

Mapping the dominant genic male-sterile gene *Ms* in *Brassica napus* L. with AFLP markers

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ABSTRACT

From a survey of 480 AFLP primer combinations, five markers associated with the male sterility in *Brassica napus* L. had been identified in a BC₁ population generated from a cross between GMS line Rs1046A and cv. Samourai. Co-segregating analysis revealed that these markers were tightly linked to the dominant genic male-sterile gene (*Ms*) in a coupling phase. Marker P10M13₃₅₀, P13M8₄₀₀, E7M1₂₃₅ and E3M15₉₉ located at one side of the *Ms* with a map distance of 8.9cM, 5.9cM, 5.2cM and 3.7cM respectively, whereas P6M6₄₁₀ resided 5.9cM away from *Ms* at the other side. The markers identified in this study provided a very useful tool for molecular tagging of the *Ms* allele in GMS three-line breeding selection.

Key words: *Brassica Napus* L.– Dominant genic male-sterile gene (*Ms*) - AFLP markers

INTRODUCTION

The genic male sterility (GMS) found in *Brassica napus* L. was an effective pollen-controlling system for hybrid seed production. So far, several types of GMS have been discovered and the sterility was reported to be governed by one gene (Mathias, 1985), two genes (Li et al., 1988) and three genes (Chen et al., 1993). Among them, one GMS line whose sterility was controlled by the interaction of dominant genic male-sterile gene (*Ms*) and fertility restorer gene (*Rf*) was suggested to be a promising approach in hybrid seed production (Li et al., 1990). Indeed, successful applications using this system had been reported (Li et al., 1990; Zhou et al., 1999). Unfortunately, it is very difficult to improve the male sterile line with the homozygous genotype ($1/2MsMsrf+1/2MsMsRfRf$) by conventional breeding method. Therefore, markers linked to the *Ms* and *Rf* are required to facilitate and accelerate the genotype identification in breeding program. So far, no phenotypic or DNA markers associated with these genes is available for canola breeders. Here we presented the first report on molecular mapping of the *Ms* gene in a BC₁ population.

MATERIALS AND METHODS

A backcross population was generated by crossing GMS line Rs1046A (*MsMsrf*) with winter rapeseed cv. Samourai (*msmsrf*) as a recurrent parent. All plants in the F₁ generation (*Msmmsrf*) were completely male sterile and the segregation of fertility to sterility in the BC₁ population followed the expected 1:1 ratio ($\chi^2 = 0.8653$, $P > 0.250$) ($1/2Msmmsrf+1/2msmsrf$). Sixty eight fertile plants and 68 sterile plants were randomly selected from the BC₁ generation for AFLP analysis. DNA from each individual, along with both parents, was extracted using a modified SDS method (Li et al, 1994). For

bulked segregant analysis, equivalent amounts of DNA from ten fertile plants and ten sterile plants were pooled to create fertile bulk (BF) and sterile bulk (BS), respectively. Both bulks were then subjected to AFLP screening.

AFLP analysis was performed following the procedure described by Vos et al. (1995) with minor modifications. The AFLP markers were prefixed with the primer combination followed by the molecular weight of specific fragment in a lower case.

Co-segregation analysis was performed with MAPMAKER/EXP V3.0. The genetic linkage map was generated using a minimum LOD of 3.0 and a maximum recombination fraction of 0.3. Map distances were converted to cM using the Kosambi function.

RESULTS

A total of 480 AFLP primer combinations (256 for E+3/M+3 and 224 for P+3/M+3) were screened on BS and BF, leading to the identification of five putative markers associated with male sterility. These primer pairs were then tested on the parents, bulks and twenty individuals contributed to the bulks. The result showed that primer combination E3M15, E7M1, P6M6, P10M13 and P13M8 generated a specific band only present in Rs1046A, BS and most of the sterile plants but absent in Samourai, BF and most of the fertile plants, thus could be signed as candidate markers linked to the *Ms* gene (table1). Then, these markers were tested on a larger sample of 136 plants. The linkage analysis revealed that all candidate markers were tightly linked to the *Ms* gene in a coupling phase, with a recombinant frequencies from minimum of 3.7% to maximum of 8.8% (table1), and marker P10M13₃₅₀, P13M8₄₀₀, E7M1₂₃₅ and E3M15₉₉ located at one side of *Ms* with a map distance of 8.9cM, 5.9cM, 5.2cM and 3.7cM respectively, whereas P6M6₄₁₀ resided 5.9cM away from *Ms* at the opposite side (calculated from two point analysis) (Fig.1). The markers identified in this study provided a very useful tool for molecular tagging of the *Ms* gene in breeding selection.

DISCUSSION

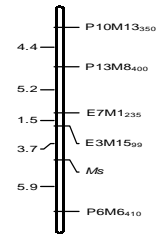
This is the first report on molecular tagging of the dominant genic male-sterile gene (*Ms*) in *Brassica napus* L. The discovery and application of the dominant genic male sterility conditioned by epistatic interaction of genes at two loci will facilitate the application of three-line system in hybrid seeds production. These five markers, especially E3M15₉₉ and P6M6₄₁₀, residing the closest to the *MS* gene at opposite side, should prove useful for the development of new GMS three lines and permit an assessment of the amount of flanking DNA that accompanies the *Ms* gene during the introgression procedures. These are also important landmarks to isolate the genic male-sterile gene in a map-base cloning strategy. Mapping and cloning of the *Ms* gene in *Brassica napus* L. will promote the study on the molecular mechanism of the dominant male sterility.

Table 1 The AFLP fragments linked to the *Ms* gene and specific for the bulks

Primer combination	Approximate size of markers (bp)	AFLP marker designation	Recombinant value between marker and <i>Ms</i> locus (%)	Specific for
E3M15	99	E3M15 ₉₉	3.7	BS
E7M1	235	E7M1 ₂₃₅	5.2	BS
P6M6	410	P6M6 ₄₁₀	5.9	BS
P10M13	350	P10M13 ₃₅₀	8.8	BS
P13M8	400	P13M8 ₄₀₀	5.9	BS

Fig.1 A linkage map of AFLP markers surrounding the dominant genic male-sterile gene (*Ms*) in *Brassica napus* L. AFLP markers (right) are designated by the primer combination followed by molecular weight of specific fragment in lower case. Dominant genic male-sterile gene (*Ms*) is in italics.

Dist.
cM Markers



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