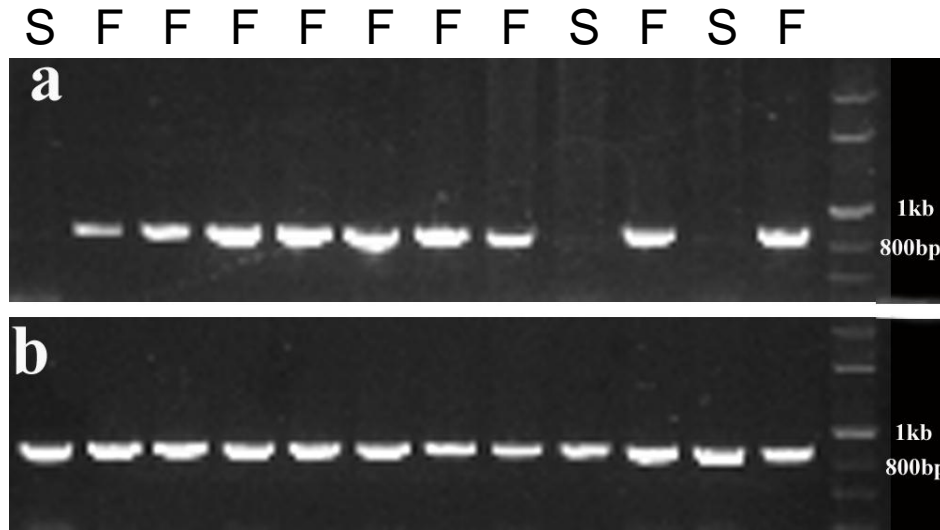
A

B

A: pol CMS

B: T0 containing the transgenic *Rfp*
Complementation construct

Co-segregated analysis



No.T1 progeny	Phenotype
19	Sterility
43	Fertility

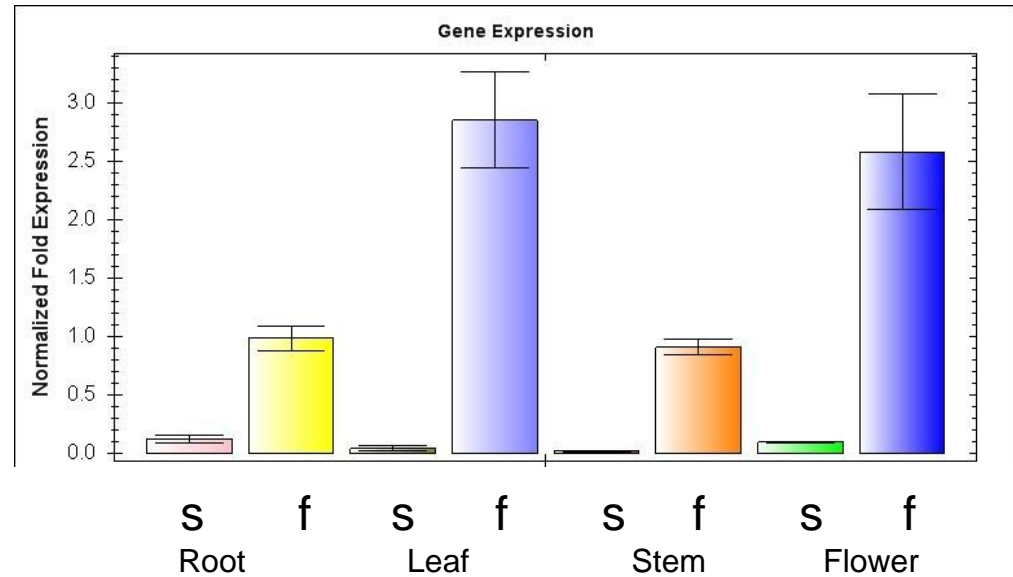
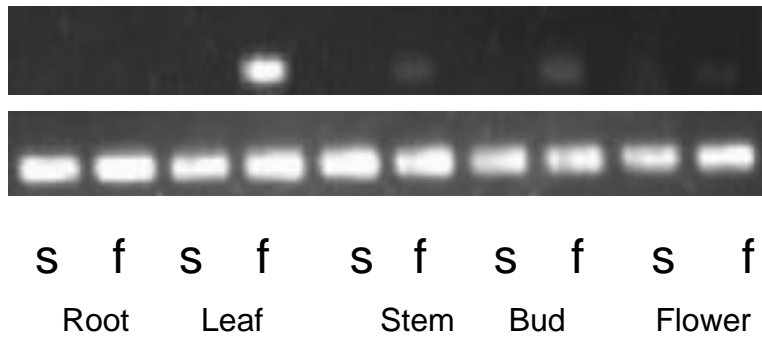
$\chi^2=0.774$ for 1:3, $P<0.05$

a: PCR with transgenic vector specific primers

b: PCR with *orf224* specific primers

The recovered fertility of the T1 progeny plants could be co-segregated with the introduced DNA.

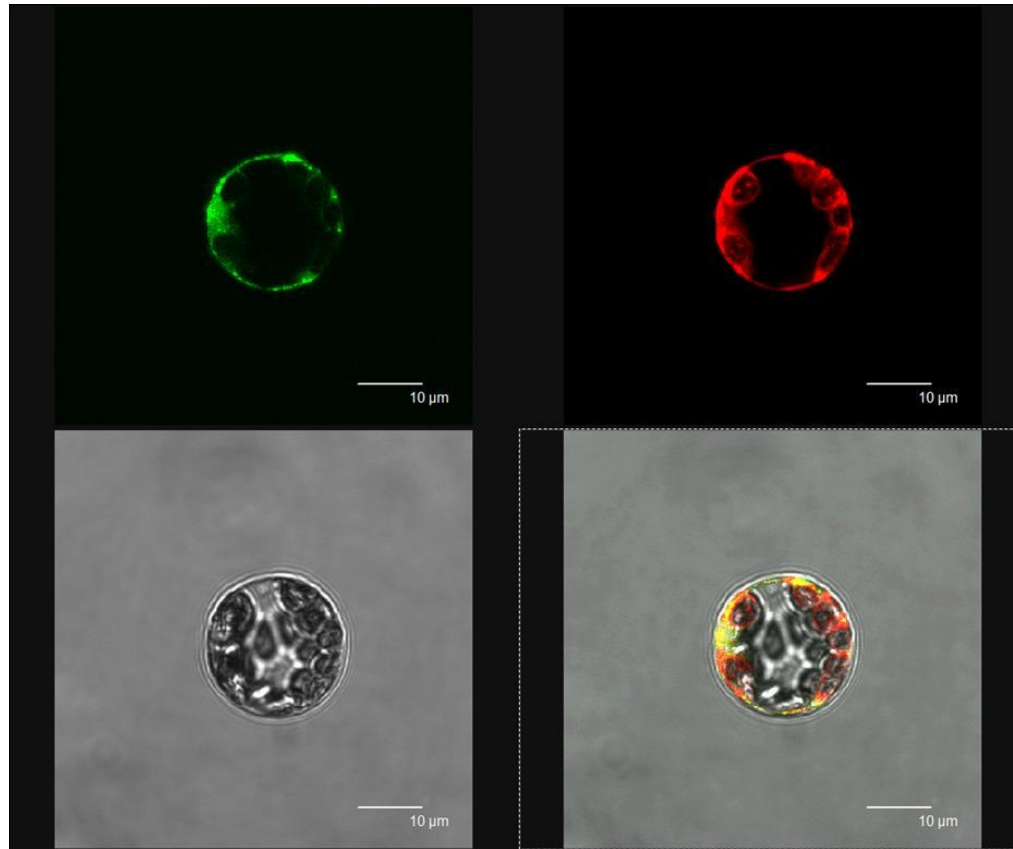
Detection of *Rfp* transcript by RT-PCR and qRT-PCR



Subcellular localization of the *Rfp* gene.

Rfp::GFP

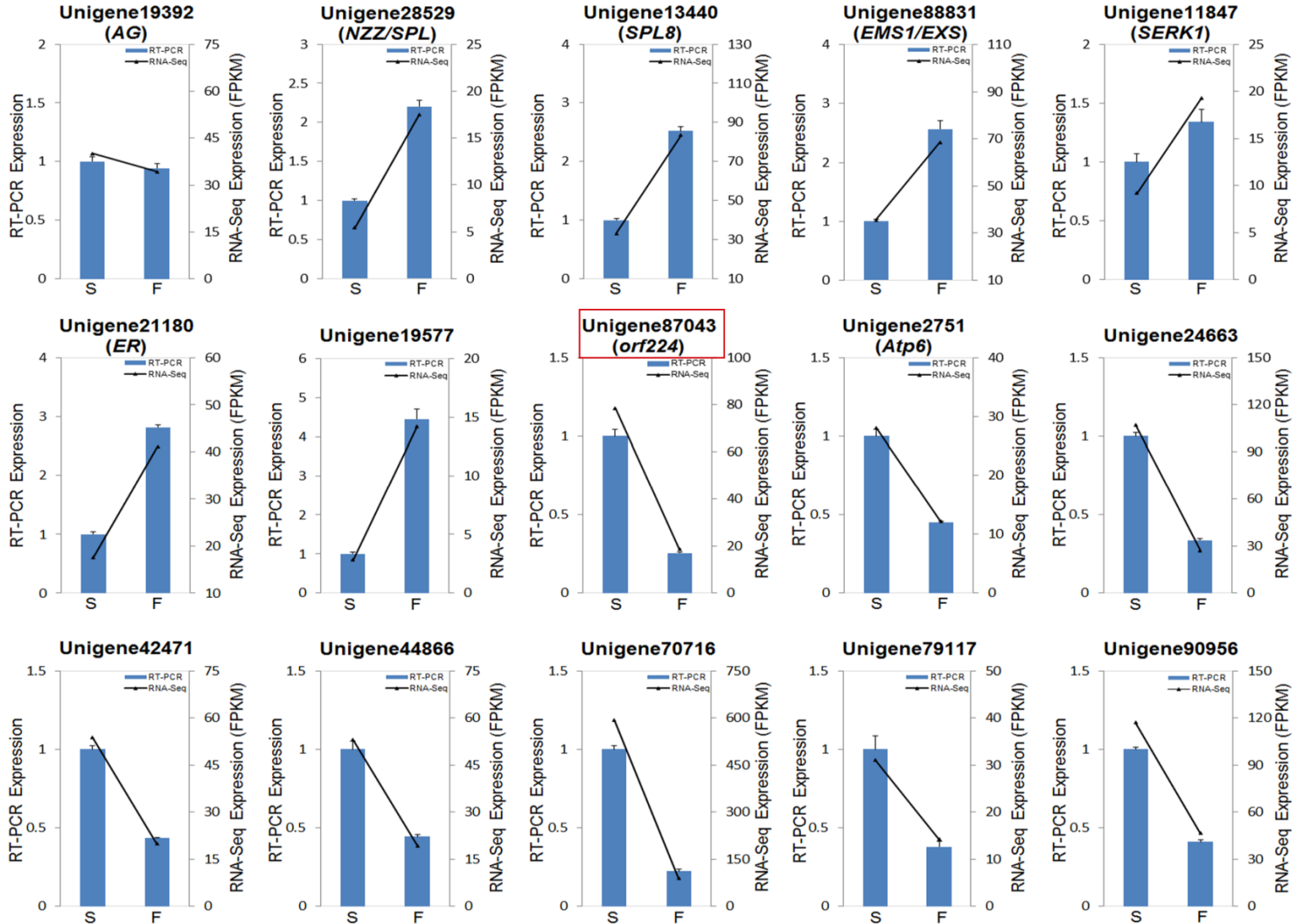
Mitotracker Red CMX-Ros



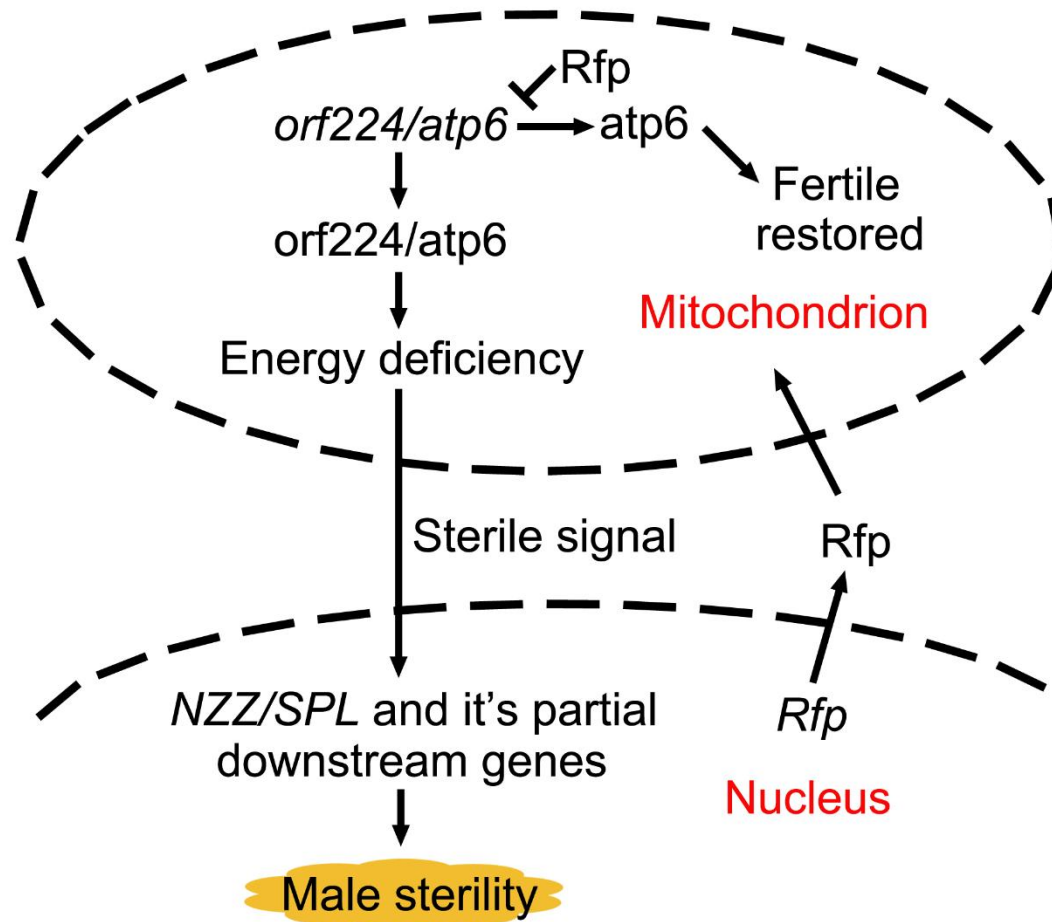
Bright

Merged

RNA-seq



Proposed model for sterile and restored mechanism of *po/CMS*



Summary

- ❖ The differences between the pol CMS line and the wild-type are first observed at the stage 4 of pollen development.
- ❖ The *Rfp* locus was narrowed down to a 29.2-kb genomic region of linkage group N9.
- ❖ The complementation test confirmed that the candidate gene ORF2, encoding a PPR protein, could recovered the fertility of pol CMS.
- ❖ Subcellular localization analysis indicated that Rfp is a mitochondrial-localized protein. the *orf224-atp6* transcript is cleaved by the action of Rfp, and the resulting *orf224* RNA fragment is degraded at the Posttranscriptional Level.

Acknowledgements

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Thank you!

