Inheritance of two rapeseed mutants with apetalous flowers and molecular mapping of the gene(s) controlling petal-loss trait in *Brassica napus*

CHEN Biyun, WU Xiaoming, LU Guangyuan, GAO Guizhen, XU Kun, LI Xiangzhi

Oil Crops Research Institute of CAAS, Key Laboratory of Genetic Improvement of Oil Crops, Wuhan 430062, China
Email:luwiz@oil crops.cn

Abstract

In this study, APT02, a petal-loss mutant in *Brassica napus* and AM032, a petal-stamenoid mutant in *Brassica campestris,* were used to construct F_2 and BC_1 populations by crossed respectively with normal petalous rapeseed of *Brassica napus* and *Brassica campestris*. Mode of inheritance for two kinds of rapeseed mutants with apetalous flowers was studied.Based on the observation of PDgr (petalous degree) segregation, It was hypothesized that the petal-loss trait of APT02 was controlled by two pairs of recessive major genes and one pair of recessive modifying genes, and the petal-stamenoid trait of AM032 was controlled by a pair of recessive genes. Maternal effect wasn't found in apetalous trait of APT02 and AM032.An F_2 population derived from a cross between apetalous line' APT02 'and normal petalled cultivar 'ZS NO.4' was used for molecular marker searching and chromosomal mapping of the gene(s) controlling petal-loss trait in *Brassica napus*.Twenty pairs of AFLP primers and 170 pairs of SRAP primers were selected and screened from two parents. In further selection through bulked segregant analysis(BSA)approach, one SRAP marker e8m3_4 (600 bp) and one AFLP marker E3247_15(150 bp)were obtained and found to be linked to the gene(s) controlling petal-loss trait, with the genetic distance of 5 cM and 13.5 cM. A linkage map in *Brassica napus* was constructed. It consisted of 213 AFLP, 56 SSR loci and a morphology marker throughout 17 main linkage groups, two triplet and four linkage pairs. Total length of the map covered 2487.1 cM, and average interval between markers was 10.09 cM. By genetic mapping, the gene(s) controlling petal-loss trait (*WHB*) was mapped in LG4.

Key words: *Brassica napus*, *Brassica* campestris, mutant, petal-loss, petal-stamenoid, inheritance, AFLP marker, SRAP marker, map construction

1. Introduction

The third whorl floral organs' variation of rapeseed has mainly two kinds of that one apetalous phenotype was petal-loss type another apetalous type was petal-stameniod type. The petal-loss genotypes may be more effective in photosynthesis and reallocation of assimilates due to the removal of the yellow flower layer (Chapman 1984, Yates & Steven 1987, Mendham *et al*. 1991, Fray *et al*. 1996). Moreover, the petal-loss types may avoid some diseases, especially rapeseed stem rot (*Sclerotinia sclerotiorum*) or downy mildew (Peronospora parastica)(Mc Lean 1958, Krüger 1975, Larmarque 1983).The apetalous genotypes have different origins. There are different genetic models of the apetalous trait. Apetalous character controlled by from one to four pairs genes was found (Singh 1961, Zhao *et al*. 2004, Buzza *et al*. 1983, Kelly *et al*. 1995, Singh *et al*. 1991).therefore the inheritance mode of apetalous character couldn't be confirmed up to the present.Fray *et al*. (1997) using RFLP technology had performed map-making to the petal-stameniod character and got five RFLP markers. In order to perform marker-assisted breeding, Robinson *et al*. (1999) mapped three genes of petal-loss character (Apet-1,Apet-2,Apet-3).Tan *et al*. (2003) had obtained RAPD marker S_{352_580} that was linked closely to the genes controlling petal character. In rapeseed germplasm resources research, two kinds of mutants were obtained of that one apetalous phenotype was a petal-loss mutant in *Brassica napus* another apetalous type was a petal-stamenoid mutant in *Brassica campestris*. Above two kinds of mutants' genetic regulation wasn't unknown. Mapping of the Gene(s) Controlling Petal-loss Trait in *Brassica napus*, hadn't been reported in China. In this paper, through studying above two kinds of mutants' genetic models and chromosomal mapping of the gene(s) controlling petal-loss trait in *Brassica napus*, we hope to be helpful to make full use of apetalous special germplasm resources and breed apetalous breeding in *Brassica napus*.

2. Materials and Methods

Plant materials: Table 1 showed Plant materials used in the present study.

Population construction: Planting and field management of experimentation materials was performed on common program. The populations were constructed on the crossing strategy that Fig.1 revealed.

Petalous Degree (PDgr) was calculated according to Buzza (1983):

$$PDgr(\%) = (\sum_{i=1}^{k} Pi / 4N) \times 100$$

with,Pi: the number of petals on the i-th flower
N: total number of the flowers counted

At least 25 open flowers of each plant were counted for number of petals at initial flowering stage. The apetalous, intermediate and normal petalled genotypes are defined as PDgr between 0-10 %, 10-90% and 90-100%, respectively. The

accordance between expected and observed segregation was tested by Chi-Square test (χ^2).

Table 1 Plant materials used in the present study

Name	Type	Origin	Discription
APT02	*B. napus*.L	Ausralia	mutant, petal-loss, steady
ZS NO.4	*B. napus*.L	China	normal-petalled
SC95-16	*B. napus*.L	Canada	normal-petalled
Yunyou NO.11	*B. napus*.L	China	normal-petalled
AM032	*B.campestris*.L	America	mutant, petal-stameniod, steady
Guiding sweet rape	*B.campestris*.L	China	cultivating-breed, normal-petalled, steady
Nantong yellow seed	*B.campestris*.L	China	cultivating-breed, normal-petalled, steady

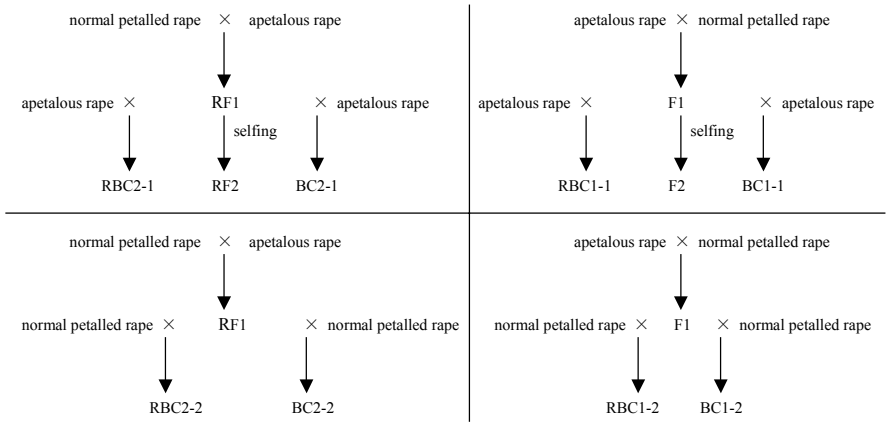

Fig.1 The crossing strategy used to analyze Genetic mode of apetalous trait.

Mapping-population construction and DNA extraction

An F_2 population derived from a cross between apetalous line "APT02' and normal petalled cultivar 'ZS NO.4' was used for molecular marker. Genomic DNA was isolated from leaves using the modified SDS method of Li J,*et al*(1994).

AFLP and SRAP Analysis

AFLP analysis was performed according to the protocol described by (Vos *et al*.1995). SRAP analysis was performed according to the protocol described by (Li and Quiros 2001). The separated DNA fragments was detected using siliver-staining method(Yong *et a*l. 1996).

Linkage-map construction

Linkage-genetic analysis was performed according to Mapmaker/Exp 3.0 soft (Lincoln *et al* 1992).

3. Results

Genetic analyzing apetalous trait in Brassica napus

At three crosses(APT02×ZS NO.4, APT02×SC95-16, APT02×Yunyou NO.11), PDgr segregated in BC1-1, RBC1-1, BC2-1, RBC2-1, F_2 and RF_2. The generations F_1, RF_1 were uniform with normal petals. Among three wild type *Brassica napus* varieties, approximately one-eighth of the plants in BC1-1, RBC1-1, BC2-1, RBC2-1 displayed apetalous trait, approximately one-eighth of the plants had an intermediate number of petals and the rest had normal petal number. Around 1/64 of the plants in F_2, RF_2 displayed apetalous trait, approximately 3/64 of the plants had an intermediate number of petals and the rest had normal petal number. At segregation populations, about 1:1:6 for apetalous, intermediate and normal petalled plants segregated in BC1-1, RBC1-1, BC2-1 and RBC2-1. About 1:3:60 for apetalous, intermediate and normal petalled plants segregated in F_2 and RF_2.The results suggest that apetalous trait of 'APT02' was controlled by two major recessive gene pairs and a third modifying recessive gene pair, maternal effect wasn't found in this trait. Normal petalled plants have six alleles in nucleus coding normal petals (AABBCC), whereas the apetalous ' APT02' mutant has six alleles coding the absence of petals (aabbcc).

Chi-Square test (χ^2) shows that all observed segregations agree with the expected ratios. Thus, we conclude that the apetalous trait of 'APT02' was controlled by two major recessive gene pairs and a third modifying recessive gene pair.

Genetic analyzing petal-stameniod trait in Brassica campestris

At two crosses(AM032× Guiding sweet rape, Nantong yellow seed×AM032), PDgr segregated in BC1-1, F_2 and RF_2. The generations F_1, RF_1 were uniform with normal petals. Among two wild type *Brassica campestris* varieties, approximately 1/2 of the plants in BC1-1 displayed apetalous trait, approximately 1/2 of the plants had normal petal number. Around 1/4 of

the plants in F_2, RF_2 displayed apetalous trait, approximately 3/4 of the plants had had normal petal number. At segregation populations, about 1:1 for apetalous and normal petalled plants segregated in BC1-1. About 1:3 for apetalous and normal petalled plants segregated in F_2 and RF_2.The results suggest that apetalous trait of 'AM032' was controlled by a pair of recessive genes, maternal effect wasn't found in this trait. Normal petalled plants have two alleles in nucleus coding normal petals (PP), whereas the apetalous ' AM032' mutant has two alleles coding the absence of petals (pp).

Chi-Square test (χ^2) shows that all observed segregations agree with the expected ratios. Thus, we conclude that the apetalous trait of 'AM032' was controlled by a pair of recessive genes.

AFLP and SRAP Analysis

Twenty pairs of AFLP primers and 170 pairs of SRAP primers were selected and screened from two parents. In further selection through bulked segregant analysis(BSA)approach, one SRAP marker e8m3_4 (600 bp) and one AFLP marker E3247_15(150 bp)were obtained and found to be linked to the gene(s) controlling petal-loss trait, with the genetic distance of 5 cM and 13.5 cM.

Linkage-map construction

A linkage map in *Brassica napus* was constructed. It consisted of 213 AFLP, 56 SSR loci and a morphology marker throughout 17 main linkage groups, two triplet and four linkage pairs. Total length of the map covered 2487.1 cM, and average interval between markers was 10.09 cM. By genetic mapping, the gene(s) controlling petal-loss trait (*WHB*)was mapped in LG4.

4. Discussion

There are different genetic models of the apetalous trait in *B. napus*(Buzza,1983;Kelly *et al*.,1995).Present study showed that the petal-loss trait of APT02 was controlled by two pairs of recessive major genes and one pair of recessive modifying genes. This result was in conformity with Kelly *et al*. (1995).

'AM032' is a mutant in *Brassica campestris* where the petals are converted to sterile stamen. Present study showed that the petal-stamenoid trait of AM032 was controlled by a pair of recessive genes. Fray *et al*. (1997) described an apetalous variant in a spring oilseed rape where the petals are converted to sterile stamen. This phenotype is also under the control of two loci. Present study result wasn't in conformity with Fray *et al*. (1997).This verified that 'AM032',a petal-stamenoid mutant in *Brassica campestris* was a new type mutant material.

Through analyzing two kinds of mutants' genetic models, we knew that two kinds of mutants' genetic models. This will provide theoretic instruction to creating and being utilized of new mutants.In order to perform marker-assisted breeding, Robinson *et al*. (1999) got multi-RFLP markers between the APET loci and their flanking markers. Present study obtaining one SRAP marker e8m3_4 (600 bp),that was found to be linked to the gene(s) controlling petal-loss trait, could be used to direct marker-assisted breeding and had been utilized to marker-assisted breeding apetalous *B. napus* line.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China(No.30170585, 30170585) and Key Technologies R&D Programme of China(No. 2001BA511B07).

References

Buzza G C. Crucifer Newseletter, 1983, 8: 11-12.
Chapman J F, Daniels R W, Scarisbrick D H. Journal of Agricultural Science, 1984, 102: 23-31.
Fray M J, Puangsomlee P. Thero Appl Gent, 1997, 94(6): 731-736.
Kelly A,Fray M,Arthur E A. Murphy D.*Proc.9th international rapeseed congress.Dorchester:* 1995,732-734.
Krüger W., 1975. PflKrankh. 2: 101-108
Larmarque C., 1983. Proc. 6th International Congress Paris France: 957-962
Li G, Quiros C F. Theor Appl Genet, 2001, 103(3): 455-461
LI Jia, SHEN Bing-Zhang, HAN Ji-Xiang, GAN Li. Journal of HuaZhong Agricultural University, 1994, 13(5): 521-523.
Lincoln S, Daly M, Lander E. Whitehead Institute Technical Report, Cambridge, MA, USA, 1992.
Mc Lean D. M., 1958. Plant Disease Rep. 42: 663-666
Mendham N. J., Rao M. S. S., and Buzza G. C.,. GCIRC Congress. 1991: 596-600
Robinson S, Arthur E. The 10th International Rape Congress, Australia, 1999, Sept 26-29.
Singh D, Singh H,Rana P S. *Crop Research*, 1991,4:293-295.
Singh D. *Current Science*,1961,30:62-63.
TAN Xiao-Li, LI Dian-Rong, WANG Hao, GUO Ai-Guang. Acta Bot Boreal Occident Sin, 2003, 23(4):56l-565.
Vos P, Hogers R, Bleeker M, *et al*. Nucl Acid Res, 1995, 23(21): 4407-4414.
Yates D. J. and Steven M. D.,. J. Agric. Sci., Cambridge. 1987,109: 495-502
Yong G C, Matthew W B, Olivier P. Genome, 1996, 39:373- 378.
Zhao Y, Wang M L. Euphytica,2004,137: 381–386.