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Full Length Research Paper

Simple sequence repeat (SSR) markers analysis of genetic diversity among *Brassica napus* inbred lines based on correlation between seed quality traits and seed pigments content

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Information regarding diversity and relationships among different quality traits and seed coat characterization of breeding material is necessary for hybrid *Brassica napus* breeding and seed coat color selecting. Simple-sequence repeat (SSR) analysis of the 62 loci distributed uniformly throughout the *Brassica napus* genome was carried out for 90 inbred lines which were derived from the black-seeded coat and the yellow-seeded coat parents in order to assess genetic diversity among the inbred lines and correlation to different traits with seed coat and seed pigment. The average-linkage (UPGMA) cluster analysis yellow-seeded coat lines and black-seeded coat lines were divided in two major groups; SSR clustering analysis results is linkage with the traits clustering analysis results. The correlation studies showed that seed coat color, anthocyanidin content, total phenol content, melanin content and flavonoid content in seed coat had significant negative correlation with oil content in different environments. The anthocyanidin content, flavonoid content, total phenol content and melanin content had significant positive correlation in seed coat. The results indicate that SSR analysis is effective for the assessment of genetic diversity among *Brassica napus* inbred lines and seed coat color is one of the most important traits to breeding of *B. napus* quality improvement.

Keywords: *Brassica napus* L, quality traits, agronomic traits, correlation analysis.

INTRODUCTION

Brassica napus L. is the predominant rapeseed species grown worldwide. Improvements in the yield as well as in the quality of oil and meal of rapeseed are two important objectives for rapeseed breeders (Liu, 1985). The successful development of *B. napus L.* cultivars with low erucic acid in the oil and low glucosinolate content in the meal has made rapeseed a valuable source of high quality oil for people and nutritional protein for live-stock (Qiu et al., 2006). Previous studies have demonstrated that yellow seeds have a thinner seed coat than black seeds in the same genetic background. In the other hand, thinner seed coat has been associated with higher oil content in the seed and higher protein and lower fiber contents in the meal (Xiao and Liu, 1982; Shirzadegan and Robbelen, 1985; Rashid and Rakow, 1995). Other

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Abbreviations: SSR, Simple-sequence repeat; **UPGMA,** unweighted pair group method with arithmetic mean; **AFLP,** amplified fragment length polymorphism; **RAPD,** random amplification polymorphic DNA.

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research has demonstrated that the toxic substances were concentrated in the seed coat during the maturation (Yuan et al., 2003). Therefore, the quality of rapeseed oil may be greatly affected by seed coat pigments, most of which are fat-soluble and remain in the rapeseed oil. As genetic linkage maps in *B. napus L.* have become available, some major quantitative trait locus (QTL) of seed coat color and linked markers were found in recent years. Xiao et al. (2007) identified four amplified fragment length polymorphism (AFLP) markers linked to seed coat color in *B. napus L*. Somers et al. (2001) reported that in *B. napus L.* a single major gene (*pigment 1*) and 2 minor genes controlled seed color and that these genes were flanked by 8, 1, and 2 random amplification polymorphic DNA (RAPD) markers, respectively. Liu et al. (2005) found that AFLP and RAPD markers were linked to the Y gene in a DH population with the yellow-seeded parent line, No.2127-17. Badani et al. (2006) and Fu et al. (2007) reported 3 and 19 QTLs for seed colour in different populations.

In comparison with other molecular marker techniques, simple-sequence repeat (SSR) markers are numerous, highly polymorphic and informative, codominant, technically simple, reproducible, and relatively inexpensive when primer information is available. Furthermore, SSR markers often occur in gene-rich genome regions, increasing their potential relevance for allele-trait association studies in well-characterized genome regions containing quantitative trait loci. SSR markers have been widely used in diversity studies in maize, rice and tomatoes (Reif et al., 2006; Vigouroux et al., 2005; Warburton et al., 2005; Olsen et al., 2006; Caicedo et al., 2007; Bredemeijer et al., 2002). It has been proved that SSR markers are useful for genetic diversity and structure studies of *Brassica*. Fu and Gugel (2010) studied the genetic diversity of 300 plants by employing 22 SSR primer pairs from eight linkage groups, detecting 88 polymorphic loci. The genetic diversity in Austalian canola cultivars were analyzed by using 18 SSR primer pairs and finding 112 polymorphic loci (Wang et al., 2009). By using 15 SSR markers with known locations on the *Brassica* A, B, and C genomes, Pradhan et al. (2011) assessed genetic diversity of 180 *Brassica nigra* (L.) Koch genotypes from 60 different accessions. Soengas et al. (2011) establish the genetic relationship among eight populations and studied the genetic structure by analyzing the polymorphic alleles of 18 SSR markers.

The objectives of this study were to use a set of SSR markers to detect DNA polymorphism and analyzed the relationship of quality traits among *B. napus* RILs familly. It will provide useful information for *Brassica* breeding program and improving the seed meal in the future.

MATERIALS AND METHODS

Plant materials

A total of 90 inbred lines were analyzed in this study. They were derived from a cross between black-seeded male parent cultivar Zhongyou 821 and yellow-seeded female parent line GH06, including 45 yellow-seeded lines and 45 black-seeded lines. In 2006 and 2007, they were simultaneously grown at Beibei and Wanzhou in Chongqing. Although Beibei and Wanzhou are at almost the same latitude, the altitude of Wanzhou is about 700 m higher than that of Beibei. Each plot contained 3 rows with 15 plants per row. Field management essentially followed the normal agronomic procedures in the region. Seeds were harvested from self-pollinated plants for analysis.

Measurements for the seed coat color, and content of oil and protein in the seeds

Self seeds of all RILs were used to estimate the seed color (SC) and yellow seeded degree (YSD) following the methods described by Fu et al. (2007). The seed oil content and protein content in the seeds of RILs were measured by near infra-red reflectance spectroscopy (NIRS 5000; Denmark), in small circular cups in three parallel replications using the software WinISI II, vers. 1.50 at wave length range of 1100 to 2498 nm. All the spectra were computed at 8 cm^{-1} resolution between 4000 and 11 000 cm^{-1} with the scanning of 64 and expressed as percentage of oil or protein on total seed weight (%).

Measurement for the seed coat pigments

0.2 g seed coat was laid into a 10 ml polypropylene centrifuge tube, and then 5 ml methanolic HCl (95% methanol, 5% concentrated HCl) was added followed by an hour incubation at 80°C (supplying the solvent to maintain the stable volume). After centrifugation at 8228 G for 6 min, the supernatant was decanted and another 5 ml of methanolic HCl were added to the residue. Absorbance at 600, 530, 325 and 280 nm were recorded in the total supernatant. The anthocyanidin content was calculated as $\triangle$530 - 600 nm = 0.1 and the flavonoid content was calculated as A325 nm g^{-1} (Pirie and Mullins, 1976). The total phenol content was calculated by gallic acid to make a standard curve (concentration range of 10 to 100 µg) (Yan et al., 1998).

Two milliter (2 ml) 2% NaOH were added to the residue as explained above, and incubated at 80°C. The supernatant was decanted and another 5 ml of 2% NaOH was added to the residue. Total supernatant was measured at absorbance of 290 nm, and the melanin content was calculated by A290 nm g^{-1} (Zhou et al., 1997). Though the seed coat pigments were extracted, the residue of seed coat after drying should be white.

DNA extraction and SSR assays

Leaves from 3 to 5 seedlings for each accession were pooled together for DNA isolation. Genomic DNA was extracted according to the protocol of Doyle and Doyle (1990). The concentration and purity of each DNA sample was measured using a GeneSpecI spectrophotometer at wave-lengths of 260 and 280 nm, quantified by visual comparison to λ DNA standards on ethidium bromide-stained agarose gels.

We chose 100 SSR primers from the available literatures (Lowe et al., 2003; Piquemal et al., 2005; Rahman et al., 2007; Choi et al., 2007; Long et al., 2008; Cheng et al., 2009), and assayed their preliminary discriminatory power using the two parents. Sixty-two SSR markers were selected to analyze the genetic diversity according to their polymorphism (Table 2). All primers were synthesized by Shanghai Sangon Biological Engineering Service Co. Ltd. (China) and listed in Table 2. PCR reactions were

performed in 96-well plates with a volume of 10 µL. The composition of the mixture include the following: 20 ng/µl of DNA template, 0.5 pmol of each primer, 0.2 mM dNTP mix, 1 mM $MgCl_2$, 10×PCR reaction buffer (with 15 mM $MgCl_2$, TransGen Biotech) and 0.5 unit of *Taq* DNA polymerase (TransGen Biotech). PCR was carried out in PTC-100 and PTC-200 thermo cycler with the following program: 94°C for 4 min; 35 cycles with 94°C denaturation, 55°C annealing, 72°C elongation, which slightly modified those reported by Piquemal et al. (2005). All PCR products were detected using non-denaturing polyacrylamide gel electrophoresis (10% polyacrylamide) and silver staining (Zhang et al., 2002).

Data analysis

The PCR product by SSR primer pairs were scored based on their fragment size with comparing the fragment sizes reported in the literature (Piquemal et al., 2005; Long et al., 2008; Cheng et al., 2009; Choi et al., 2007; Lowe et al., 2003). Subsequently they were converted to a binary format matrix through coding as either present (1) or absent (0).

We estimated genetic diversity (*D*) for each SSR locus using the formulas: $Di = n\ (1-\Sigma P_{ij}^2)/n-1$, where *n* is the number of accessions analyzed, and *Pij* the frequency of the *j*th allele for the *i*th locus across all alleles at loci. Average marker diversity (*D*) was estimated as $D = \Sigma D_i/r$, where *r* was the number of loci analyzed. To see the relationship between the different RILs, we estimated the genetic similarity according to Jaccard coefficients from the alleles across all the loci in the 90 accessions using the formula: $J = N_{ij}/(N-N00)$, where N_{ij} was the number of shared alleles in both accessions *i* and *j*, *N* was the number of all alleles across all accessions investigated and the *N*00 was the number of alleles present neither in line *i* nor in line *j*. To see the relationship between lines, a dendrogram based on similarity coefficients was constructed with the unweighted pair-group method using the arithmetic averages (UPGMA; Sneath and Sokal, 1973). The estimation of genetic diversity and the cluster analysis was performed using NTSYS-pc software package Version 2.2 (Rohlf, 2005).

The mean of seed quality traits and seed coat pigments were calculated by the Microsoft office EXCEL. The Pearson correlation coefficient (*r*) and propability-value (*p*) were used to show correlation and their significance by using the software SPSS 13.0 in the present experiment. A probability value of $p < 0.05$ was considered statistically significant.

RESULTS

Quality traits analysis

The quality traits and seed coat pigments had significant differences between yellow-seeded female parent line GH06 and black-seeded male parent cultivar Zhongyou 821 (Table 1). Moreover, yellow seeds had significantly higher oil content but lower seed coat pigments than black seeds in different environments. The difference between the two parents was significant at a level of $P < 0.01$. The ninety *B. napus L.* RILs were classified into eight groups according to the k-means cluster analysis based on seed quality characters. There were significant differences among different groups ($P< 0.01$). The characteristics of each group were as follows; group 1: yellow seed with high oil and protein content, group 2: yellow seed with low oil and high protein content, group 3: yellow seed with high oil and low protein content, group 4: yellow seed with low oil and protein content, group 5 to 8 had black seeds with the same quality traits as group 1 to 4, respectively.

Correlation of quality traits in Beibei and Wanzhou in 2006 and 2007

The correlations of quality traits were analyzed in four different environments (Tables 3 and 4). The results show that the protein content and seed coat pigments had significant negative correlation with oil content, except the AC of seed coat pigments in 2006/Beibei (Tables 3 and 4). Moreover, the seed coat pigments were also significantly negatively correlated with seed coat colour in 2006/Beibei, 2006/Wanzhou and 2007/Beibei, respectively (Tables 3 and 4). However, the oil content had significant positive correlation with seed coat color in 2006/Beibei, 2006/Wanzhou and 2007/Beibei [0.41($P < 0.01$), 0.37($P < 0.01$) and 0.27($P < 0.01$) respectively] (Tables 3 and 4). The protein content was significantly positively correlated with seed coat color [0.38($P < 0.01$)], and significantly negatively correlated with seed coat pigments in 2007/Beibei (Table 3). In the seed coat, there is a significant positive correlation among the seed coat pigments, such as anthocyanidin content, flavonoid content, total phenol content and melanin content, except the anthocyanidin content in 2006/Beibei (Tables 3 and 4).

Relationship among *Brassica napus* L inbred lines

Forty eight markers had been mapped on the linkage groups according to previous researches in *B. napus.* L. (Lowe et al., 2003; Piquemal et al., 2005; Rahman et al., 2007; Choi et al., 2007; Long et al., 2008; Cheng et al., 2009), and some primers were also unknown. Sixty two primers produced 249 alleles in the 90 lines, and the allele number for the SSR loci ranged from 2 to 7 with a mean value of 4.0 (Table 2).

Genetic similarities among accessions were estimated based on the Jaccard's similarity. A UPGMA dendrogram was constructed for the two parents and the 90 RILs (Figure 1) unveiling two main groups. The yellow-seeded lines and black-seeded lines could be distinguished and the accessions in a given group have closer relationships. Group A included 46 inbred lines which were 44 yellow-seeded lines and 2 black-seeded lines (Figure 1). This cluster was subdivided into three subclusters. The yellow-seeded female parent line GH06 was classified into subcluster I. There were only two black-seeded lines (G6E434B and G7E462B) in the subclusters II. The other included only yellow-seeded lines.

In group B, there were 46 inbred lines (Figure 1), including 2 yellow-seeded lines (G3E323Y and

Table 1. Phenotypic analysis of the seed oil content, seed protein content, seed coat color and seed coat pigments in two different environments for the RIL and parents of *B. napus*

Year	Trait		Locations (Beibei / Wanzhou)								
			Zhongyou 821	GH 06	F2:7 family populations *B. napus* lines						
			Mean	Mean	Mean	Max	Min	SD	CV	Skewness	Kurtosis
2006	Oil content		34.49/43.14	37.2**/44.2**	38.5/44.67	43.94/54.72	31.79/32.53	2.52/3.63	6.33/13.18	-0.16/-0.02	-0.27/0.16
	Protein content		28.57/22.07	28.48**/21.97**	26.52/22.03	30.59/27.58	21.60/16.86	1.60/2.56	2.56/6.55	-0.13/0.08	-0.02/-0.78
	Seed coat colour (YSD)		29.31/30.94	86.42**/87.84**	67.65/64.84	90.40/87.55	32.79/33.32	17.3/15.53	299.38/241.04	-0.49/-0.39	-1.23/-1.14
	Seed coat pigments	AC	2.40/1.92	1.45**/1.07**	1.41/1.42	3.46/2.80	1.01/1.00	0.49/0.42	0.24/0.18	1.24/0.95	0.92/-0.08
		FC	1.26/1.02	0.58**/0.47**	0.74/0.78	1.69/1.60	0.19/0.11	0.29/0.3	0.08/0.09	0.93/0.84	-0.07/-0.05
		TPC	19.52/15.84	8.22**/8.42**	11.04/12.16	24.69/26.02	3.76/1.72	4.02/4.09	16.12/16.76	0.98/0.91	0.07/0.94
		MC	6.52/4.41	1.1**/1.02**	2.84/2.63	8.09/7.03	0.75/0.52	2.04/1.64	4.14/2.68	0.87/0.81	-0.7/-0.28
2007	Oil content		35.49/42.14	38.5**/43.9**	37.41/42.68	44.01/47.46	31.37/35.34	2.47/2.51	0.06/0.06	0.02/-0.42	-0.26/-0.14
	Protein content		28.15/23.17	27.98**/22.88**	28.07/24.99	31.67/29.47	22.58/21.28	1.32/1.33	0.05/0.05	-0.8/-0.31	-2.56/1.59
	Seed coat colour (YSD)		83.83/—	144.81**/—	104.05/—	159.8/—	43.92/—	25.01/—	0.24/—	-0.21/—	-1.04/—
	Seed coat pigments	AC	26.05/18.64	3.47**/0.44**	7.94/7.74	32.89/34.48	0.79/0.27	8.49/8.65	1.07/1.12	1.23/1.11	0.44/0.4
		FC	60.63/48	34.43**/26.42**	40.13/36.34	72.24/65.68	20.92/18.83	13.22/12	0.33/0.33	0.65/0.45	-0.55/-0.85
		TPC	80.71/49.17	46.57**/30.44**	42.63/37.07	88.74/61.22	27.73/20.9	2.28/10.12	0.1/0.27	0.1/0.48	-0.44/-0.74
		MC	329.3/204.14	67.88**/81.36**	160.15/179.56	431.82/466.45	69.65/69.45	92.33/104.16	0.58/0.58	1.44/0.71	2.65/-0.79

AC, Anthocyanidin content; FC, flavoid content; TPC, total phenol content; MC, melanin content; YSD, yellow seeded degree. **Ssignificant at P<0.01.

G1E322Y) and 44 black-seeded lines. The black-seeded male cultivar Zhongyou 821 was classified into subcluster I. The yellow-seeded lines were classified into different subcluster.

DISCUSSION

In the past, great efforts have been made to better understand the seed coat color, quality traits and yield, and numerous genetic resources of *B. napus L.* were developed. There are many genetic variations in genetic origins, oil content, and protein content, seed coat color and seed pigment compositions and components (Zhang et al., 2003). In this study, SSR markers revealed polymorphism in a RILs family. The distribution for the yellow-seeded and black-seeded lines in three clusters agreed fairly well with their pedigrees. The results indicate that SSR markers are effective and useful for analyzing the genetic diversity of *B. napus* genetic resources. Many other authors have also reached similar conclusions on the use of SSR markers in the breeding of rapeseed (Ahmad et al., 2011; Ana et al., 2011; Cruz et al., 2007; Li et al., 2011; Hasan et al., 2006; Tommasini et al., 2003).

In recent years, developing yellow-seeded *B. napus L.* cultivars increased output and oil content remained an important objective of oilseed rape breeding. Several lines of yellow-seed *B. napus L.* were bred by breeders in many areas (Hu et al., 1988; Wang and Liu, 1991, 1996; Chen and Liu, 1994). Despite numerous successes in modifying the genetic mechanism of the quality traits of *B. napus L.*, enhancing the seed quality traits has proven to be more difficult. A previous study revealed that yellow seeds have thinner seed coats, higher oil contents, and higher protein and lower fibre contents in the meal than black seeds with the same genetic background (Xiao and Liu, 1982). It showed that the quality of rapeseed oil and oilseed residues are greatly affected by seed coat. Some experiments indicated that the inheritance of oil content was controlled by an

Table 2. SSR primers used for the genetic diversity analysis.

SSR-primer	Linkage group	Number of allele	Number of polymorphism	Sequence		Source
Au14	Unkown	3	1	Unkown		Long et al., 2008
Au39	Unkown	2	1	Unkown		Long et al., 2008
Au8	N13	6	2	Unkown		Long et al., 2008
BN35D	N1/N11	7	4	GCAGAAGGAGGAGAAGAGTTGG	TTGAGCCGTAAAGTTGTCACCT	Et al., 2008; Cheng et al., 2009
BnGMS351	N9	4	1	ATACTCGTTATGGCGAGAGA	TGGTCAAATTCTTGAACATT	Cheng et al., 2009
BnGMS417	N3	3	2	AATGGAACGACTCAACATAGT	GGATCGACTCAAAGTCACAT	Cheng et al., 2009
CB10196	N4	4	1	TTGTAGGCAATGATGAGGA	GAGAGAAGGGCTCCTTTG	Piquemal et al., 2005
CB10199	N4	2	1	CTCATCATATTCGGCGAC	GCTTGAGTTTCCATGGTG	Piquemal et al., 2005
CB10504	N18	3	2	GGTGTCCCAACTGTTGAA	CATTGGCATAGGAACAGG	Piquemal et al., 2005
CN1	N1/N11	2	1	TGGTTGGTGCAGACTTACGA	TTTCCCGAATCCTCAGATTG	Long et al., 2008
CN17	N13	6	2	CACCATCACCACCTTCACAA	TGGTTCACTCATGTCTCCGA	Long et al., 2008
CN35	N2/N12	6	2	CGACAGAGGGTTCAAATGGT	CGGTGTGTAGGTCTGCTCAA	Long et al., 2008
CN52	N5/N14	2	1	CCGGCTTGGTTCGATACTTA	TTGCGAATCTTTAAGGGACG	Long et al., 2008
CN53	N5	4	2	CACCGAACAAAACTGAGGGT	CGTTTCACTGCGTTCTACCA	Long et al., 2008
CN57	N6	5	4	CACACCCTTACCACGTTCCT	GCAACAAAGCATACTTCGCA	
CN62	N7	3	1	AGGAAGCCCAACAGGACTTT	AATTCGATTCTCCATCGTGC	Long et al., 2008
CN67	N7	4	2	CAGATTCGGATTTGGGAAGA	GGCGGAAGAATCAAAGGAGT	Long et al., 2008
CN71	N8	4	2	CAGATGAGACAACACAGGAAACA	ACTCAATACGTTTTTCGCGG	Long et al., 2008
CN78	N9/N18	6	5	AGTCGGGCTCGTATATCTCG	GTTTCGTGGCGGAAATTAGA	Long et al., 2008
CN79	N9/N18	5	3	TCAGTCACAAAAAGTCAACTCAAA	ACGGAGTAGGAGTTGGGAGG	Long et al., 2008
cnu_ssr063	N7	3	2	GAGAGAAGAAGAAGAAGGAAGCAGAA	GATCTCGCGTGTGGCAACT	Long et al., 2008
cnu_ssr076	N9	3	1	GCCTGCACTAAAATAGCTGCAAA	GAATGGAAGGCGTCGATCAT	Cheng et al 2009
cnu_ssr149	N6	7	2	GGAAGCCTCTGTGCGAAAAA	TGCCGACGATTTGATAGAGGA	Long et al., 2008
cnu_ssr167	N7	4	2	CGAGTTTGGACCCTCGATATG	AGCCCAAGGTTTACGGTGGT	Long et al., 2008
cnu_ssr223	N3	6	2	ACCCGAAAAGAGAATATGGCCT	ACAGTGGCGTTAGGTGGGG	Cheng et al., 2009
cnu_ssr235	N1	3	2	CAACCACATGAGATTGGTTTAGTT	GAAATGGTTTTGGAGCGGTA	Long et al., 2008
cnu_ssr257	N5	3	2	TGCATGATGTTCATGTCTTGTAAA	TCCTTCTGTAAACCGGTTGTAATTT	Long et al., 2008
cnu_ssr269	Unkown	4	2	GTCCATCTCCTACCTGCTCCA	GTTTTGAGCCGAATAATGGTTG	
cnu_ssr288	N3	6	3	GCGTTTCGTCCTCTTCTCAC	TTACCCACCTTGGCTTCATC	Long et al., 2008
cnu_ssr343	Unkown	3	1	TGGATGATTTCGTCGTCTGG	TGAAAGCCAAAACTAATAAAAGTCACA	
cnu_ssr361	Unkown	2	1	TTCTGCACATGAGAGCACAAGA	TCGATAAAAGAAACTCAAATGACTGC	
cnu_ssr370	N3	4	2	CAAATCGGGCATTGTTCCAT	CAATCAAGGAAAAATCTGTACCAATC	Long et al., 2008
cnu_ssr398	Unkown	4	1	TGACATTCGCATCAGATTTGT	TTGGGCTTCACGCATAAGAT	Long et al., 2008
cnu_ssr409	Unkown	2	1	TTCCGGTCACTTCTAGCTTCA	TTTTGGTGGTTAGTATGTCGCTAT	
EJU1	N9	4	2	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	Choi et al., 2007

Table 2. Contd.

EJU2	N9	2	1	TTCACATCTTCTTCATCTTCC	TTGCTATTCGTTCTCAGTCTC	Choi et al., 2007
EJU4	N8	5	3	CACCTTATCATCTCTCTATCCC	CCTCTGTTTCTCTCCTTGTG	Choi et al., 2007
ENA10	N5	3	2	ATCGTCTCCTCTCATCTCAA	ATTACATCCTCCACCTTCTTC	Choi et al., 2007
ENA14	N7	5	2	CTTACGGTGGAAATGCTG	TCGCTGGTGCTAAACTTG	Choi et al., 2007
ENA22	N9	4	3	TTTGTAGACGAACAGCCACG	AGAATCGCATTTGATGGAGG	Choi et al., 2007
ENA23	N2,N3	4	1	GCTGTGCCAGTTCCTCTTTC	TCATTCCAAATGGCCTTACC	Choi et al., 2007
ENA27	N9	3	1	AAAGGACAAAGAGGAAGGGC	TTGAAATCAAATGAGAGTGACG	Choi et al., 2007
ENA6	N7	2	2	CTCGTCTTCTTCACCTACAAC	CTGACATCTTTCTCACCCAC	Choi et al., 2007
ES-b09-1	N6	6	4			Long et al., 2008
GOL2	N5	4	1	AGACATCCCACATCGGCTAC	GACCCAAGACCCAAGACTCA	Choi et al., 2007
GOL3	N8	3	2	ACTCACTTTTGTTGGGCGTC	GGAGCCGCTTTCTCTACCTT	Choi et al., 2007
MR119	N5	5	4	GCTGAAACGCGTAGAGACTAA	GCTGGGAAATACGTTGAAA	Long et al., 2008
MR47MR32	SCAR	4	1	TGAACTGTGGAAGCCAAGC	TCACCACTACGCGGTAACTG	Rahman et al., 2007
niab_ssr106		2	1	GTCTCAAGCCAACATCCATC	AACGGAACCATAAGGAGACC	
niab_ssr003		3	2	TGTGTCGCTCGTCTACGTCT	ACCATCGACTTCGTGGAAAC	
niab_ssr013	N5	5	4	GGAACCGTCCTTACTTTCTCTGT	AGGATTGTGTTTTCCACATTGTC	Long et al., 2008
niab_ssr022	N19	8	5	CTCTCGTCTCGGAGGATCTAAA	GTGAGAGTGGTTGCTGAGTGAG	Long et al., 2008
niab_ssr037	N6	6	2	GCGGTTAATAGGTTCCGGTT	CCAATTGCATCGATCTGTCA	Long et al., 2008
niab_ssr046	Unkown	5	2	AACCATTGATCACAAAATTTTCAA	CCGTGGGCCTTTATCTTGTA	
niab_ssr082	Unkown	3	1	CATTTCCCCGTGACTATCTG	CGTCTTCATCTCAATCTCGC	
niab_ssr090	N8	4	2	GCTGATTTCTCCGCTATCAC	AAGACACCGTTTGTGAATTT	Long et al., 2008
niab_ssr091	N1,N11	3	1	TGGTTCTGCTATTGCTGTCA	GAAGTTTGTGAGCCAGGAAA	Cheng et al., 2009
niab_ssr097	Unkown	4	2	TTCTTTGGAGATGGTGTGGT	CAATCTTGTGGTGAGGGAAG	
niab_ssr120	Unkown	2	1	AAGAAAACTTATTTGATGGTACG	CTAAATCCAAACCAGAATTGA	
Ra2-A01	N7	4	2	TTCAAAGGATAAGGGCATCG	TCTTCTTCTTTTGTTGTCTTCCG	Lowe et al., 2003
SA29	N3	4	1	TTGTTGTTGCGCTTTCTGTC	AATTGCGACCCAAGTAGGTG	
SA63	N1	7	5	AGCCGTGTAGCACCAGAACT	CGTGTAGTGTGCGCATCTTT	Long et al., 2008

additive-dominant genetic model and that is controlled by maternal genes instead of embryo genes (Liu et al., 1990; Steffansson et al., 1961; Zhang et al., 1996). Previous studies also showed the importance of environment and genotype x environment effects in the expression of oil content (Pritchard et al., 2000; Si et al., 2003). Other studies also showed that the protein had a significant negative correlation with the seed total oil content (Sugimoto et al., 1989, 1992; Vazquez et al., 1993; Si et al., 2003). There were some research reports on the basic properties of protein and accumulation of storage protein subunits (Hoglund et al., 1992). Other studies indicate that the oil content in F_1 is affected by genes of both maternal and embryo (Fu, 2004) but in F_2 is mainly controlled by the gene effects of the individual itself (Liu et al., 1990; Lickfett et al., 1999). In this study, correlation analysis was carried out among the quality traits of a RILS family in *B. napus L*. The results show that protein content and seed coat pigments had significant negative correlation with the oil content in different environments. This is in accordance with the results of previous researches (Sugimoto et al., 1989; Zhao et al., 2006) which show that the protein content had a significant negative

Table 3. Correlation coefficient among all traits of $F_{2:7}$ family populations in Beibei, in 2006 and 2007.

Year	Trait		Oil content	Protein content	Seed colour	Seed coat pigment			
						AC	FC	TPC	MC
	Oil content		1						
	Protein content		-0.71**	1					
	Seed color		0.41**	0.08	1				
2006									
		AC	-0.01	-0.09	-0.04**	1			
	Seed coat	FC	-0.30**	-0.03	-0.67**	-0.05	1		
	Pigments	TPC	-0.32**	-0.01	-0.66**	-0.06	0.96**	1	
		MC	-0.30**	-0.10	-0.80**	-0.01	0.86**	0.86**	1
	Oil content		1						
	Protein content		-0.44**	1					
	Seed color		0.27**	0.38**	1				
2007									
		AC	-0.42**	-0.32**	-0.66**	1			
	Seed coat	FC	-0.36**	-0.27**	-0.64**	0.81**	1		
	Pigments	TPC	-0.36**	-0.31**	-0.65**	0.80**	0.88**	1	
		MC	-0.46**	-0.32**	-0.68**	0.94**	0.85**	0.84**	1

AC, Anthocyanidin content; FC, flavoid content; TPC, total phenol content; MC, melanin content; *, **: significant at P<0.05 and P<0.01, respectively.

Table 4. Correlation coefficient among all traits of $F_{2:7}$ family populations in Wanzhou, in 2006 and 2007.

Year	Trait		Oil Content	Protein content	Seed color	Seed coat pigment			
						AC	FC	TPC	MC
	Oil content		1						
	Protein content		-0.77**	1					
	Seed color		0.37**	0.02	1				
2006									
	Seed coat Pigments	AC	-0.23**	-0.05	-0.67**	1			
		FC	-0.22**	-0.03	-0.63**	0.88**	1		
		TPC	-0.24**	-0.02	-0.56**	0.86**	0.88**	1	
		MC	-0.21**	-0.10	-0.73**	0.82**	0.75**	0.76**	1
	Oil content		1						
	Protein content		-0.37**	1					
	Seed color		-	-	1				
2007									
		AC	-0.56**	-0.07	-	1			
	Seed coat	FC	-0.46**	-0.11	-	0.82**	1		
	pigments	TPC	-0.32**	-0.18*	-	0.54**	0.73**	1	
		MC	-0.57**	-0.11	-	0.93**	0.87**	0.64**	1

AC, Anthocyanidin content; FC, flavoid content; TPC, total phenol content; MC, melanin content; *, **: significant at P<0.05 and P<0.01, respectively.

correlation with the oil content. Different fields' environments have no influence on the trade-off of oil content and protein content. Whereas, there is significant negative correlation between the seed color and seed coat pigments in different environments in two years. The results show that seed coat pigments were involved in the seed coat color. So we can change the seed pigment components to arrive at the objectives of high oil content and protein content in *B. napus L.*

In our study, we used SSR markers to assess the

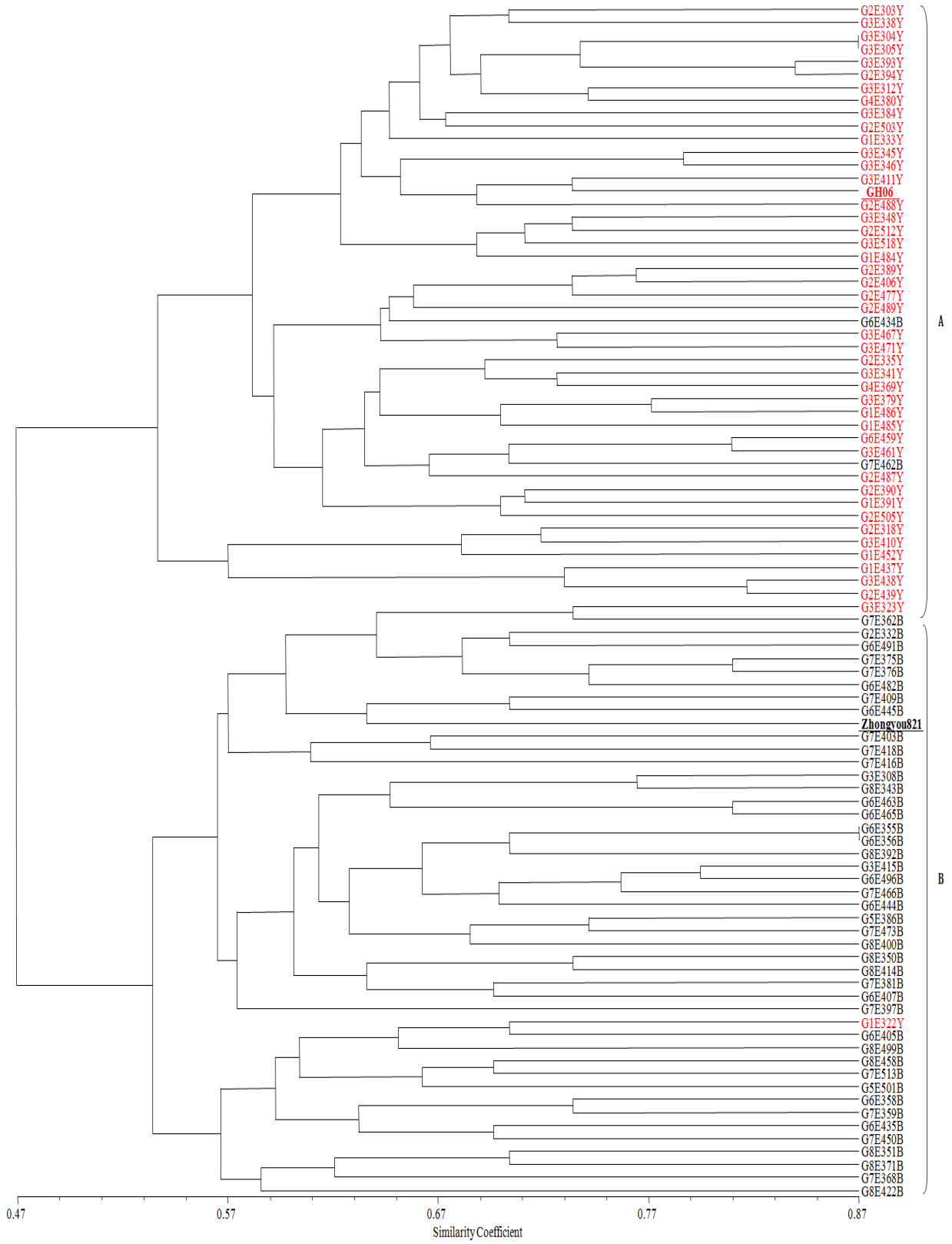

Figure 1. Relationship between 90 RILs of *B. napus* as revealed by hierarchical cluster analysis of SSR based genetic distance estimates; prefixes G1, G2, G3, …, G4 in genotype designations indicate the group membership of genotypes according to the k-means cluster analysis. Red color indicates the yellow-seeded lines and black color indicates the black-seeded lines.

genetic diversity, genetic structure and genetic relationship in a *B. napus L.* RILs familly. The correlation of seed coat colour, the oil content, protein content and seed pigment components in different environments were also analyzed. The grouping of 90 inbred lines by cluster analysis was generally consistent with known pedigrees. SSR marker information described in this work provides a useful starting point for structure-based association analyses of phenotypic traits in *B. napus*. The results of correlations between seed coat pigment content and seed coat colour, oil content, and protein content provided definitive information on different seed coat color germplasm characterization of *B. napus* RILs family. These results can give us more clues in the improving quality traits of rapeseed. In addition, the information of markers could be applied in association mapping analyses of quality traits or marker-assisted selection of yellow-seeded in *B. napus*.

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