

Inheritance and molecular mechanism of the genic male sterility accession “Shaan-GMS” in *Brassica napus* L.

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Abstract

Genic male sterility (GMS) is one of the most important ways to utilize the heterosis in *B. napus* and the exploitation of the new source of male sterility is always its important basic work. Shaan-GMS was a newly discovered male sterility accession in *B. napus* in 1994, which fertility was very stably expressed in various genetic backgrounds and environments. In this paper, 83 *B. napus* accessions from China and abroad were tested for their maintaining or restoring ability for Shaan-GMS. Among them, two accessions were screened out with the restoring ability for Shaan-GMS. The genetic pattern of fertility restoration of male sterility of Shaan-GMS was also investigated. Homozygous male sterile line 803AB was developed from the progeny of the cross between Shaan-GMS and its restorer 96-803. Near isogenic line 220AB derived from Shaan-GMS was characterized by isozymes, protein and RAPD molecular markers. PCR technique was employed to isolate gene homologous to the *MS2Bnap* (X99922.1) from two rapeseed dominant digenic male sterile lines, namely 220A (male sterile) and 220B (male fertile), 6A (male sterile) and 6C (male fertile). It can be concluded that Shaan-GMS had the similar maintaining and restoring relationship with DGMS line 6CA from Shanghai Academy of Agricultural Sciences. However, Both DGMS lines had some differences in the molecular level. Shaan-GMS may be a potentially valuable male sterile resource in population improvement and heterosis utilization in *B. napus*.

Key words: *Brassica napus* L.; Digenic male sterility; Inheritance; Molecular mechanism

Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops in the world. Today, *B. napus* becomes the most important oil crops in China, which occupied about 80% of total production area of Brassica oil crops (Liu, 2000). Significant heterosis for seed yield and other agronomic traits had been well documented in oilseed rape (Morice 1979; Sernyk and Stefansson 1983). Many approaches were exploited to utilize the heterosis, among which, genic male sterility (GMS) was one of the most successful ones in China. The exploitation and study of the new source of male sterility is always its important basic work. Up to date, several types of GMS have been reported in rapeseed (reviewed by Fu et al. 2000), such as monogenic dominant GMS, digenic dominant GMS, monogenic recessive GMS, digenic recessive GMS and multigenic recessive GMS.

So far, four digenic dominant GMS (DDGMS) accessions have been reported in *B. napus* (Li et al. 1985; Dong and Du 1993; Wang et al. 1999; Hu 2003). Shaan-GMS was a newly discovered male sterility accession in *B. napus* in 1994, which fertility was very stably expressed in various genetic backgrounds and environments. Eighty-three *B. napus* accessions from China and abroad were tested for their maintaining or restoring ability for Shaan-GMS. Among which, two accessions were screened out with the restoring ability for Shaan-GMS. The genetic pattern of fertility restoration of male sterility of Shaan-GMS was also investigated. Homozygous male sterile line 803AB was developed from the progeny of the cross between Shaan-GMS and its restorer 96-803. Near isogenic line 220AB derived from Shaan-GMS was characterized by isozymes, protein and RAPD molecular markers. PCR technique was employed to isolate gene homologous to the *MS2Bnap* (X99922.1) from two rapeseed dominant digenic male sterile lines, namely 220A (male sterile) and 220B (male fertile), 6A (male sterile) and 6C (male fertile). In this paper, we will summary the research works in the above areas.

The Discovery of Shaan-GMS

Shaan-GMS was a newly discovered male sterility accession in *B. napus* in 1994, which fertility was very stably expressed in various genetic backgrounds and environments. Compared with their fertile sibs, the male sterile plants were characterized by blooming later, flower bud being small, especially flower buds abscission occurring at the bottoms of the inflorescence, no pollen in it at all, stamen being fully degeneration, however, pistil being normal.

Inheritance of Male sterility of Shaan-GMS

Eighty-three *B. napus* accessions from China and abroad were tested for their maintaining or restoring ability for Shaan-GMS. Among them, two accessions were screened out with the restoring ability for Shaan-GMS. Allelic test showed that the restorer genes in these two restorers were allelic. Genetic experiments designed by Liu (1991) were used to investigate the genetic pattern of fertility restoration of male sterility of Shaan-GMS. The results showed that male fertility of Shaan-GMS was controlled by two pair of nuclear genes. If male sterility gene was designated as Ms, its allele recessive gene as ms, dominant inhibition gene as Rf, which can inhibit the expression of the Ms, resulting in the restoration of F₁, its allele recessive gene as rf. The phenotype of MS_rff was male sterility, and the phenotypes of other seven genotypes were male fertility. So

the genotype of Shaan-GMS, 96-803 and 84004 were Msmsrfrf, msmRfRf and msmsrfrf respectively.

Development of homozygous male sterile line 803AB

We used DGMS (dominant genic male sterility) Shaan-GMS(Msmsrfrf) as female to cross with its restorer 96-803 (msmsRfRf). F₂ were obtained by bug-selfing F₁. Fertile plants in F₂ family lines were selfed. Male fertility plants of F₃ family lines with fertility segregating, were test-crossed with temporary maintainer 84004 (msmsrfrf). Based on the results of testcross with 84004, Sib-mating was made in F₃ family lines in which F₁ of ms plants with 84004 were all sterile. Using this method, homozygous ms line 803AB was successfully developed.

Isozymes and soluble proteins

The zymograms of some isozymes and soluble proteins (Prot) in different size buds and stamens of two dominant genic male sterile (DGMS) lines, 220AB(derived from Shaan-GMS) and the control F₁(6CA×220) (*B.napus* L.) were analyzed using PAGE (polyacrylamide gel electrophoresis).The results showed that Esterase(EST) had 13 bands, Prot had more than 20 bands, CAT had 8 bands, and α -Amylase(α -AMY) had 12 bands. The band number of EST and Prot in the 3-4mm long buds and their stamens were less than smaller buds and fertile plants, both in 220AB and F₁ (6CA×220). Some bands of EST, Prot, and Catalase(CAT) moved forward and their Rf values increased due to degradation of the molecular. The change of CAT was similar in the two DGMS types. Compared with the control, electrophoresis pattern of α -AMY showed 5 new additional bands in 4-5mm length buds of sterile plants in 220AB, which meant that the starches was degraded and energy was deficient in the stamen.

RAPD markers for the DGMS gene in Shaan-GMS

Totally 825 random 10-mer primers were screened on the DNA samples of fertile and sterile bulks of 220AB derived from Shaan-GMS and F₁(6CA×220),among which 632 primers produced 2043 bands, One primer,BA1102,produced repeatable polymorphic band BA1102.500 between the paired bulk of 220AB,however, it didn't produce polymorphic band between the paired bulk of F₁(6CA×220),Furthermore, 30 individuals of 220AB were screened by primer BA1102,BA1102.500 band can be produced in 15 male sterile plants, but no PCR products were detected for 15 fertile plants, which showing that BA1102.500 have close relationship with the dominant male sterile gene Ms in Shaan-GMS.

MS2Bnap genomic DNA homologous to MS2 gene from *Arabidopsis thaliana*

PCR technique was employed to isolate gene homologous to the *MS2Bnap*(X99922.1) from two rapeseed (*Brassica napus* L) dominant digenic male sterile lines, namely 220A (male sterile) and 220B (male fertile), 6A (male sterile) and 6C (male fertile). The isolated 2581bp sequences from 220A (named 220A-gDNA, GenBank accession number AY288778), 220B (220B-gDNA, AY257490), 6A (6A-gDNA, DQ060318) and 6C (6C-gDNA, DQ060319) all contained six introns. Forty-one single nucleotide polymorphism (SNP) sites were detected by alignment among these four sequences, 7 of them dispersed in the exon regions. Two SNPs (1247,1656) were detected between 220A-gDNA and 220B-gDNA, and the one at nucleotide 1247 of 220A-gDNA with A replaced by C was a missense mutation, which may be the putative male sterility site in 220A. All 8 SNPs identified between 6A-gDNA and 6C-gDNA were located in the third intron, so the proteins encoded by them are the same. The one SNP between 6A-/6C-gDNA and 220A-/220B-gDNA at nucleotide 2474 of 220A-/220B-gDNA with C replaced by G was a missense mutation. Mutation site of BNMS2PROT (CAA68190.1) encoded by *MS2Bnap* in 220A(254) and 6A/6C(584) is different, which indicated dominant digenic male sterile line 220AB and 6CA have some difference in the molecular level. Comparison of structure of *MS2Bnap* in *B. napus* with that of *MS2* in *Arabidopsis thaliana* revealed that the similarity of exons between these two genes is higher than that of introns.

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