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Full Length Research Paper

Genetic diversity and relationship analysis of the *Brassica napus* germplasm using simple sequence repeat (SSR) markers

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Oilseed rape (*Brassica napus* L.) is an important oilseed crop worldwide. The objective of this research was to study the genetic diversity and relationships of *B. napus* accessions using simple sequence repeat (SSR). A set of 217 genotypes was characterized using 37 SSR markers of mapping on the *B. napus* genome. The detected alleles were 2 to 11 at each of the 37 markers, with an average of 5.29 per marker. Unweighted pair group method with arithmetic mean (UPGMA) clustering enabled the identification of two general groups with increasing genetic diversity as follows: (1) group I was further divided into three groups (A, B and C), group A included 121 accessions, and consisted of the yellow-seeded and black-seeded cultivars and breeding lines. The group B included 70 accessions and consisted mainly of the yellow-seeded cultivars and breeding lines, which were mostly cultivated in China. The group C included 10 accessions and consisted of the black-seeded cultivars and breeding lines with low levels of erucic acid. (2) Group II included 16 accessions consisted mainly of breeding lines and German cultivars, which were black-seeded lines with high levels of oleic acid (>80%) and low erucic acid and seed glucosinolate. The grouping of accessions by cluster analysis was generally consistent with known pedigrees, which included the grouping of lines derived both by backcrossing or self-pollination with their parents. The molecular genetic information gained enables also help breeders and geneticists to understand the structure of *B. napus* germplasm and to predict which combinations would produce the best off-spring which is potentially interesting with respect to increasing heterosis in oilseed rape hybrids.

Key words: *Brassica napus* L., genetic diversity, microsatellites, SSR markers.

INTRODUCTION

Oilseed rape (*Brassica napus*, genome AACC, 2n = 38) is the most important source of edible vegetable oil in China and the second most important oilseed crop in the world after soybean. It originated in a limited geographic region through spontaneous hybridizations between turnip rape (*Brassica rapa*, AA, 2n = 20) and cabbage (*Brassica oleracea*, CC, 2n = 18) genotypes (Kimber and McGregor, 1995). Like most agricultural crops, the first step in *Brassica* improvement is full assessment of the local materials, including collection, evaluation and molecular characterization of germplasm lines. Usually, local varieties of oil seed crops are of excellent quality and flavor also have a good level of resistance to pests and diseases and may be superior to exotic materials. So, the enhancement of genetically diverse gene pools is an essential requirement in plant breeding.

However, the challenges that face modern plant breeders are to develop higher yielding, nutritious and environmentally friendly varieties that improve our quality

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of life without harnessing additional natural habitats to agricultural production (Zamir, 2001). Without a broad base of heterogeneous plant material, it is impossible for plant breeders to produce cultivars that meet the changing needs regarding adaptation to growing conditions, resistance to biotic and abiotic stresses, produce yield or specific quality requirements (Friedt et al., 2007). Therefore, the most efficient way to further improve the performance of crop varieties is to access to large diverse pool of genetic diversity. Moreover, information on the genetic diversity of *B. napus* germplasm collections can provide breeders and geneticists important information on the allelic diversity present in *B. napus* materials and may help to identify genetically diverse pools for use in cross combinations to improve important agronomic traits or to better exploit heterosis (Diers and Osborn, 1994).

Traditionally, morphological, phenological and agronomical traits have been employed as criteria for the introgression of new variation into oilseed rape breeding lines. In comparison with other molecular marker techniques, simple-sequence repeat (SSR) markers are numerous, highly polymorphic and informative, co-dominant, 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 proven 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 Australian canola cultivars were analysed by using 18 SSR primer pairs, which produced 112 polymorphic loci (Wang et a., 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) also established 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 among cultivated *B. napus* accessions and the genetic diversity *B. napus* accessions appropriately. This will provide useful information for *Brassica* breeding program in the future.

MATERIALS AND METHODS

The plant materials for this study comprised 217 genotypes, which were selected and used for rapeseed breeding lines or hybrid breeding. Most of the 217 accessions were selected by the Rapeseed Engineering Research Center of Southwest University in Chongqing or provided by different breeding institutes in China. Some of these have consistent pedigrees, which were derived both by backcrossing or self-pollination with their parents. The other was widely grown in German. The accessions investigated and their origins are listed in Table 1. Although they had a little range of morphological types and geographical origins, there were many hybrid rapeseed with higher yield and quality from these accessions and widely cultivated. All genotypes were grown in Beibei, Chongqing, China, in the growing seasons of 2009 and 2010.

DNA extraction

The plants of all accessions were cultivated for one month in the field. 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) with some modifications. The concentration and purity of each DNA sample were measured using a GeneSpec I spectrophotometer at wave-lengths of 260 and 280 nm quantified by visual comparison to λ DNA standards on ethidium bromide-stained agarose gels.

SSR assays

We used 37 SSR markers that were selected genome wide primer combinations, and then analyzed the genetic diversity which were selected from the collection available in the public domain obtained from five sources: John Innes Centre, UK (http://www.brassica.bbsrc.ac.uk/BrassicaDB/); National Institute of Vegetable and Tea Science, Japan (http://vegetea.naro.affrc.go.jp); Agriculture and Agri-Food, Canada (http://brassica.agr.gc.ca/index_e.shtml), Plant Biotechnology Centre, La Trobe University, Australia (http://www.hornbill.cspp.latrobe.edu.au) and *Brassica rapa* Genome Project (http://www.brassica-rapa.org/BRGP/status.jsp); These were synthesized by Shanghai Sangon Biological Engineering Service Co. Ltd. (China) and listed in Table 2.

Polymerase chain reactions (PCR) were performed in 96-well plates with a volume of 10 µL. The composition of the mixture was as follows: 20 ng/µL of DNA template, 0.5 pmol of each primer, 0.2 mM dNTP mix, 2.5 µL 10×PCR reaction buffer (with 15 mM $MgCl_2$) and 0.5 U of *Taq* DNA polymerase (TransGen Biotech, China). PCR was carried out in PTC-100 and PTC-200 thermo cycler with the following program: 94°C for 5 min; 35 cycles with 94°C denaturation 45 s, annealing for 45 s, 72°C elongation for 1 min, elongation for 10 min (Table 2). All PCR products were detected using non-denaturing polyacrylamide gel electrophoresis (10% polyacrylamide) using DYCZ-30 electrophoresis cell and silver staining (Zhang et al., 2002).

Analysis of genetic relationship

The analysis of genetic diversity was based on discrete variables of binary data matrix that consist of the presence (1) and absence (0) of an allele per SSR locus for each accession. Additionally, we estimated genetic diversity (*D*) for each SSR locus using the formulas: $Di = n\,(1-\Sigma Pij2)/n-1$, where *n* is the number of accessions analyzed, and *Pij* is the frequency of the *j*th allele for the *i*th locus across all alleles at loci. Average marker diversity (*D*) was estimated as $D = \Sigma Di/r$, where *r* was the number of loci analyzed. To detect the relationship between accession studied, we estimated the genetic similarity according to Jaccard's coefficients from the alleles across all the loci in the 217 accessions using the formula: $J = Nij/(N-N00)$, where *Nij* was the number of shared alleles in both accessions *i* and *j*, *N* was the number of all alleles across all

Table 1. A list of tested oilseed lines and their pedigree.

Number of field	Pedigree/source	Origin	Number of field	Pedigree/source	Origin	Number of field	Pedigree/source	Origin
H1	[(GH01/Yuanza 1)/GH01]F_2/02P208(F_7)	SWU of CN	L295	-	SWU of CN	L567	GH01/(Pin901871/Zhongshuang 1)	SWU of CN
H2	-	SWU of CN	L296	-	SWU of CN	L568	GH06	SWU of CN
H3	[(GH01/99A227)F_6/(GH16/Chuanyou 18)F_5]F_5	SWU of CN	L297	SC94005/GH01	SWU of CN	L569	Zhongshuang 10	SWU of CN
H4	-	SWU of CN	L298	-	SWU of CN	L570	94005	SWU of CN
H5	-	SWU of CN	L299	GH01/Pin93－496	SWU of CN	L583	R54-4	SWU of CN
H6	-	SWU of CN	L300	-	SWU of CN	L585	R71-1	SWU of CN
H7	[(GH01/851)F_6/(Ⅲ-227/Zhongshuang 1)F7]F_5	SWU of CN	L301	SC94005/GH16	SWU of CN	L588	05E26-2	SWU of CN
H8	-	SWU of CN	L302	-	SWU of CN	L589	05E105-2	SWU of CN
H9	{(GH01/851)F_6/[(7018/*Brassica oleracea*)/(Zhongyou 821/D2)]F_7}F_6	SWU of CN	L303	(GH01/3529-5)F_4/(Aisipeide/74-317)	SWU of CN	L590	05E105-3	SWU of CN
H10	-	SWU of CN	L304	Pin93-496/[GH01/(Pin901871/Zhongshuang 1)]	SWU of CN	L591	05E159-1	SWU of CN
H11	[Andor/(Altex/96V44)F_8]F_5	SWU of CN	L305	-	SWU of CN	L592	05E159-2	SWU of CN
H12	Yuhuang 2	Dianjiang of CN	L383	07H40-1	SWU of CN	L593	05E258-1	SWU of CN
L01	GH01//(Pin901871/Zhongshuang 1)	SWU of CN	L384	07H46-1	SWU of CN	L594	-	SWU of CN
L02	-	SWU of CN	L385	07H89-3	SWU of CN	L595	-	SWU of CN
L03	-	SWU of CN	L386	07R51-2	SWU of CN	L83	Zhongshuang 9/06R6	SWU of CN
L04	-	SWU of CN	L387	-	SWU of CN	L85	-	SWU of CN
L05	-	SWU of CN	L388	07R52-3	SWU of CN	L86	-	SWU of CN
L06	-	SWU of CN	L389	07R53-4	SWU of CN	L87	-	SWU of CN
L07	-	SWU of CN	L390	07R54-4	SWU of CN	P1	(Aisipeide/74-317)/(821/Pin93-496)F_8	SWU of CN
L08	-	SWU of CN	L391	07R55-5	SWU of CN	P3	[(821/Pin93-496)F_8/(821/97V27)F_8]F_5	SWU of CN
L09	-	SWU of CN	L392	07R56-2	SWU of CN	P4	[(821/Pin93-496)F_8/(Altex/96V44)F_8]F_5	SWU of CN
L10	GH01/3529-5	SWU of CN	L393	07R58-4	SWU of CN	P8	[(Altex/96V44)F_8/Wanxian158]F_5	SWU of CN
L11	-	SWU of CN	L394	07R60-4	SWU of CN	P10	[{Yellow *Brassica oleraceal* [194/(Aisipeide /74-317)]}F_6/(GH01/GH03)F_6] F_5	SWU of CN

Table 1 Contd

L12	-	SWU of CN	L395	07R61-1	SWU of CN	P16	[(GH01/GH03)F_6/{Yellow *Brassica oleracea*/ [194/(Aisipeide/74-317)]}F_6] F_5	SWU of CN
L13	-	SWU of CN	L396	07R62-2	SWU of CN	P19	[(Aisipeide/74-317)/Pin93-496]F6/Zhongshuang 9 F5	SWU of CN
L14	-	SWU of CN	L397	07R63-2	SWU of CN	P42	[Zhongshuang 9/(Youyan 2/Pin93-496)F_6]F_5	SWU of CN
L15	[(D57/O)/85-64]/84-24016	SWU of CN	L398	07R64-4	SWU of CN	P30	[(Aisipeide/74-317)/Pin93-496]F_6/Zhongshuang 9] F_5	SWU of CN
L16	-	SWU of CN	L399	07R64-3	SWU of CN	P40	{Zhongshuang 9/[(Aisipeide /74-317) /Pin93-496]F_6} F_5	SWU of CN
L17	Ningyou 10	SWU of CN	L400	07R65-4	SWU of CN	P46	{Zhongshuang9/[{97V38/[(Siban/*Brassica oleracea* var italica)/Primor]/2328} /97V38] F_1}F_5	SWU of CN
L18	GH01//(Pin901871/Zhongshuang 1)	SWU of CN	L401	R66-4	SWU of CN	P56	-	SWU of CN
L19	GH05/GH02	SWU of CN	L402	R67-2	SWU of CN	P58	[Zhongshuang 9/96V44]F_5	SWU of CN
L20	GH16/Mixed powder	SWU of CN	L403	R68-4	SWU of CN	P60	[(Pin901871/Zhongshuang 1)F10/964222S] F_5	SWU of CN
L21	(GH16/SC94005)F_3/SC94005	SWU of CN	L404	R68-3	SWU of CN	P61	[{[(D57/Oro)/85-64]/84-24016}F6/96V44]F_1/[(821/Pin93-496)F_7/Zhongshuang 9]F_1	SWU of CN
L22	Pin93-496/[GH01/((Pin901871/Zhongshuang 1))]	SWU of CN	L405	R69-3	SWU of CN	P65	(94005/Mixed powder) F_5	SWU of CN
L23	[(Aisipeide/74-317)/Pin93-496]F_6/(GH01/99A227)	SWU of CN	L406	R69-4	SWU of CN	P70	[(Pin901871/Zhongshuang 1)F_{11}/(94005/Mixed powder)F_4] F_5	SWU of CN
L24	[GH01/(Pin901871/Zhongshuang 1)]F_6/(GH01/851)	SWU of CN	L407	-	SWU of CN	P72	Pin93-496	SWU of CN
L25	(GH01/99A227)F_6/(GH01/851)	SWU of CN	L408	R70-1	SWU of CN	P73	Zhongshuang 220	SWU of CN
L26	[GH01/(Pin901871/Zhongshuang 1)]F_6/(GH01/851)F_6	SWU of CN	L409	-	SWU of CN	P74	Zhongshuang 1	SWU of CN

Table 1 Contd

L27	[GH01/(Pin901871/Zhongshuang 1)]F_6/(GH01/99A227)F_6	SWU of CN	L410	R71-1	SWU of CN	P75	Zhongshuang 4	SWU of CN
L28	-	SWU of CN	L411	R72-2	SWU of CN	P76	Zhongshuang 5	SWU of CN
L110	Zhongshuang 9/06E25	SWU of CN	L412	R73-1	SWU of CN	P77	Zhongshuang 6	SWU of CN
L111	-	SWU of CN	L413	R73-4	SWU of CN	P78	Zhongshuang 7	SWU of CN
L112	-	SWU of CN	L414	R74-1	SWU of CN	P79	Zhongshuang 9	SWU of CN
L113	-	SWU of CN	L426	Westar		P80	Zhongshuang 10	SWU of CN
L114	Zhongshuang 9/06E47	SWU of CN	L427	[GH01/(Pin901871/Zhongshuang1)]F_6 /(GH01/99A227)	SWU of CN	P81	Huashuang 4	SWU of CN
L115	-	SWU of CN	L428	06-634-4	SWU of CN	P82	Huashuang 5	SWU of CN
L116	Zhongshuang 9/06E85	SWU of CN	L429	Holliday		P84	Huyou 18	SWU of CN
L117	Zhongshuang 9/06E98	SWU of CN	L430	-		P85	Zhongnongyou 136	SWU of CN
L118	-	SWU of CN	L431	Zhongshuang 9	SWU of CN	P86	94005	SWU of CN
L198	Express	Germany	L432	Y511-7	SWU of CN	P87	Youyan 2	SWU of CN
L199	Campino	Germany	L433	Y511-11	SWU of CN	P88	851	SWU of CN
L200	Aragon	Germany	L434	Y520-5	SWU of CN	P89	Zheyou 6001	SWU of CN
L201	Viking	Germany	L435	Y520-11	SWU of CN	P91	56602	SWU of CN
L212	04SH145/04P17(06M16)	SWU of CN	L436	Y539-1	SWU of CN	P92	Yang 6614	SWU of CN
L213	04SH254/04P35(06M58)	SWU of CN	L437	Y539-3	SWU of CN	P110	(96V44/Zhongshuang 9) F7	SWU of CN
L214	04SH243/04P35(06M49)	SWU of CN	L438	Y539-4	SWU of CN	P122	[(GH01/851)F_6/Zhongshuang 9]F_7	SWU of CN
L215	04SH32/04P17(06M121)	SWU of CN	L551	GH01/851	SWU of CN	P145	2007R343	SWU of CN
L216	-	SWU of CN	L552	GH01/3529-5	SWU of CN	P205	P214-1	SWU of CN
L217	04SH145/04P17(06M124)	SWU of CN	L553	-	SWU of CN	P208	P219-1	SWU of CN
L218	-	SWU of CN	L554	GH16/SC94005	SWU of CN	P217	P235-2	SWU of CN
L219	-	SWU of CN	L555	-	SWU of CN	P222	P237-2	SWU of CN
L220	04SH32/04P17(06M120)	SWU of CN	L556	SC94005/GH16	SWU of CN	P226	P243-1	SWU of CN
L221	-	SWU of CN	L557	-	SWU of CN	W1		SWU of CN
L285	GH01/3529-5	SWU of CN	L558	-	SWU of CN	W2		SWU of CN
L286	-	SWU of CN	L559	[(D57/O)/85-64]/84-24016	SWU of CN	W3		SWU of CN
L287	GH01/851	SWU of CN	L560	Zhongshuang 9/06E123	SWU of CN	W406		SWU of CN
L288	GH16/SC94005	SWU of CN	L561	GH16/SC94005//K127	SWU of CN	W423		SWU of CN
L289	-	SWU of CN	L562	06P243/Zhongshuang 9	SWU of CN	W434		SWU of CN
L290	-	SWU of CN	L563	Zhongshuang 9	SWU of CN	W488		SWU of CN
L291	-	SWU of CN	L564	GH16/SC94005	SWU of CN	W514		SWU of CN
L292	SC94005/GH16	SWU of CN	L565	-	SWU of CN	W635		SWU of CN
L293	-	SWU of CN	L566	-	SWU of CN	W7		SWU of CN
L294	-	SWU of CN						

Negative sign (-) indicated the same to the last one; SWU: indicating the Southwest University; CN: indicating the China.

Table 2. Allelic diversity at SSR loci amplified by primer used for the genetic diversity analysis.

SSR Primer	Forward sequence	Reverse sequence	Tm (°C)	Number of alleles detected	Polymorphic Loci detected	Polymorphic rate (%)	Reported
sR12387	5'-GGGTCTGGGTTTTTCTGTGA-3'	5'-GATTGGGCCGTGTAATATCG-3'	55	4	1	25.00	Cheng et al. (2009)
sNRA59	5'-CAGATTCGGATTTGGGAAGA-3'	5'-GGCGGAAGAATCAAAGGAGT-3'	55	6	1	16.67	Long et al. (2007)
sR3688	5'-GGAGTCCACTTCATGGAGGA-3'	5'-CTCTTGCTCGTAGGTTTCCG-3'	55	7	2	28.57	Choi et al. (2007)
Au39	Unknown	Unknown	56	5	4	80.00	Long et al. (2007)
BRAS051	5'-GAATAGCCTCGCAGAAGTAGC-3'	5'-CGACGGCGATAAAACGAA-3'	55	7	6	85.71	Lowe et al. (2004); Piquemal et al. (2005); Choi et al. (2007); Cheng et al. (2009)
BRMS075	5'-GTTTCACATATTTTCTCTGTTTATT-3'	5'-ACCTTAAATGTTAAGTAAGCTAAAC-3'	55	3	2	66.67	Suwabe et al.(2008)
BRMS093	5'-TCCAAGTAGACCGAATCAAGAGAGT-3'	5'-ATAAATCGAACCTGAAACCATGTCT-3'	55	5	3	60.00	Suwabe et al. (2008); Cheng et al. (2009)
BRMS098	5'-TGCTTGAGACGCTGCCACTTTGTTC-3'	5'-CATTCCTCCCCACCACCTTCACATC-3'	55	7	4	57.14	Choi et al. (2007); Suwabe et al. (2008)
BRMS106	5'-ACCAAACGACGCAAACAAACAAATA-3'	5'-TGACTTCGGAACGTGCAATAGAGAT-3'	55	4	4	100.00	Choi et al. (2007); Cheng et al. (2009)
BRMS129	5'-TGAGGTTAGACATGGCGCTGCTTGC-3'	5'-TTTGATCATTGTGGTCGCGAGTTCG-3'	55	6	3	50.00	Suwabe et al. (2006)
BRMS175	5'-GTGATACTGAAAGGGAGAGAGTGAG-3'	5'-AATCCTCATGAGCAAATCAACTAAC-3'	55	7	2	28.57	Suwabe et al. (2008)
BRMS232	5'-AAAACAATACGACTGATTGAACCAT-3'	5'-CAAATCATAGTCGAAACTAGCTAAAA-3'	55	4	4	100.00	Suwabe et al. (2008)
BRMS240	5'-CAAGAGTATTTGTGTGGGTTGACTC-3'	5'-AAATAACGAACGGAGAGAGAGAGAG-3'	55	4	4	100.00	Suwabe et al. (2006)
BRMS246	5'-ACATGTGCTTTATGAGAGAGAGAGA-3'	5'-TCTTTGTCACATTAATCCTTCCACT-3'	55	3	2	66.67	Choi et al. (2007); Cheng et al. (2009)
BRMS324	5'-AACTTAACCGAAACCGAGATAGGTG-3'	5'-AATCTCGAAATTCATCGACTTCCTC-3'	55	11	7	63.64	Suwabe et al.(2008)
CB10022	5'-AACAACCAAACATAGTCCC-3'	5'-GTTGACTTTGACCTTGACTT-3'	55	6	5	83.33	Piquemal et al. (2005); Long et al. (2007); Cheng et al. (2009)
CB10065	5'-CGGCAATAATGGACCACTGG-3'	5'-CGGCTTTCACGCAGACTTCG-3'	55	4	2	50.00	Piquemal et al. (2005); Long et al. (2007); Cheng et al. (2009)

Table 2. Cond.

CB10278	5'-TGAAGAAGCTGGGACAAG-3'	5'-CAATGCAATACAGCACCA-3'	55	4	1	25.00	Piquemal et al. (2005); Long et al. (2007)
CB10302	5'-CGATACTTGGAGCGTGTC-3'	5'-CTGGTGTCTTAACCACGC-3'	55	3	1	33.33	Piquemal et al. (2005)
CN52	5'-CCGGCTTGGTTCGATACTTA-3'	5'-TTGCGAATCTTTAAGGGACG-3'	56	4	3	75.00	Long et al. (2007)
EJU5	5'-GGCACGTACATGGAGGATTC-3'	5'-TGTTGGTCGAGCTGTTTCAG-3'	56	8	7	87.50	Choi et al. (2007)
ENA19	5'-AAGTTACCAAGGAGAGGACAG-3'	5'-AAAGGGACGCTACAAGTCA-3'	56	4	1	25.00	Choi et al. (2007)
FITO 040	5'-GATTGTTTGTTTCTAACTGTGG-3'	5'-TAGGATGTGACTTGGTCTTTC-3'	55	3	3	100.00	Long et al. (2007)
MR119	5'-GCTGAAACGCGTAGAGACTAA-3'	5'-GCTGGGAAATACGTTGAAA-3'	55	6	5	83.33	Long et al. (2007)
niab_ssr022	5'-CTCTCGTCTCGGAGGATCTAAA-3'	5'-GTGAGAGTGGTTGCTGAGTGAG-3'	60	6	6	100.00	Long et al. (2007)
niab_ssr091	5'-TGGTTCTGCTATTGCTGTCA-3'	5'-GAAGTTTGTGAGCCAGGAAA-3'	60	2	1	50.00	Cheng et al. (2009)
niab_ssr112	5'-TCACGAGACTACCCTTGGAG-3'	5'-GCAACAGTGCTTTTCTTGGT-3'	60	6	4	66.67	Cheng et al. (2009)
SA63	5'-AGCCGTGTAGCACCAGAACT-3'	5'-CGTGTAGTGTGCGCATCTTT-3'	56	7	4	57.14	Long et al. (2007)
sN11722	5'-CGATCTGAGCGTTGTTGCTA-3'	5'-GCGCGACTCAAAGAAGAAGT-3'	55	5	1	20.00	Cheng et al. (2009)
sNRD03	5'-GAAGATTCGAGCTCTTTCGG-3'	5'-CGTTTCAGAATCATATTGTATTTTGCT-3'	55	5	3	60.00	Cheng et al. (2009)
sORF73	5'-CGTGGGCCAAGCTTAGATTA-3'	5'-CGTTCAAGAAGACACAGATCAAA-3'	55	10	5	50.00	Long et al. (2007)
sR12777	5'-CAAGCAGTTTAAGGAACCGC-3'	5'-ATAATTGCATTTTGCTCCGC-3'	55	5	4	80.00	Cheng et al. (2009)
sR7223	5'-AGGACCCGACTTTCCTTGTT-3'	5'-ACCAAACTCGGCGTACAAAT-3'	55	7	3	42.86	Long et al. (2007)
sR9222	5'-CACCGAACAAAACTGAGGGT-3'	5'-CGTTTCACTGCGTTCTACCA-3'	55	6	3	50.00	Long et al. (2007)
sR94102	5'-ATCCCCAAACTACCCTCACC-3'	5'-AGGATGAGCAAAGGAAAGCA-3'	55	2	1	50.00	Long et al. (2007)
sR9447	5'-AAATTCGAAAATGCAAACGG-3'	5'-CCAATCTTGGAACAATAGAAGATG-3'	55	7	3	42.86	Long et al. (2007)
OI10-C05	5'-GGCTACAAAATGTTTGATAAGCTCT-3'	5'-ACCTGAAAGAGAGGCTACACAT-3'	55	3	2	66.67	Lowe et al. (2004); Cheng et al. (2009)
Total				196	117		

accessions investigated, while the *N*00 was the number of alleles present neither in accession *i* nor in accession *j*. In addition, to investigate the relationship between accessions, a dendrogram based on similarity coefficients, was constructed with the unweighted pair-group method with arithmetic averages (UPGMA; Sneath and Sokal, 1973). The estimation of genetic diversity and the cluster analysis were performed using NTSYS-pc software package (Rohlf, 2005).

RESULTS

Assessment of polymorphism by SSR markers in *B. napus* accessions

The markers covered each of the linkage groups according to previous research done in *B. napus* (Lowe et al., 2004; Piquemal et al., 2005; Choi et al., 2007; Long et al., 2007; Suwabe et al., 2006, 2008; Cheng et al., 2009). Among the 37 primers used in the present study, a total of 117 scorable polymorphic loci with 196 alleles were amplified in the 217 genotypes. The polymorphic loci gave unique genetic fingerprints for all 217 accessions. Eight primers yielded on average minimum number of bands (1.00), while primers BRMS324 and EJU5 yielded maximum (7.00) number of alleles per genotype on average (Table 2). The average number of alleles per loci was 5.29. Level of polymorphism rate were calculated and observed in this study, it was in the range of 16.67 to 100.00%.

Genetic relationship of *B. napus* accessions

Genetic similarities among accessions were estimated based on Jaccard's similarity (1908). An UPGMA phenogram was constructed for all 217 accessions and the similarity coefficient ranged from 0.00 to 0.91. Then 217 accessions were classified into two groups at the similarity coefficient 0.04 (Figure 1). Group I included 201 accessions, group II included 16 accessions

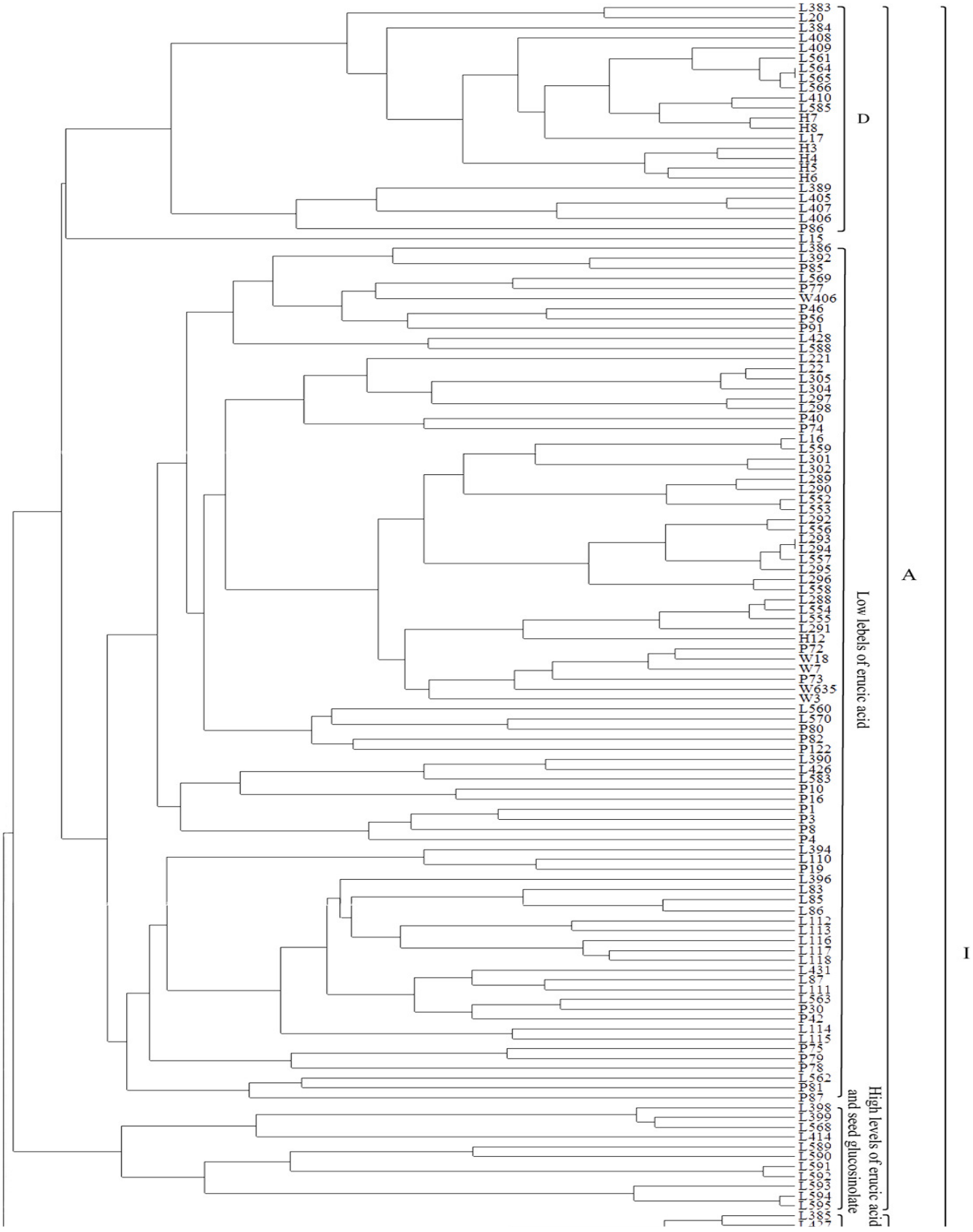

Figure 1. Phenogram showing Jaccard's genetic similarity coefficients for a diverse set of 217 oilseed rape accessions revealed by UPGMA clustering based on genetic fingerprints calculated from 37 SSR primer combinations.

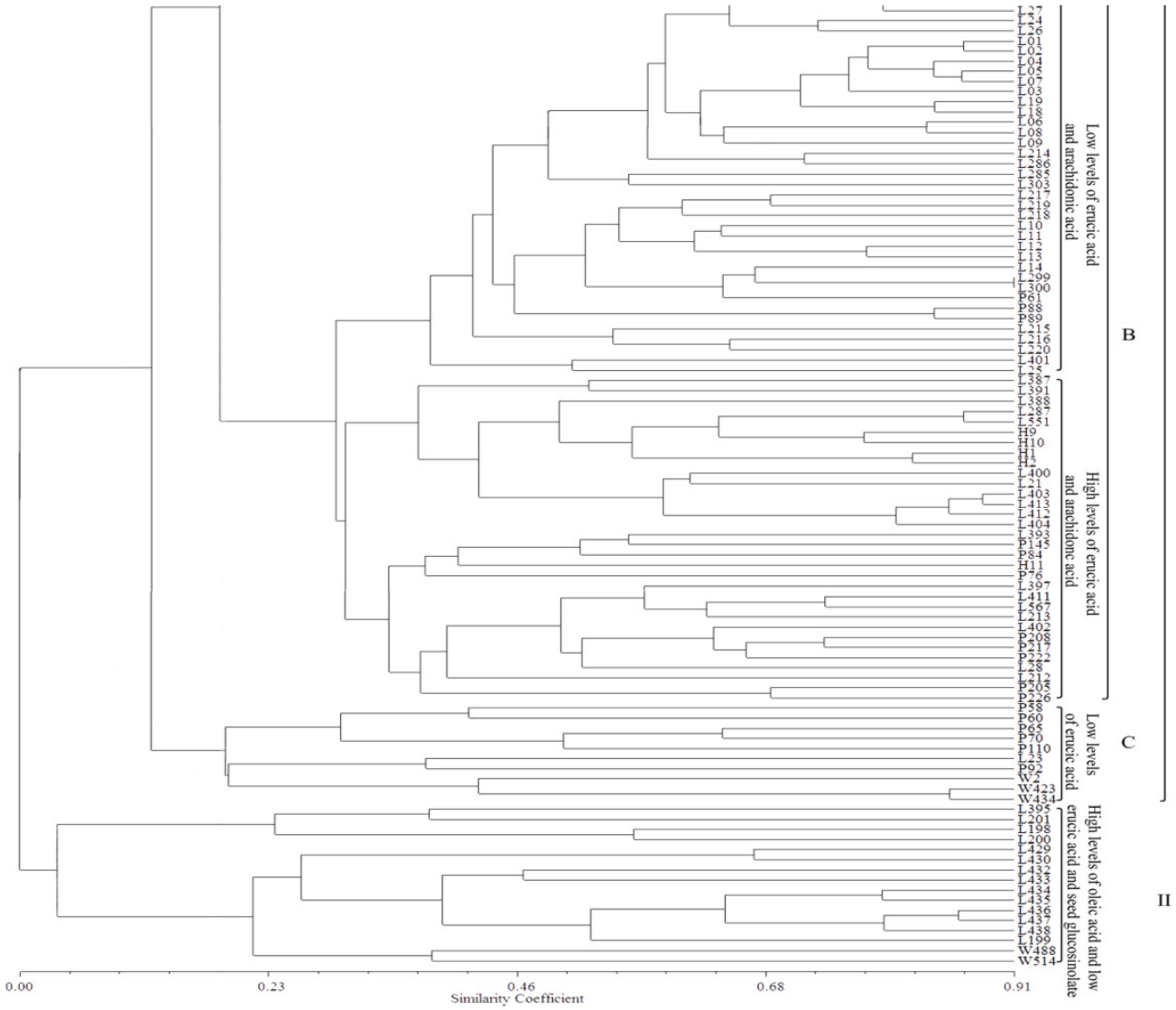

Figure 1 Contd

consisting mainly of breeding lines and German cultivars, which were black-seeded lines with high levels of oleic acid (>80%) and low erucic acid and seed glucosinolate. The grouping of accessions by cluster analysis was generally consistent with known pedigrees. Moreover, the group I was further divided into three groups with a genetic similarity coefficient of only around 0.18 (A, B and C). The first group A included 121 accessions and consisted of the yellow-seeded, black-seeded cultivars and breeding lines. The second group (B) included 70 accessions and consisted mainly of the yellow-seeded cultivars and breeding lines, which were mostly cultivated in China. The group C included 10 accessions and consisted of the black-seeded cultivars and breeding lines with low levels of erucic acid.

The group A was also further divided into three sub-clusters. The first group I consisted of yellow-seeded cultivars and breeding lines with the high or low levels of erucic acid, seed glucosinolate and arachidonic acid. The second group consisted of low levels of erucic acid with the yellow-seeded or black-seeded cultivars and breeding lines. The near-isogenic lines or the derivation of off-spring of Zhongshuang No.9 were located on this region between the L394 and the P78 (Figure 1). The last group included the high levels of erucic acid, seed glucosinolate and arachidonic acid. In addition, the group B was further divided into two groups including the 38 and 32 accessions, which each showed a similarity index of around 0.24 to their respective cluster. The first group consisted mainly of the local cultivars and breeding lines with low levels of erucic acid and arachidonic acid derivation from the GH01. While the second group included 32 accessions with high levels of erucic and arachidonic acid.

DISCUSSION

In our study, the 37 SSR markers showed sufficiently high sensitivity to detect DNA polymorphisms among the 217 *B. napus* accessions. The results obtained in this study will also demonstrate that SSR markers can be suitable and efficient tool for genetic characterization of many plant species including oilseed rape (Hasan et al., 2006, Naito et al., 2008). The SSR markers information could provide a useful starting point for structure-based association analyses of phenotypic traits in this *B. napus* core collection and the theoretical basis for the hybrid-dization and selecting parents in oilseed breeding pro-grams. Local materials, including collections, evaluation and molecular characterization of germplasm lines were also the mainly genetical resources of parental varieties to oilseed rape breeders. Some previous reports have also deeply researched Brassicaceae, such as the differences between the spring and the winter of oilseed, the China and Europe accessions (Hu et al., 2003, Hasan et al., 2006), significant yield increases in spring oilseed rape hybrids (Butruille et al., 1999; Cruz et al., 2007; Quijada et al., 2004; Udall et al., 2006) and genetic diversity of rapeseed cultivars and germplasm (Ahmad et al., 2011; Ana et al., 2011; Moghaddam et al., 2009). Moreover, knowledge about germplasm diversity and genetic relationship among local cultivars and the main breeding lines could be an invaluable aid in crop improvement strategies.

In our study, the grouping of accessions by cluster analysis was generally consistent with known pedigrees. This consistency included the grouping of lines derived both by backcrossing or self-pollination with their parents. First, the most accessions were classified into group I, including both the higher or lower levels of erucic acid, seed glucosinolate and arachidonic acid of yellow-seeded and black-seeded cultivars and breeding lines or the local cultivars and the near-isogenic lines of Zhongshuang No. 9 and GH01. They have been developed from cultivars of diverse origins. Some lines are sister inbred lines developed from the same F_2 population. Secondly, group II consists mostly of black-seeded lines with high levels of oleic acid (>80%) and low erucic acid and seed gluco-sinolate. The few materials in cluster II originated from Germany cultivars, such as L198, L199, L200 and L201 (Figure 1). The results obtained herein therefore 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 (Cruz et al., 2007; Li et al., 2011; Hasan et al., 2006; Tommasini et al., 2003).

In addition, the findings of this preliminary study indicate that a set of microsatellite primers could be used for several important aspects of various breeding strategies, example organizing the germplasm of oilseed genetic resources, identification of cultivars, selecting appropriate parents for *B. napus* hybrids and for monitoring hybridity level, and ultimately to assist the development of molecular markers for marker-assisted breeding. Genome-wide SSR marker data described in this work provides a useful starting point for structure-based association analyses of phenotypic traits in this *B. napus* core collection.

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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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