



Construction of Restorer Lines and Molecular Mapping for Restorer Gene of *hau* Cytoplasmic Male Sterility in *Brassica napus*

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Abstract

Cytoplasmic male sterility is a general phenomenon in almost 200 species, and the interaction between chimeric genes in mitochondria and restorer genes in nucleus may be responsible for restoration of male fertility. *Orf288* has been identified as a CMS-associated gene in the *hau* CMS line of *Brassica napus* and *Brassica juncea*, however, the restorer lines/genes have not been found yet. We have successfully constructed two restorer lines in *B. napus* by extensive testcrossing and have mapped a major restorer gene *Rfh* to a physical distance of 94 kb on chromosome A03 by whole-genome resequencing and molecular markers. We found that the restorer line is indeed restored to male fertility at histological level. Comparative genomics and collinearity analysis between close relatives revealed that rearrangements and recombination may have happened and thus caused the production of *Rfh* or components of the restoration of fertility complex. Meanwhile, nuclear backgrounds with multiple loci and temperature were related to the variation and instability of restoration of fertility in three different populations. Our study provides new sights into the coevolution between restorer genes and CMS-associated genes as well as the cultivation of superior hybrids via molecular breeding.

Results

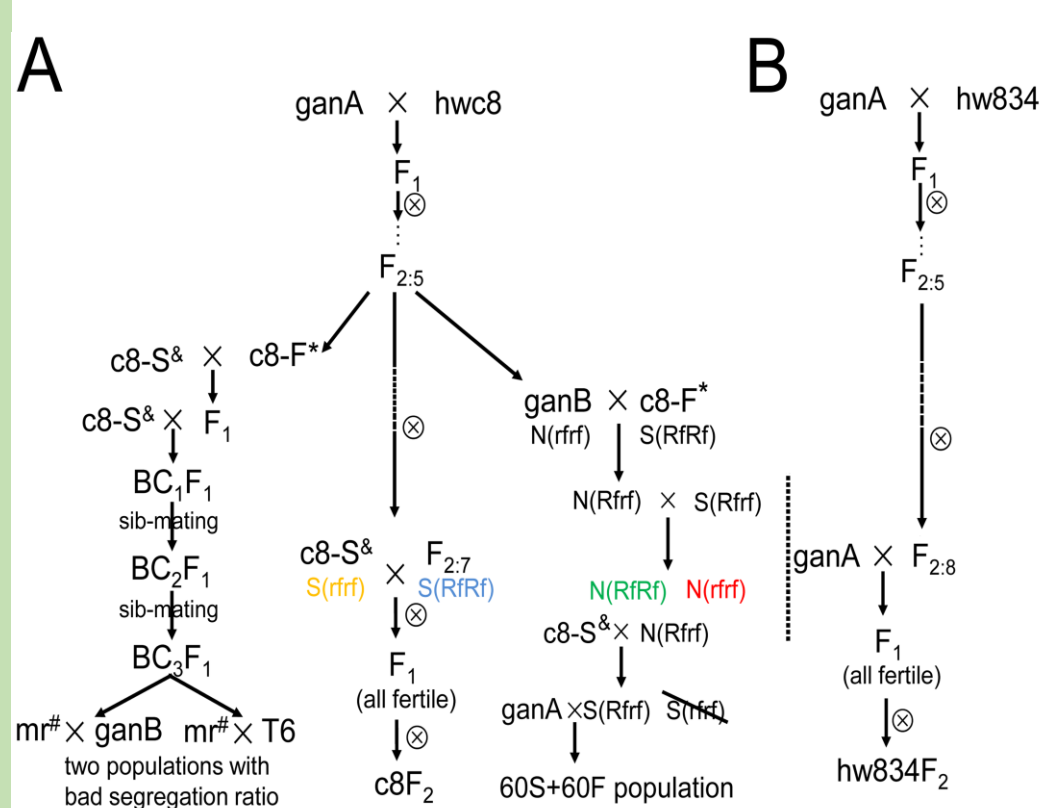


Fig. 1 Procedures for constructing two restorer lines and several segregation populations for *hau* CMS in *B. napus*.

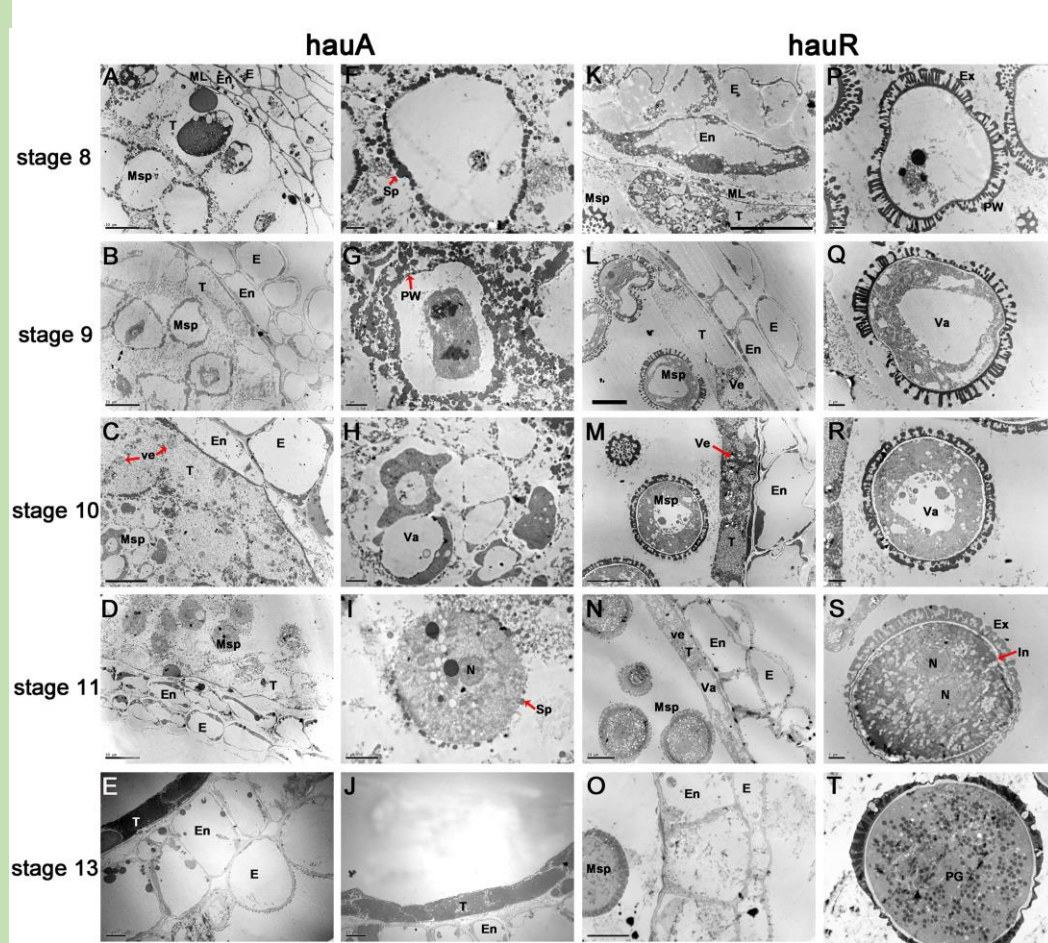


Fig. 2 TEM analyses of tapetal structure and pollen development of *hau* CMS line *hauA* and restorer line *hauR*.

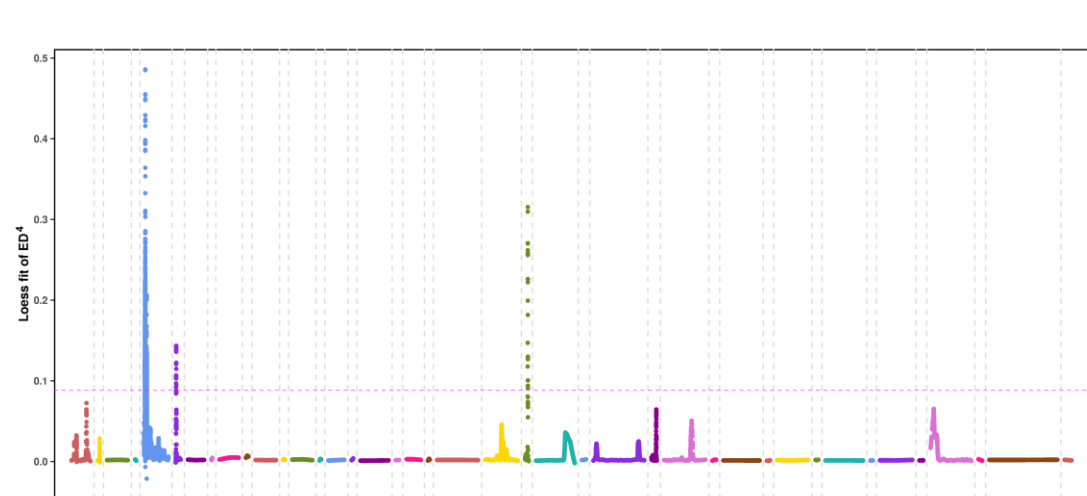


Fig. 3 Map of loss of fit of SNP ED4 based on 78,171 SNPs from resequencing data between the *hauA* and *hauR* pools. The threshold of loss of fit is set at median + 3SD (standard deviation).

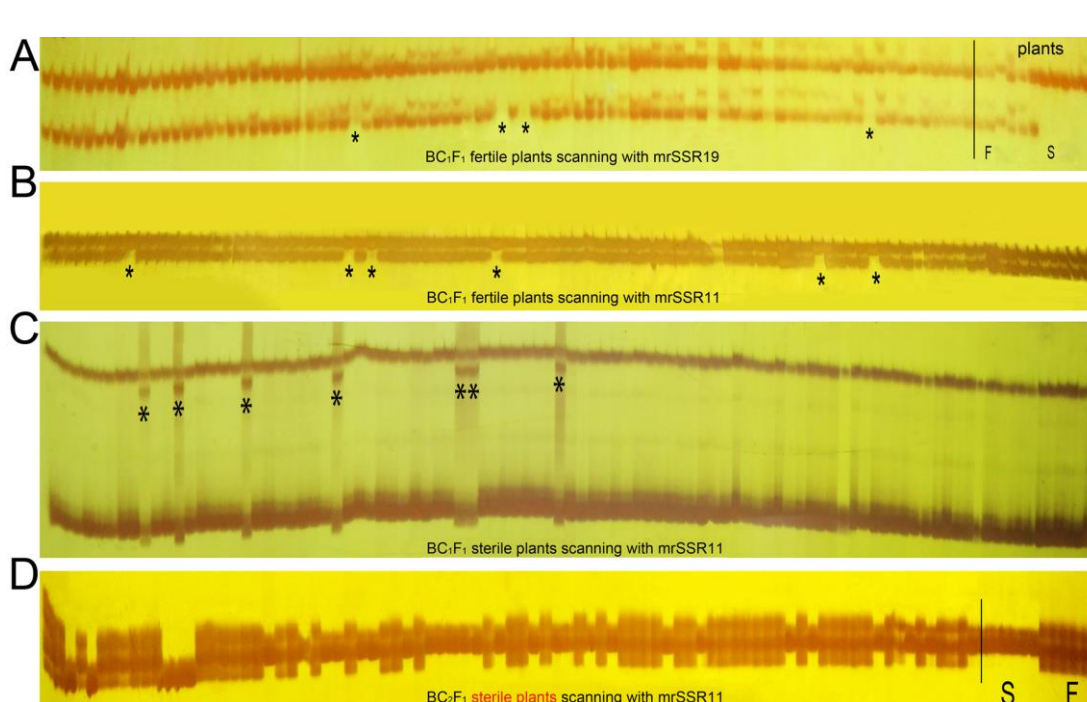


Fig. 4 Molecular markers scanning and recombinants detection. (A) 84 BC_1F_1 fertile plants scanning with *mrSSR19* marker. (B) 96 BC_1F_1 fertile plants scanning with *mrSSR11* marker. (C) 96 BC_1F_1 sterile plants scanning with *mrSSR11* marker. (D) 84 BC_1F_1 sterile plants scanning with *mrSSR11* marker.

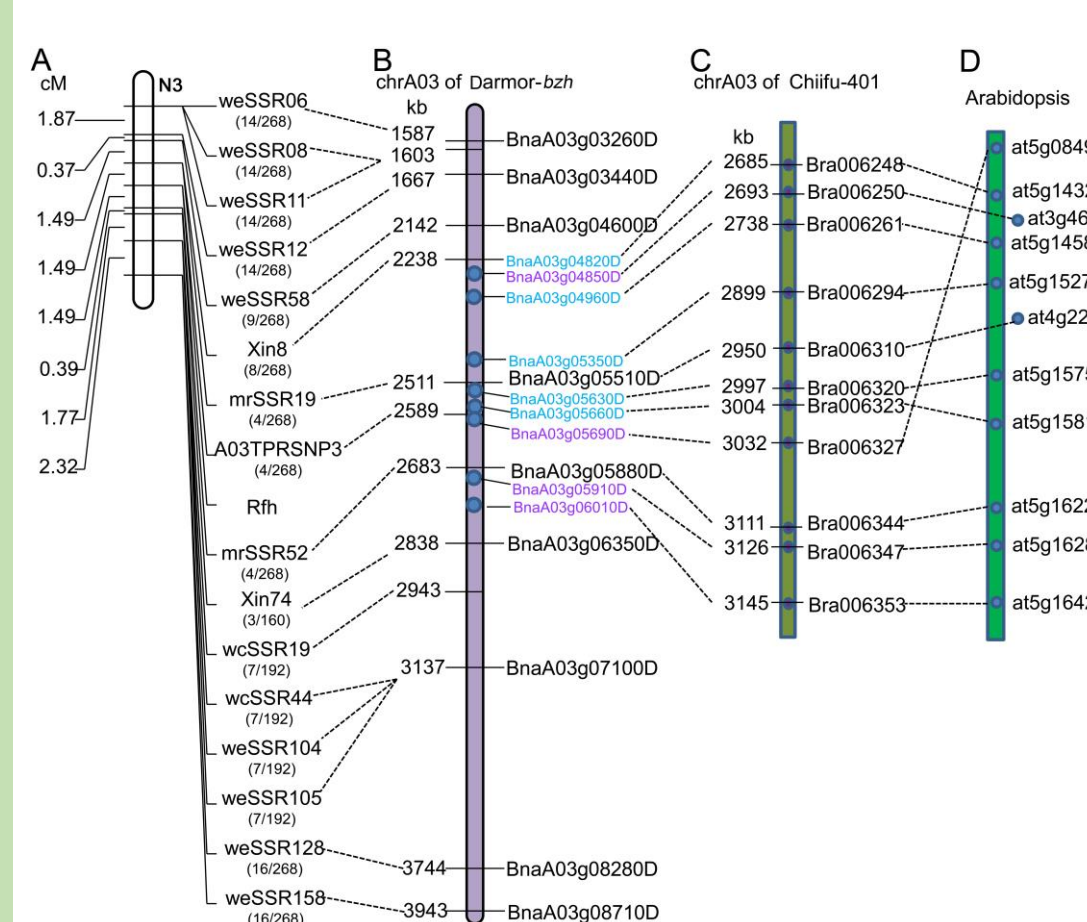


Fig. 5 Genetic and physical mapping of the predicted candidate region in *B. napus* compared to *B. rapa* and *Arabidopsis* genome.

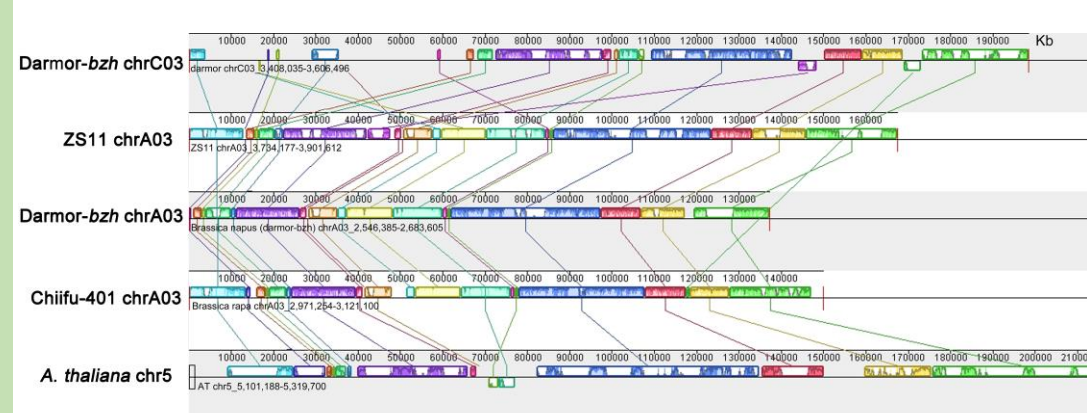


Fig. 6 Syntenic analyses of the homologous regions from chrA03 or chrC03 of Darmor-bzh, ZS11, *B. rapa* Chiifu-401, and *A. thaliana*. Continuously colored region is a locally collinear block (LCB) region without homologous sequence rearrangement. Lines with different colors trace each orthologous LCB through each genome.

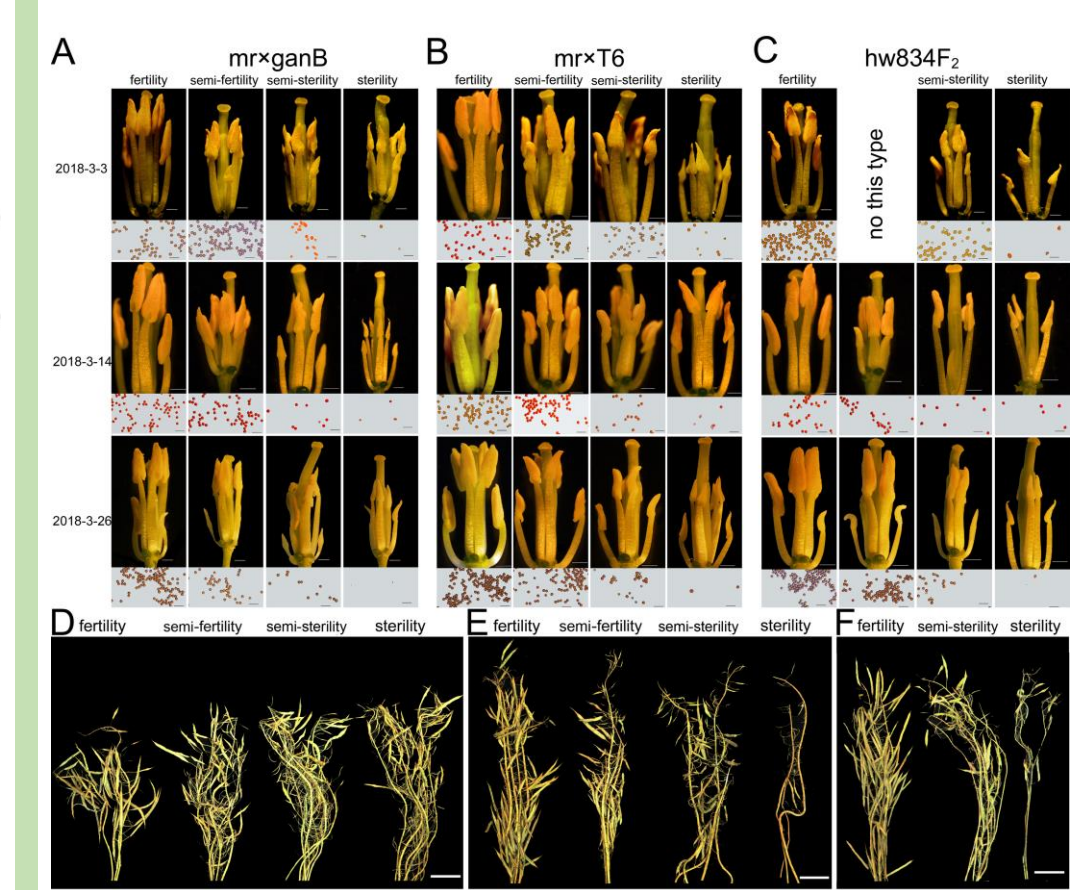


Fig. 7 Observations of anthers development, pollen vigor and seed-setting of the '*mr* × *ganB*', '*mr* × *T6*' and *hw834F_2* populations at different temperatures.

Table 1 Fertility segregation in several populations

Population	Total number ^a	Fertile plants	Sterile plants	Expected ratio	χ^2 value
BC_1F_1	268	140	128	1:1	0.537
BC_2F_1	890	461	429	1:1	1.15
BC_3F_1	539	273	266	1:1	0.09
60S+60F	533	242	291	1:1	4.5
$c8F_2$	292	224	68	3:1	0.457
<i>hw834F_2</i>	248	188	60	3:1	0.086

^a $\chi^2_{0.05,1}=3.84$; ^aplants hard to be classified were filtered out.

Conclusion

Two restorer lines of *hau* CMS in *B. napus* have been constructed and one major gene *Rfh* is responsible for fertility restoration. After histologic inspections, we found that the restoration of fertility happened. Then we have mapped *Rfh* to a 94 kb region on chrA03 with 2.98 cM by resequencing data and molecular markers. Finally, we have discussed multiple loci and temperature besides the restorer gene lead to instability and variation of male fertility in different populations with different nuclear backgrounds. Next we should enlarge population size and find closer markers to fine mapping of the restorer gene as well as RFC components to explore the model for fertility restoration of *hau* CMS line. Our finding may provide a good case for nuclear-mitochondrial interaction and contribute to the utilization of heterosis by introducing ideal agronomic traits into the restorer lines.