

分子标记辅助选育甘蓝型黄籽波里马恢复系

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摘要: 将分子标记辅助选择(MAS)和一个略作修改的轮回选择育种计划结合起来, 目的将来自一个春性、稳定和纯黄 DH 品系 No. 2127-17 的黄籽基因转育到一个半冬性的波里马恢复系恢 5148-2 中, 以选育黄籽波里马恢复系。在本育种计划中, 首先应用 142 条 RAPD 引物扫描 12 个优良黄籽 DH 系, 获得 2 个与恢 5148-2 背景恢复率最高的 DH 系。此后应用显性 SCAR 标记 SCS1130 和共显性标记 SCA1 分别分析 BC_1F_1 和 BC_2F_1 分离群体, 选择黄籽的单株。为了更快的将所选单株的遗传背景恢复到恢 5148-2 在 BC_1F_1 和 BC_2F_1 分离群体中分别应用 88 条 RAPD 和 60 对 AFLP 引物进行遗传背景分析, 为了节约成本和简化分析, 应用两步法进行此分析, 逐步缩小分析群体样本, 每次选择与恢 5148-2 遗传距离最小的 3 个单株作进一步的分析。最终, 经共显性标记 SCA1 分析, 从 9 个纯合黄籽单株中选择 5 株优良的恢复系作下一步分析, 背景分析表明它们与恢 5148-2 的遗传距离小, 为 0.015 7~0.036 4, 遗传背景得到较好的恢复。

关键词: 分子标记辅助选择(MAS); 甘蓝型油菜; 黄籽恢复系

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Molecular Marker-assisted Selection for Development of Yellow-seeded Pol CMS Restorer Lines in *Brassica napus* L.

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Abstract: Marker-assisted selection (MAS) was used in a modified recurrent backcross program to transfer the yellow-seeded gene derived from the stable, pure and spring-type yellow-seeded *Brassica napus* double haploid (DH) line No. 2127-17 to the semi-winter-type Polima cytoplasmic male sterility (CMS) restorer line Hui5148-2 for breeding Polima cms restorer lines. In this scheme, 142 random amplified polymorphic DNA (RAPD) primers were used to select the yellow-seeded DH lines with high recovery of Hui5148-2. Then, SCS1130, a dominant sequence-characterized amplified region (SCAR) marker, and SCA1, a co-dominant cleaved amplified polymorphic sequence (CAPS) marker, were screened in BC_1F_1 and BC_2F_1 segregating progeny to select individuals carrying the yellow seed gene. In order to recover the recurrent parent background and simplify the analysis, 88 RAPD primers and 60 amplified fragment length polymorphism (AFLP) primer pair assays were used to select lines with the least genetic distance (GD) to Hui5148-2 using a two-step selected approach in yellow-seeded BC_1F_1 and BC_2F_1 plants. As a result, only 5 elite restorer lines were selected with GD values of 0.015 7~0.036 4 to Hui5148-2 from 9 BC_2F_2 homozygous lines identified by SCA1 and background analysis.

Key words: Marker-assisted selection (MAS); *Brassica napus*; Yellow-seeded restorer lines

The genus *Brassica* includes important oilseed crop species and oilseed rape (*Brassica napus*) is the most economically important worldwide. The primary objectives in *B. napus* breeding are to improve the oil quality and yield, which is determined by the seed yield potential and

the oil content in the seeds. A common strategy used to enhance the yield potential is to develop hybrid varieties, and Polima cytoplasmic male sterility (CMS) is the most important hybrid system used in China. One approach to enhancing the oil content of the seed is to increase the oil

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content in the embryos or to reduce the hull proportion of the seeds. Compared with black oilseed rape seeds, yellow seeds have a significantly thinner seed coat, i. e. a lower hull proportion, and consequently a potentially greater oil content. The yellow seeds have some other advantages, including a clearer oil, a higher protein content and a lower fiber content of the meal^[1]. Thus, yellow-seeded hybrid cultivars bred by the development of yellow-seeded Polima restorer lines can potentially improve the oil quality and increase the oil yield.

The most critical step for breeding such a line is to transfer the yellow seed gene into a desirable genetic background and to select progeny with a homozygous genotype. However, only limited success has been achieved, which is partly due to the lack of elite and stable yellow seed germplasm or the complex inheritance and the environmental effect on the yellow-seeded trait in *B. napus* L.^[2,3]. Recently, a yellow-seeded DH line No. 2127-17 that can produce stable and pure yellow seeds has been developed in our laboratory. The yellow-seed trait in this line has a maternal genotype, monogenic control and partial dominance^[3]. The use of genetic markers tightly associated with the yellow seed gene can trace it efficiently at any stage of growth in a segregating population, regardless of environmental conditions. In addition, molecular fingerprints can help the selection of individuals that are genetically closer to the recurrent parent for expediting the recovery of the recurrent parent in a backcross breeding program^[4-6].

The primary objective of this study was to transfer the yellow-seeded gene from No. 2127-17 to Hui5148-2 for developing stable, homozygous yellow-seeded Polima cms restorer lines using molecular marker-assisted selection (MAS).

1 Materials and methods

1.1 Plant Materials and DNA isolation

The recurrent parental line Hui5148-2 is an elite semi-winter-type black-seeded Polima cms restorer line of hybrid *B. napus*. Its hybrid with 1141A has been registered as Huaza No. 7 and is widely grown in China. The spring-type yellow-seeded donor parental line No. 2127-17 was derived from a resynthesized *B. napus* line derived from the interspecific cross between an oilseed *B. rapa* L and vegetable *B. alboglabra* (a subspecies of *B. oleracea*). It has greater oil and protein content compared

with the black-seeded restorers, but based on a broad testcross it has no restorer ability, along with some other undesirable agronomic traits (Table 1). The elite Polima cms line 1141A was used as the donor of sterility cytoplasm.

Total DNA was extracted from fresh leaves of seedlings in different generations and quantified as described by Liu^[7].

1.2 Marker-assisted selection scheme (MAS) and analysis of genetic distance (GD)

In a modified MAS backcross breeding scheme, the 142 most polymorphic RAPD decamer primers from 810 were used to select yellow-seeded F₁-derived (Hui5148-2 No. 2127-17) DH lines with a high recovery of Hui5148-2. The progeny of each backcross was inspected with the dominant SCAR marker SCS1130 and the co-dominant CAPS marker SCA1 to determine whether the yellow seed gene was present (Fig. 1).

The GD of the selected yellow-seeded restorer lines relative to Hui5148-2 was determined by analysis with 88 RAPD primers from the 142 used above and 60 AFLP primer pairs showing high polymorphism were selected from a set of 256 E+3/MC+2 and 256 E+3/MG+2 primer pairs as described by Liu^[2], using a two-step selected approach in BC₁F₁ and BC₂F₁, respectively. Firstly, we selected 30 RAPD or 20 AFLP markers to analyze the progenies and to eliminate about half the individuals, and then 58 RAPD or 40 AFLP markers to analyze the remaining plants. As a result, three individuals with least GD were selected to backcross with Hui5148-2 in BC₁F₁ and self-pollinate in BC₂F₁. Only strong and distinguishable DNA bands were considered for GD analysis. The presence of a band was recorded as 1, and the absence of the band was recorded as zero. Polymorphic data were used to estimate GD using the equation described by Nei and Li^[8]. This was followed by selection of homozygous plants in the later BC₂F₂ progeny using SCA1. Lastly, the percentage of the Hui5148-2 background in the selected lines was evaluated by 80 RAPD and 90 AFLP markers.

1.3 Seed color classification and analysis of seed quality

The seed color was analyzed using a method described by Liu^[2]. Quality analysis of seeds was done using near-infrared reflectance spectroscopy (NIRS) as described by Gan^[9].

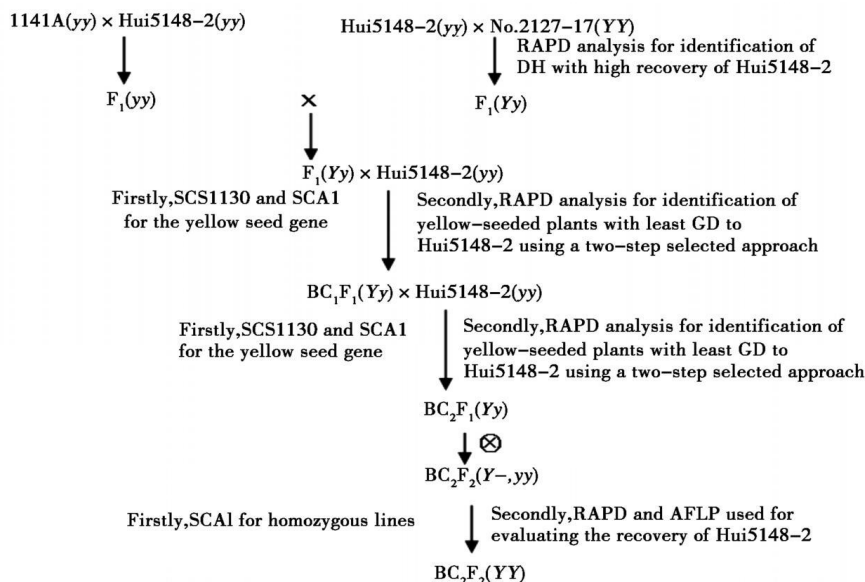


Fig. 1 The modified molecular marker-assisted backcross breeding scheme for Hui5148-2 with the yellow-seeded gene derived from No. 2127-17

2 Results

2.1 Background analysis in the DH population

Among 12 superior yellow-seeded DH lines, GD analysis using 314 polymorphic bands obtained by 142 RAPD primers (2.2 bands/primer) demonstrated that DH₁₄₆ and DH₂₁ were the accessions with the least GD values (0.432 9 and 0.502 2, respectively). As a result, the two tri-crosses were backcrossed with Hui5148-2 to obtain BC₁F₁ populations, which were designated as BC₁F₁₋₂₁ and BC₁F₁₋₁₄₆, respectively, and selected for further analysis.

2.2 MAS analysis in BC₁F₁

Based on the field performance, among the two BC₁F₁ families, one superior and most similar to the Hui5148-2 family (BC₁F₁₋₁₄₆) with 353 plants along with the parental lines was selected for MAS analysis. Genotyping these plants using SCS1130 or SCA1 separately confirmed that 169 plants possessed the yellow-seeded gene. However, when they were used in combination, 161 were proven to contain the yellow-seeded gene. From these 161 plants, 142 with vigorous growth were chosen for RAPD background analysis. Initially, 30 primers pre-screened all these 142 plants, and a total of 51 polymorphic bands (1.7 bands/primer) were detected. After GD analysis using the initial data, 46 plants were selected to create a sub-BC₁F₁ for further analysis. Subsequently, using the remaining 58 primers, 85 polymorphic bands were amplified with an average of 1.5 bands/primer and

the GD values among the 46 lines ranged from 0.246 9 to 0.381 5. Accordingly, three putative BC₂F₁ (BC₂F₁₋₂₅, BC₂F₁₋₈₅ and BC₂F₁₋₄₉) were generated.

2.3 MAS analysis in BC₂F₁

On the basis of the BC₂F₁ field performance, only the BC₂F₁₋₈₅ family composed of 223 individuals was further analyzed via SCS1130 and SCA1. As a result, 98 plants were identified to contain the yellow-seeded gene because of simultaneous detection of the two markers. Because AFLP techniques usually exhibited greater efficiency than other markers in advanced generations^[10], the background of the selected lines was conducted in the same manner. Out of the 98 plants, 94 were selected to pre-conduct with a total of 20 pairs. The results showed that 91 out of 1 800 intense and reproducible bands could be scored as polymorphisms with a mean of 5.5 per primer pair. As a result, 46 plants were identified to survey and with the remaining 40 pairs, 160 polymorphic bands (4.0 bands/pair) from a total of 3 400 bands were detected. GD analysis demonstrated that the value of minimum GD was 0.08. Finally, 3 BC₂F₂ (BC₂F₂₋₂₇₈, BC₂F₂₋₂₄₈ and BC₂F₂₋₂₄₃) progenies were produced.

2.4 Further analysis in BC₂F₂

For a successful backcross breeding program, it is necessary to identify the BC₂F₂ population genotype to obtain homozygous lines, since they segregated for the yellow seed gene. For this purpose, we used co-dominant SCA1, which can simplify this process without an additional generation of progeny testing to make this distinc-

tion. On the basis of the field appearance, BC₂F₂-248 containing 49 individuals was finally selected and subjected to SCA1 analysis; 9 plants were homozygous dominant (YY), 23 were heterozygous (Yy), and 17 were homozygous recessive (yy) in a segregation of 1 ♀ : 1 with $\chi^2=2.80$.

Finally, only 5 dominant yellow-seeded elite lines based on the field appearance with higher oil and protein content were further selected (Fig. 2, Tab. 1). Background analysis showed that GD values to Hui5148-2 ranged from 0.015 7 to 0.036 4, implying that the recovery of the Hui5148-2 alleles was very high.

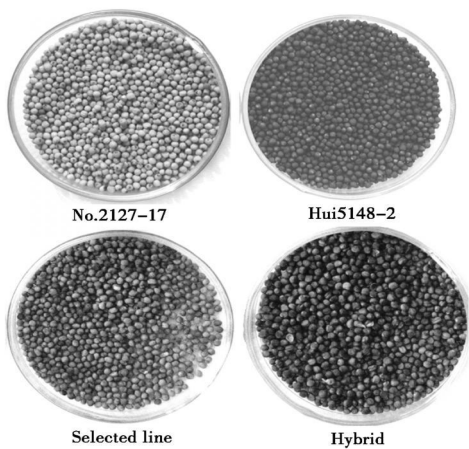


Fig. 2 The seed color of two parents
one selected line and commodity seeds of the hybrid

Tab. 1 Quality analysis of the parents and 5 selected lines

Materials	Oil / %	Protein / %	Erucic acid / %	Oleic acid / %	Glucosinolate / (mol/g)
No. 2127- 17	47. 10	19. 7	35. 8	29. 3	140. 5
Hui5148- 2	42. 50	18. 3	0. 5	64. 5	32. 1
5 selected lines	45. 54- 46. 79	18. 84- 19. 44	0. 58- 1. 45	62. 64- 67. 88	55. 15- 90. 54

3 Discussion

In the present study, high selection efficiency and low environmental limitation were obtained for the yellow-seeded gene derived from No.2127-17 using markers SCS1130 and SCA1 together. Simultaneously, the stepwise RAPD or AFLP assays rapidly determine the relative GD to Hui5148-2 and reduce the cost of MAS through progressive reduction in the number of individuals subjected to final marker analysis.

Moreover, the selected restorer lines have particular advantages in combination with hybrid breeding. If they were crossed with a black-seeded male sterile line, breeders would be able to produce certified black hybrid seeds for planting that will yield yellow commodity seeds for the crushing industry because of the maternal genotype and partial dominance of the yellow-seeded trait.

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