# QTL mapping of seed coat color of yellow-seeded *Brassica napus*

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**Abstract**

The development of yellow-seeded varieties of *Brassica napus* for improving the oilseed quality characteristics of lower fibers content and higher protein and oil content has been a major focus of breeding researches worldwide in recent years. With the black-seeded 'Youyan 2' as male and the yellow-seeded GH06 as female parents respectively, $F_2$ population of 132 individuals was obtained. A linkage map was constructed with 164 markers including 125 AFLP, 37 SSR, 1 RAPD and 1 SCAR markers distributed over 19 linkage groups covering approximately 2549.8 cM with an average spacing of 15.55 cM. Two loci located on the 5th and 19th group were detected for the trait of seed coat color based on the linkage group using multiple interval mapping method and explained 46% and 30.9% of the phenotypic variation, respectively.

**Key words:** *Brassica napus*, seed coat color, SSR, AFLP, linkage map, QTL

## Introduction

As one of the most important oil crops in the world, rape (*Brassica napus*) contributes to appropriately 50% of the vegetable oil consumption in China. At present, the most important task for rape breeders is to breed new rape varieties with high oilseed output and good quality. The seed coat color of yellow-seeded *B. napus* is of particular interest for oilseed rape breeding, not only due to its increased content of oil and protein in yellow seeds compared with black seeds, but also because of the reduced crude fiber content in the meal (Ochodzki P et al., 2003). For the purpose of increasing oil content, the improvement of yellow-seeded *B. napus* has been conducted for many years in China(Liu H L et al., 1991), and the first yellow-seeded *B. napus* cultivar 'Hua-yellow No. 1' was registered in 1990.

In recent years, there are many researchers focusing on the yellow-seeded *B. napus* breeding (Rahman M H, 2001; Meng J et al., 1998; Tang Z L et al., 1997). Shirazdegan et al. (1986) reported that the seed coat color of *B. napus* was determined by three homozygous recessive genes at three loci, but other researches showed different results (LI Jia-Na et al., 1998). Yellow-seeded materials from different genetic backgrounds have different locus controlling yellow seed coat color. Furthermore, other researches also revealed that the seed coat color of *B. napus* was affected by temperature (Van Deynze et al., 1993).

By now, a number of agronomic traits in *Brassica* species have been mapped using molecular markers. These include boron efficiency (Xu F S et al., 2001), Sclerotinia sclerotiorum resistance (Zhao J W et al., 2003), blackleg resistance (Pilet M L et al., 1998), erucic content (LIU Xue-Ping et al., 2004) and club root resistance(Manzanares-Dauleux M J et al., 2000). The mapping of seed coat color in *Brassica* had been extensively studied because it is an important character for *Brassica* quality breeding. In *B. napus*, a chalcone synthase gene was identified tightly linked to seed coat color (Van Deynze et al., 1995). In another study, a RAPD marker co-segregated with yellow seed color in B. napus was discovered (Somers D J et al., 2001). In *Brassica juncea*, an AFLP marker linked to seed coat color was identified and converted to SCAR marker for rapid selection (Negi M S et al., 2000).

The objectives of this study are to construct a linkage map of B. napus mainly using AFLP and SSR markers with yellow-seeded GH06 and black-seeded 'Youyan2' as parents and to analyze seed coat color QTL of yellow-seeded B. napus based on the linkage map.

## Materials and Methods

*Brassica napus* 'GH06', with light-yellow seed coat color, bred by Rapeseed Centre in Southwest Agricultural University, was used as female parent in cross and black-brown seeded 'Youyan2' as male parent. The black-brown hila of $F_1$ was bigger than yellow-seeded parent GH06 and some seeds looked like the black-brown seeds when their hila turn upward. One $F_1$ with light-yellow seed was self-pollinated to produce the $F_2$ population, about one-fourth of the individual was black-brown, and three-fourth was yellow which represent a continual distribution from brown-yellow to light-yellow. One hundred and thirty two $F_2$ individuals were randomly selected and self-pollinated to generate $F_2$:3 families. One-fourth of the family was black-brown, and 11 families was light-yellow seed coat color, the seed coat color of the rest showed segregation. The seed coat color was measured with dissecting-microscope under the same illumination and calculated using the formula: 10×(1-EX/EYouyan2), in which EX referred to Individual exposure time and EYouyan2 referred to parent 'Youyan2' exposure time respectively (YIN Jia-Ming et al., 2004).

Total DNA for AFLP, SSR, RAPD and SCAR analysis was extracted from young leaves of individual $F_2$ plants and their parents, using the SDS method described by Li et al. (1994). The AFLP analysis was conducted as described by Vos et al. (1995), with minor modifications by our laboratory. Total DNA (100ng) was double-digested with restriction enzymes EcoRI

and MseI. The PCR reaction was undertaken by pre-amplification and selective amplification with one and three anchor bases at the 3'end, respectively. Eight pairs of selective primers of combination were used in the selective amplification. The primers included: EcoRI end primers E1 (E+AAC), E2 (E+AAG), E3 (E+ACC), E4 (E+ACT), E5 (E+AGG), E6 (E+AGT), E7 (E+AGC) and E8 (E+ACA), as well as MseI end primers M1 (M+CAA), M2 (M+CAC), M3 (M+CAG), M4 (M+CTC), M5 (M+CTG), M6 (M+CTA), M7 (M+CAT) and M8 (+CGT). After denaturalization, the PCR products were subsequently separated in denaturing polyacrylamide gel and silver-stained following the method of Bassam et al.(Bassam B J et al., 1991).Primer sequences were downloaded from *Brassica* database (www.ukcrop.net) and synthesized by Shanghai Sangon Biological Engineering Service Co. Ltd. (China). PCR was carried out in PTC-100 thermocycler with a total volume of 20μL per reaction containing 50ng of genomic DNA, 50ng of primers, 0.2 mmol/L of each dNTPs, 1×PCR Buffer and 1.5 mmol/L of MgCl2 plus 1U of Taq DNA polymerase. The PCR reaction conditions were: 94°C for 1 min; 10 cycles of 94°C for 30s, 63°C with 0.5°C lowered after each cycle, for 1min, 72°Cfor 1min followed by 30 cycles of 94°C for 30s, 58°C for 1min, 72°C for 1min. The PCR products were detected using silver staining.RAPD and SCAR were performed according to the descriptions of Somers (Somers D J et al., 2001)and Negi (Negi M S., 2000) respectively.The linkage map was obtained using Map Manager QTX (Meer J M et al., 2002)(P=1e-6). QTL for seed coat color were resolved by multiple interval mapping (Kao C H et al., 1999) using WinQTLCart (Basten C J, et al., 2001)with a LOD threshold of 2.7.

## Results

A linkage map was constructed using 132 individuals of the $F_2$ population. The map consists of 164 markers including 125 AFLP, 37 SSR, 1 SCAR and 1 RAPD marker distributed over 19 linkage groups covering approximately 2549.8 cM with an average spacing of 15.55 cM between adjacent markers (Fig.1). Of the 164 markers, 17 markers of segregation distortion were detected ($P<0.05$), but they did not skewed to one parent collectively. In addition, the number of bands and polymorphic alleles scored with different AFLP primer combinations were different. AFLP markers were considered as allelic markers when they appeared steadily in the two parents for three times in the amplification with the same primer combination. In SSR analysis, we employed *B. napus*, *B. rapa* and some *Arabidopsis* SSR primers, and found great differences in efficiency of polymorphism among them. The polymorphic frequency for the three types of SSR primers was 58%, 43% and 10%, respectively. Finally, 37 SSR markers including 20 *B. napus*, 12 *B. rapa* and 5 *Arabidopsis* SSR markers were detected. Furthermore, a RAPD (rapd1) and SCAR (scar1) marker from Somers (Somers D J et al.,2001) and Negi(Negi M S et al., 2000) were located in the 17th and 2nd linkage group in the analysis of map construction.

Seed coat color of $F_{2:3}$ families were analyzed with dissecting-microscope based on the exposure time. Despite the continuous variation in the exposure time of different lines in the $F_{2:3}$ families, two peaks and one valley at 4.424 appeared in the frequency distribution (Fig.3). Ninety two lines with relative values of seed coat color higher than 4.424 were classified as a group of yellow seed coat phenotype and 29 lines with lower values than 4.424 were grouped into black seed coat phenotype. Furthermore, the ratio of yellow seed coat lines to black seed coat lines in the $F_{2:3}$ families was 92: 29 (11 data missing), fitting well to the expected Mendelian 3:1 ratio ($x^2=0.0248$, $x^2 \leqslant x^2_{0.01,1}$), which provided strong evidence for a major quantitative trait locus controlling the yellow seed coat color phenotype.

For seed coat color, we detected 2 QTL using multiple interval mapping method with LOD score of 2.7. They explained 46% and 30.9% of the phenotypic variation, and distributed within the 5th and 19th linkage group, respectively (Table 1). One major quantitative trait locus, Sc1, located in the interval e7m4.150-Na12-E02.550 on LG5 and explained 46% of phenotypic variation. The positive additive effects of Sc1 and Sc2 represent the alleles from the yellow-seeded parent $GH_{06}$ and have positive effects on the seed coat color.

## Discussion

$F_1$ plants were yellow, being quite similar to their female parent $GH_{06}$, but significantly different from their male parent Youyan 2, which demonstrated that the seed coat color was controlled by dominant yellow seeded genes. The separate rate between the black and yellow seed in $F_2$ individuals and $F_{2:3}$ families displayed that the seed coat color was controlled by one major locus in this *B. napus* population. Furthermore, the seed coat color may be affected by the epistatic and environmental interaction effects. The yellow-seeded *B. napus* parent $GH_{06}$ in the present study was developed by multi-crossing (black-seeded *B. napus* × yellow-seeded *B. juncea)* × yellow-seeded *B. napus* followed by self-pollinating for 10 generations. $GH_{06}$ is greatly different from other yellow-seeded *B. napus* in which the seed color is a dominant character and inherits stably, while the seed coat color of the yellow-seeded *B. napus* reported before was controlled by 3-4 recessive loci (Van Deynze et al., 1995). The most possible reason is that the yellow-seeded *B. napus* from different sources are controlled by distinct yellow-seeded loci (LI Jia-Na et al., 1998). To date, a novel yellow-seeded cultivar 'Yu-yellow No.1' originated from parent $GH_{06}$ has been widely cultivated in Yangtze River area and Southwest of China.

The seed coat color measurement method of *Brassica* differed greatly in different researches. Many researchers identified the seed coat color with eye observation (Rahman M H et al., 2001), which will bring about subjectivity usually. But Technicon near-infrared reflectance (NIR) spectrophotometer (Van Deynze et al., 1993) and colorimeter (Somers D J et al., 2001) were also used to determine the *Brassica* seed coat color. In our research, the dissecting-microscope was used to scale the seed coat color based on the different exposure time, which would reduce the subjectivity and simplify the evaluation.

Developing molecular markers associated with the seed coat color QTL in these cultivars may accelerate breeding progress for seed coat color improvement in *B. napus*. Field screening of breeding materials is difficult because of the effects

of temperature and other environmental factors. Based on the linkage map, markers tightly linked to major locus of seed coat color can be converted into SCAR markers and used for selection in our yellow-seeded B. napus breeding programs. DNA marker technologies such as RFLP and SSR have been widely used in genotyping and molecular mapping in B. napus (Saal B et al., 2001). However, low polymorphism, low throughput, and high cost of RFLP make it difficult to use them for MAS (Marker Assistant Selection) in B. napus. In contrast to RFLP, SSR is simple and able to detect a high level of polymorphism in B. napus. Once identified, some SSR markers are well adapted to large-scale, locus-specific application. Unfortunately, the number of SSR primers is still limited in *Brassica*, and the development of SSR primers requires fairly sophisticated training and resources. So far, genetic mapping of microsatellites in *Brassica* species has been scarcely reported (Uzunova M I et al., 1999). At the same time, AFLP markers are multiplex and highly polymorphic in *Brassica*. In our research, we could get five polymorphic loci averagely for each AFLP primer combination which could appear steadily thrice between the two parents. It is a rich marker source for molecular mapping and QTL tagging of important *Brassica* traits.

Two QTL were detected in our study, and explained 30.9% and 46% of the phenotypic variation, respectively. The unexplained part of the genetic variation for seed coat color may result from the incomplete coverage of the linkage map and from the somewhat limited size of our sample pool, which impeded us to detect very low effect QTL or the digenic interactions. Besides RAPD and SCAR markers, no QTL related to seed coat color was detected to date. The most possible reasons are the different origin and genome composition of different yellow-seeded materials. In addition, a good coverage of the genome is a prerequisite to identify markers linked to genes controlling important agronomic characters.

## Conclusions

The seed coat color was controlled by a major dominant locus and can be used in molecular assisted selection for yellow seeded B. napus breeding program.


Fig. 2 The seed coat color of the two parents and $F_1$

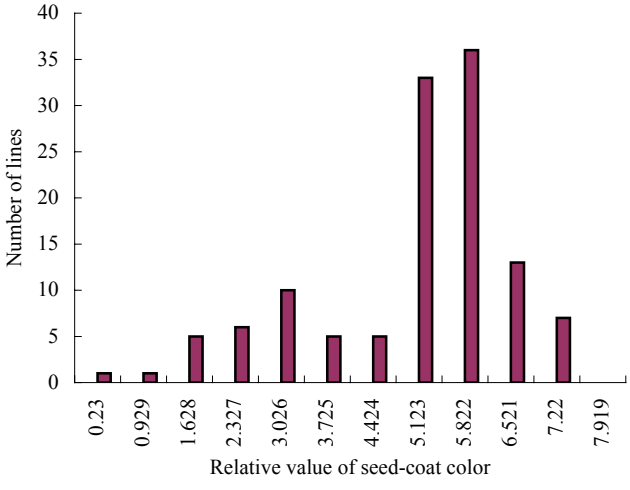


Fig. 3 The frequency distribution of seed coat color determined by exposure time

**Table 1 Seed coat color QTL detected by multiple interval mapping using software WinQTLCart**

| QTL | LG | Interval | LOD | A | D | Contribution | Distance |
|---|---|---|---|---|---|---|---|
| Sc1 | 5 | e7m4.150-Na12-E02.550 | 3.5 | 3.72 | 0.04 | 46% | 9cM |
| Sc2 | 19 | Na14-D07.500- Na14-D07.550 | 2.7 | 0.17 | -3.58 | 30.9% | 2cM |

LG: linkage group; LOD: Log likelihood ratio; A: additive; D: dominance; Distance: QTL peak to it's corresponding right marker.

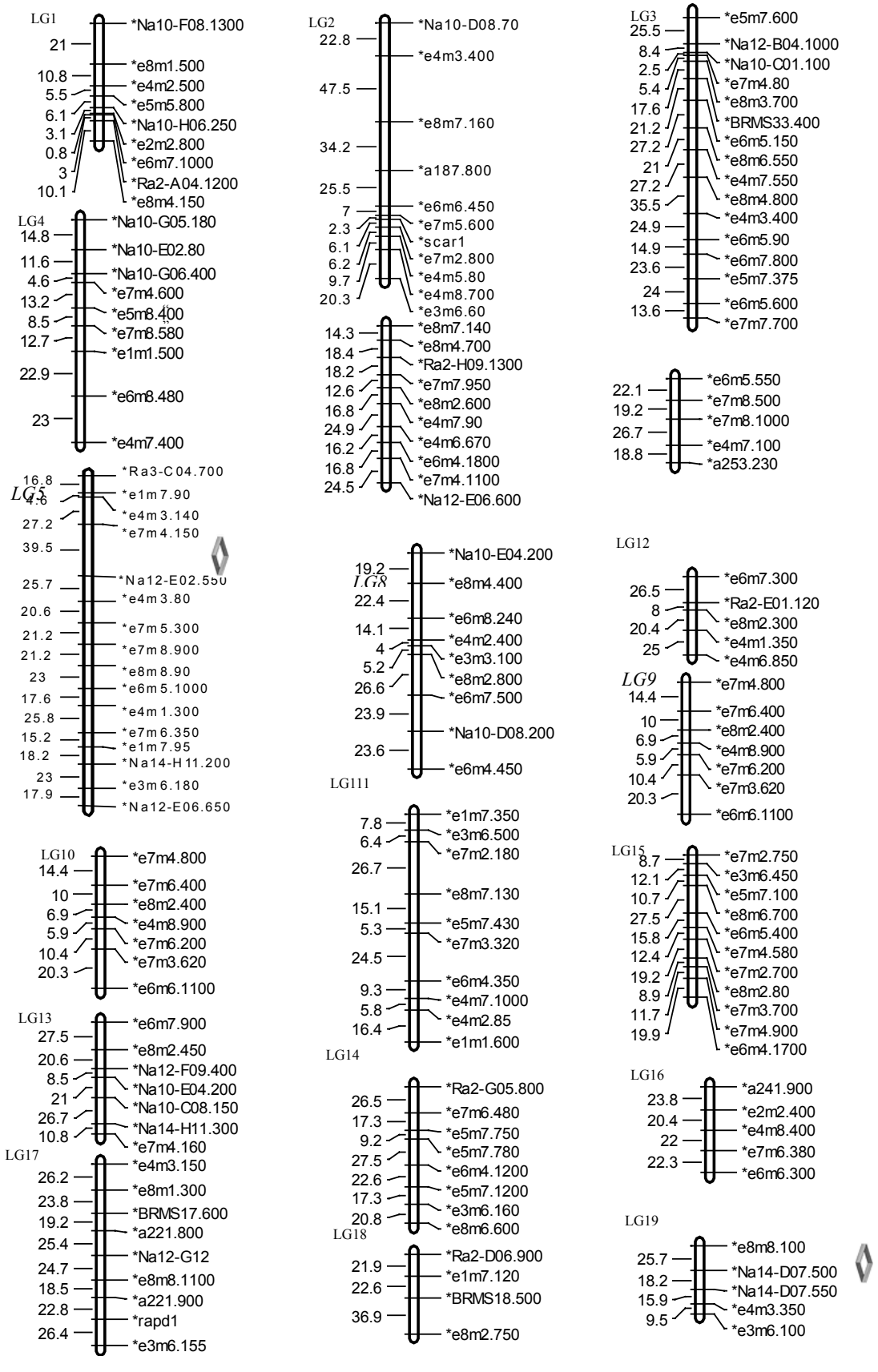


Fig. 1 Linkage map of *B. napus* and the locations of quantitative traits loci (QTL) for yellow- seeded trait in *B. napus*. The symbol indicates seed coat color QTL. Nomenclature of the AFLP markers is based on their primer combinations. E.g., 'e8m1' indicates the combination of AFLP primer E8 ($E^{+ACA}$) with M1 ($M^{+CAA}$). 'Ra' and 'BMRS' represent *B. rapa* SSR markers from www. ukcrop. net and K. Suwabe (2002) respectively, while 'Na' represents *B. napus* SSR markers from www.ukcrop.net. 'a' indicates *Arabidopsis* SSR makers.

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