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ORIGINAL ARTICLE

Genetic diversity in Chinese and exotic *Brassica rapa* L. accessions revealed by SSR and SRAP markers

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Abstract The genetic diversity and relationship was studied in a collection of 65 *Brassica* accessions, which included 54 *B. rapa* and 11 of five other *Brassica* species used as references. These 54 *B. rapa* accessions included 42 Chinese accessions and 12 exotic accessions. All accessions were analyzed by using 36 simple sequence repeats (SSR) and 43 sequence-related amplified polymorphism (SRAP) primers, and 401 polymorphic fragments were detected by SSR and SRAP markers. The average number of polymorphic fragments detected by SRAP markers was 6.23 ranging from 2 to 11 and that revealed by SSR was 3.69 ranging from 2 to 7. The unweighted pair-group method with arithmetic mean cluster analysis indicated that all accessions could be divided into five major clusters except three accessions as outliers. The exotic *B. rapa* accessions appeared in Cluster I except one yellow sarson accession from India, Chinese *B. rapa* revealed in Cluster II, and 11 accessions of other *Brassica* expressed them Cluster III, Cluster IV, and Cluster V. The results of principal component analysis and population structure analysis were in accordance with the cluster analysis. Molecular variance analysis revealed that the genetic variation was 26.10% among populations and 73.90% within *Brassica* species, which indicated existence of considerable genetic variation among exotic and Chinese *B. rapa* species, and exotic *B. rapa* can be used for broadening the genetic background of Chinese *B. napus*, and *vice versa*.

Keywords Turnip rape · Genetic variation · Simple sequence repeat · Sequence-related amplified polymorphism

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Introduction

The genus *Brassica* includes three basic diploid species *Brassica rapa* L. (AA genome, $n = 10$), *B. oleracea* L. (CC genome, $n = 9$), *B. nigra* L. (BB genome, $n = 8$), and three compound species *B. napus* L. (AACC genome, $n = 19$), *B. juncea* (L.) *Czern & Coss* (AABB genome, $n = 18$), and *B. carinata* A. Braun (BBCC genome, $n = 17$) (U N 1935). *B. rapa* is one of the most economically important oilseed crops worldwide, and it is also grown as a major vegetable and fodder crop (Prakash and Hinata 1980; Liu 1984). The oleiferous form of *B. rapa*, namely *B. rapa* subsp. *oleifera* (turnip rape) is the third

most important *Brassica* oilseed crop, and it is grown in China, Canada, India, and Northern Europe (Ramchiary and Lim 2011). A large number of subspecies in *B. rapa* are recognized as oilseed type which are distributed in Europe, Central Asia, and India, and leaf vegetable type is generally spread in East Asia (Song et al. 1988; Zhao et al. 2005). All the subspecies in *B. rapa* have a wide range of variation and genetic diversity (McGrath and Quiros 1992; Zhao et al. 2005; Takuno et al. 2007; Warwick et al. 2008; Fu and Gugel 2009; Annisa and Cowling 2013). *B. rapa* has broad geographical distribution than *B. napus* and is widely distributed throughout Europe and East to South Asia. The classical Old World is one of center of origin of *B. rapa*, with several centers of diversity in East Asia owing to ancient trade routes in Asia and recent migration to the New World (Song et al. 1990; Guo et al. 2014).

Due to extensive era of domestication, natural selection and breeding, *B. rapa* possesses more desirable characters for instance, drought resistance, cold resistance, heat tolerance, early maturity, and many other characteristics (He et al. 2002; Chen et al. 2010). *B. napus* is an allopolyploid species that resulted from interspecific hybridization between *B. rapa* (A genome) and *B. oleracea* (C genome) (Jesske et al. 2013). Nowadays, *B. napus* is grown worldwide for its high yield and disease resistance. The availability of plentiful germplasm and great genetic diversity makes *B. rapa* the ideal prospect for improvement of *B. napus* (Zhao et al. 2005; Liu and Meng 2006). Therefore, new alleles can be easily transferred into *B. napus* genome for broadening the gene pool and increasing genetic diversity of *B. napus* for future breeding programs. Rapeseed breeders in China have used indigenous *B. rapa* and exotic *B. napus* introductions in breeding programs and have developed semi-winter *B. napus* varieties adapted to local conditions (Liu 1984; Qian et al. 2006; Li et al. 2013). However, there are no reports about using the foreign *B. rapa* accessions to widening the genetic background of Chinese *B. napus*.

Evaluation of genetic diversity and relationship among Chinese and exotic *B. rapa* has significant implications for genetic improvement and incorporation of novel alleles from the *B. rapa* gene pool into *B. napus* (Liu and Meng 2006; Allender and King 2010). Various molecular marker's approaches have been employed to investigate the genetic diversity in *B. rapa* accessions worldwide. Zhao and Becker (1998) used isozyme markers to analyze genetic variation among 12 Chinese and 24 European oilseed *B. rapa* accessions. Their results revealed that existence of two clear-cut groups of Chinese and European origin in *B. rapa*. Zhao et al. (2005, 2007) used amplified fragment length polymorphism (AFLP) markers to discover genetic diversity of 161 *B. rapa* including traditional and modern cultivars, and breeding materials from different geographical locations of the world (10 of 27 *B. rapa* subsp. *oleifera* used from China), and 96 accessions of leafy vegetable types cultivated in China. Their results revealed that different morphotypes (oilseed, root, or leafy types) are often more related to other morphotypes from the same region (East Asia vs. Europe) than similar morphotypes from different regions, suggesting either an independent origin in both regions and/or a long and separate domestication and breeding history in both regions. Tanhuanpää et al. (2016) analyzed genetic diversity in 61 accessions of *B. rapa* using single nucleotide polymorphism (SNP) markers and reported great variation in the diversity indices between accessions and divided them into three groups that correspond to morphotypes and flowering habit but not to geography. Pino Del Carpio et al. (2011) used phenotypic, metabolic and molecular markers to assess 168 accessions of *B. rapa* and concluded that metabolic data provided a similar classification as the genotypic AFLP data (Zhao et al. 2005, 2007). Warwick et al. (2008) evaluated patterns and levels of genetic diversity in 93 Canadian spring turnip rape accessions by AFLP markers and revealed three main clusters corresponding to European (I), Indian (III), and a mixed Asian/European/Indian (II) proposed origins of the taxa. Fu and Gugel (2009) investigated genetic variability of Canadian elite cultivars of summer turnip rape by simple sequence repeat (SSR) markers. They discovered two distinct groups of nine cultivars separated by high vs. low glucosinates content. Annisa and Cowling (2013) studied genetic diversity in a global collection of 164 oilseed *B. rapa* accessions (including only one Chinese *B. rapa* accession) using SSR markers, in context with the cytological and morphological methods. On the basis of SSR data, their results indicated three distinct groups belonging to South Asia, Southern Europe, and Northern Europe. Guo et al. (2014) studied the genetic diversity in a global collection of 173 *B. rapa* accessions by using SSR markers, and reported three molecular genetic groups, one from the classical Old World (Europe and West Asia–North Africa), second from East Asia, and third from East, Central, South, and Southeast Asia. Takahashi et al. (2016) proposed that Central Asia is the sole geographical origin of turnips and most of Japanese turnips were derived from Central Asia.

Brassica rapa also showed cytoplasmic diversity based on PCR products using primers specific to mitochondrial genomes or plasmids (Zhang et al. 2013) and chloroplast SSR markers (Zamani-Nour et al. 2013). All above studies suggested that *B. rapa* is a genetically diverse species in both genomic and cytoplasmic DNA. However, detailed research on comparison of the genetic diversity and genetic relationship between Chinese and exotic *B. rapa* has not been carried out by combining SSR and sequence-related amplified polymorphism (SRAP) technology. Therefore, the present study was designed for investigation of genetic

diversity among Chinese and some exotic oilseed *B. rapa* accessions for providing significant insights for oilseed *B. rapa* and *B. napus* breeding.

The aims of the present study were (1) to characterize the levels and patterns of genetic diversity among a set of Chinese and some exotic *B. rapa* accessions and (2) to compare the diversity between Chinese and the exotic *B. rapa* accessions.

Materials and methods

Plant samples – A collection of 65 accessions including 42 Chinese and 12 exotic *B. rapa* along with three *B. napus*, two each of *B. juncea*, *B. carinata*, *B. nigra,* and *B. oleracea* were analyzed in this study. They contained 41 accessions of *B. rapa* subsp. *oleifera* (turnip rape), eight *B. rapa* subsp. *chinensis*, three *B. rapa* subsp. *pekinensis*, one each of *B. rapa* subsp. *chinensis* var. *tai-tsai* Lin and ssp. *trilocularis* (Table 1). Eleven other *Brassica* accessions of *B. napus*, *B. juncea*, *B. carinata*, *B. nigra,* and *B. oleracea* were included as reference. All these accessions were planted in the experimental field of Northwest A&F University at Yangling, Shaanxi, PR China, on September 20, 2013.

Genomic DNA extraction – Young leaves of 15 plants of each accession were randomly collected from plants grown in the field and ground in liquid nitrogen. Total genomic DNA was isolated using the cetyltrimethylammonium bromide (CTAB) method as described previously (Murray and Thompson 1980). The DNA pellet was dissolved in 100 µL TE buffer and detected for integrity and quality on 0.8% agarose gel. The concentration of the DNA samples was calculated by using Epoch Microplate Spectrophotometer (Epoch, USA), and the final dilution was done before use at 100 ng μL^{-1}.

Sequence-related amplified polymorphism (SRAP) analysis – Forty-three pairs of SRAP primers (Table S1) were chosen to analyze the tested accessions because of their superior polymorphism shown in previous work of our laboratory. These 43 selected SRAP primer combinations were synthesized by BioAsia (Shanghai, China). PCR reactions were performed in a 10 µL volume containing 2 µL DNA template, 0.6 µL (10 µM) of each primer, 5 µL PCR MIX containing 1 × PCR buffer, 500 µM dNTP each, 20 mM Tris-HCL (pH 8.3), 100 mM KCl, 3 mM $MgCl_2$; Golden DNA Polymerase; 2.5 U μl^{-1} (Tiangen Biotech Co., Ltd, Beijing, China), and sterile distilled water. The amplification program was performed in C1000 thermal cycler (Bio-Rad Co. Ltd. America) following these steps: 5 min at 94 °C; 30 s at 94 °C, 60 s at 56 °C, 45 s at 72 °C, 40 cycles, and a final extension at 72 °C for 5 min. Polymorphism at each locus was assessed by electrophoresis of PCR products on 8% polyacrylamide (w/v) gel in 1 × Tris–borate–ethylenediaminetetraacetic acid (EDTA) (TBE) and visualized by silver staining.

Simple sequence repeats (SSR) analysis – SSR analysis was performed by PCR with 36 pairs of SSR primers (Table S2). The primers were selected for high polymorphism and easy to score quality. The SSR reaction system is same as described for SRAP experiment. The PCR reaction was performed as follows: 1 min at 95 °C; 1 min at 94 °C, 1 min at 35 °C, and 1 min at 72 °C for 5 cycles; 1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C for 34 cycles, and 7 min incubation at 72 °C.

All the experiments for SRAP and SSR were carried out for twice, and only the reproducible bands for each sample were recorded in both replications.

Data collection and analysis – In the SRAP molecular analysis only strong, reproducible and clearly distinguished polymorphic fragments among all accessions were collected and used in the data analysis. The profile data produced by SRAP markers were scored manually for each fragment in every accession for each primer pair and recorded as 1 representing the presence of a fragment, 0 representing the absence of a fragment, and 9 as the missing of a fragment. SSR results were analyzed by loci, and alleles were detected for each locus. For jointly analyzing the data of SRAP and SSR, we also used the same scores as 1, 0, and 9 to record the SSR results as to record SRAP data (scoring of a representative gel of SSR is shown in Table S3). The following formula was used to calculate the polymorphic information content (PIC) per marker: $\mathrm{PIC} = 1 - \sum_{j=1}^{n} P_{ij}^2$, where P_{ij} is the frequency of the jth allele of the ith marker locus and n is the total number of alleles.

The data were analyzed using the qualitative routine to generate simple matching coefficients (SMC), calculated as $\mathrm{SMC} = a^{-1}(n - d)$, where a is the number of fragments in common between two accessions, n is the number of fragments in the matrix, and d is the number of fragments absent in both accessions (Sokal and Michener 1958). SMC was used to construct a dendrogram by the unweighted pair-group method with arithmetic average (UPGMA) and the sequential, hierarchical, and nested clustering (SHAN) routine in the NTSYS-pc2.10 program (Rohlf 1998). The principal component analysis (PCA) was done by first calculating a correlation matrix among markers. Eigenvalues and eigenvectors were then obtained from the correlation matrix and were used to calculate the coordinates

Table 1 List of 65 *Brassica* accessions used in this study

No.	Accession name	Origin	Taxa
1	Parkland	Canada	*B. rapa* L. subsp. *oleifera*
2	Tobin-1	Canada	*B. rapa* L. subsp. *oleifera*
3	Tobin-2	Canada	*B. rapa* L. subsp. *oleifera*
4	Dabaicaizajiaozhong	China	*B. rapa* L. subsp. *pekinensis*
5	200	China (Gansu)	*B. rapa* L. subsp. *oleifera*
6	257	China (Gansu)	*B. rapa* L. subsp. *oleifera*
7	703	China (Gansu)	*B. rapa* L. subsp. *oleifera*
8	BaicaixingYoucai D1	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
9	BaishuiYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
10	Baiyu	China (Zhejiang)	*B. rapa* L. subsp. *oleifera*
11	BaiYeTaCai	China (Shanghai)	*B. rapa* L. subsp. *chinensis* var. *tai-tsai* Lin
12	BinxianbeijiYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
13	BinxianxinminYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
14	BinxianyimenYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
15	FenyangYoucai	China (Shanxi)	*B. rapa* L. subsp. *oleifera*
16	Hao You11	China (Gansu)	*B. rapa* L. subsp. *oleifera*
17	Hei You Bai Cai	China (Henan)	*B. rapa* L. subsp. *chinensis*
18	Huai Nan Huang Xin Cai	China (Anhui)	*B. rapa* L. subsp. *chinensis*
19	HuangzeYoucai	China (Jiangsu)	*B. rapa* L. subsp. *oleifera*
20	Jingninghongheizi	China (Zhejiang)	*B. rapa* L. subsp. *oleifera*
21	LinqiYoucai	China (Shanxi)	*B. rapa* L. subsp. *oleifera*
22	LongquanheYoucai	China (Zhejiang)	*B. rapa* L. subsp. *chinensis*
23	LongYou N. 6	China (Gansu)	*B. rapa* L. subsp. *oleifera*
24	LongYou N. 8	China (Gansu)	*B. rapa* L. subsp. *oleifera*
25	LongYou N. 9	China (Gansu)	*B. rapa* L. subsp. *oleifera*
26	Re Kang50	China (Shandong)	*B. rapa* L. subsp. *pekinensis*
27	Shang hai Ji Mao Cai	China (Shanghai)	*B. rapa* L. subsp. *chinensis*
28	Shang hai Qing (Yong an)	China (Shanghai)	*B. rapa* L. subsp. *chinensis*
29	Shang hai Qing (Yu feng)	China (Shanghai)	*B. rapa* L. subsp. *chinensis*
30	Si Ji Xiao Bai Cai	China (Henan)	*B. rapa* L. subsp. *chinensis*
31	TianYou No. 2	China (Gansu)	*B. rapa* L. subsp. *oleifera*
32	TianYou No. 8	China (Gansu)	*B. rapa* L. subsp. *oleifera*
33	Xia lv Ming Xing	China (Shandong)	*B. rapa* L. subsp. *pekinensis*
34	XinjiangxianYoucai	China (Shanxi)	*B. rapa* L. subsp. *oleifera*
35	YaYou No. 1	China (Sichuan)	*B. rapa* L. subsp. *oleifera*
36	Hei You Bai Cai	China (Guizhou)	*B. rapa* L. subsp. *chinensis*
37	YongshouhuaipingYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
38	Linyou147	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
39	LinyoutongshuwanYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
40	LinyoucuimunanbaYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
41	LinyoucuimubanpoYoucai	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
42	Yellow sarson	India	*B. rapa* L. subsp. *trilocularis*
43	DongYoucai No. 1	China (Gansu)	*B. rapa* L. subsp. *oleifera*
44	Tianxuan No. 8	China (Gansu)	*B. rapa* L. subsp. *oleifera*
45	Tianyouxinxuan	China (Gansu)	*B. rapa* L. subsp. *oleifera*
46	Gaokeyinzhong4	China (Shaanxi)	*B. rapa* L. subsp. *oleifera*
47	Opava	Czechoslovakia	*B. rapa* L. subsp. *oleifera*
48	Rapido	Sweden	*B. rapa* L. subsp. *oleifera*

Table 1 continued

No.	Accession name	Origin	Taxa
49	Chicon	France	*B. rapa* L. subsp. *oleifera*
50	Izumrudnaja K 193	Union of Soviet Socialist Republics	*B. rapa* L. subsp. *oleifera*
51	Brachina	Poland	*B. rapa* L. subsp. *oleifera*
52	Asko	Germany	*B. rapa* L. subsp. *oleifera*
53	Ante	Sweden	*B. rapa* L. subsp. *oleifera*
54	Evvisa	Union of Soviet Socialist Republics	*B. rapa* L. subsp. *oleifera*
55	New Type Youcai	China (Shaanxi)	*B. napus* L.
56	C3	China (Shaanxi)	*B. napus* L.
57	Zhongshuang No. 9	China (Hubei)	*B. napus* L.
58	Shaanjie	China (Shaanxi)	*B. juncea* L.
59	Yuanjie-1	China (Shaanxi)	*B. juncea* L.
60	09H2901003	Ethiopia	*B. carinata* A. BRAUN
61	09H2901004	Pakistan	*B. carinata* A. BRAUN
62	Hneda	Denmark	*B. nigra* L. em. KOCH
63	Sizaja	Union of Soviet Socialist Republics	*B. nigra* L. em. KOCH
64	Wanfeng	China	*B. oleracea* L. var. *capitata*
65	Zidan	China	*B. oleracea* L. var. *capitata*

of each accession. The 0, 1 matrix of SRAP and SSR markers was also used for population structure analysis by Structure version 2.3.4 (Pritchard et al. 2000). All markers were treated as dominant markers, so the recessive allele model was used. The admixture model was used in the analysis. Length of burn-in period before the start of data collection and number of Markov Chain Monte Carlo analysis repeats after burn-in were both set at 10,000. The number of groups (K) ranged from 1 to 10. For the analysis of molecular variance (AMOVA), all accessions were classified into three groups, Chinese *B. rapa*, exotic *B. rapa,* and other Brassica. The components of variance attributable to different varieties and breeding lines were estimated from the genetic distance matrix, as specified in the AMOVA procedure in ARLEQUIN version 2.000. A nonparametric permutation procedure with 3000 permutations was used to test the significance of variance components associated with the different possible levels of genetic structure in this study (Excoffier et al. 1992). The pairwise F_{st} values, a value of F statistic analogs computed from AMOVA, were used to compare genetic distances between any two groups.

Results

Marker polymorphism – A total of 401 polymorphic bands were detected by SSR and SRAP primer pairs in the all 65 accessions presented in Table 2. The average number of polymorphic fragments detected by SRAP markers was 6.23 ranging from 2 to 11, whereas that for SSR was 3.69 ranging from 2 to 7 (Table S1 and Table S2). The maximum number of 11 polymorphic bands was detected by SRAP primer pair Em14Me32, while in case of SSR molecular marker maximum number of 7 bands was revealed by primer pair BrGMS635. The average PIC value 0.61 detected by SRAP markers was higher than that of SSR markers 0.42. Genetic distance varied from 0.0261 to 0.5112 and 0.0538 to 0.5600 for SRAP and SSR analysis, respectively (Table 2).

Cluster and principal component analysis – The molecular marker data of 401 SRAP and SSR polymorphic bands were jointly used to calculate genetic similarity coefficient for all accessions, and a dendogram was constructed from these similarity coefficient data with the UPGMA method (Fig. 1). Genetic similarity estimates varied from 0.65 to 0.97 for all 65 accessions. The 65 accessions were divided into five major clusters at the coefficient value of 0.733, and three accessions (No. 30, 44, and 42) were revealed as outliers. Cluster I contained all exotic accessions of *B. rapa* subsp. *oleifera* except accession No. 42, *B. rapa* subsp. *trilocularis* (yellow sarson) from India. Cluster II contained 38 *B. rapa* accessions from China. The Chinese accessions in the cluster II could be subdivided into some small groups. Three *B. rapa* subsp. *pekinensis* accessions (No. 4, 26, and 33) were clustered together into a small group. Eight *B. rapa* subsp. *chinensis* (No. 17, 18, 22, 27, 28, 29, 30, and 36) was scattered

Table 2 Amplification description of SSR and SRAP primers

Marker types	Total primers	Polymorphic primers	Allele numbers/polymorphic bands	PIC/average	Genetic distance
SSR	36	36	133	0.12–0.61/0.42	0.0538–0.5600
SRAP	43	43	268	0.34–0.83/0.61	0.0261–0.5112

relative widely in some small groups of the cluster II, indicating that this subspecies has a relatively larger variation. Cluster III included two each of *B. juncea* and *B. nigra* accessions. Cluster IV consisted five accessions, three (No. 55, 56, and 57) *B. napus*, and two (No. 37, 41) *B. rapa* accessions. Cluster V contained four (No. 60, 61, 64, and 65) accessions, two each of *B. carinata* and *B. oleracea,* respectively. Most *Brassica* species inclined to cluster within their own varietal groups in the cluster tree. However, two *B. rapa* accessions from China "YongshouhuaipingYoucai" (No. 37) and "LinyoucuimubanpoYoucai" (No. 41) unexpectedly clustered together with *B. napus* accessions in cluster IV.

The principal component analysis (PCA) result was similar to the cluster analysis (Fig. 2). The first two principal components accounted for 15.80 and 6.79% of the total variation, respectively. All 11 other *Brassica* accessions scattered widely from the *B. rapa* accessions. PCA results divided all *B. rapa* accessions into two major groups, Chinese origin and exotic *B. rapa*, except two accessions No. 37 and 41.

Population structure analysis – The break point of the estimated Ln probability of data [Ln P (D)] was obtained when $K = 5$, suggesting the presence of five groups, GI-GV (Fig. 3). Group I contained four accessions, two of each *B. carinata, B. oleracea*, Group II included five accessions, three of *B. napus* together with two *B. rapa* accessions, Group III included four accessions, two of each *B. juncea* and *B. nigra*, respectively, Group IV formed by 41 accessions of *B. rapa* from China. Group V contained 11 exotic *B. rapa* accessions. In general, the population structure analysis demonstrated highly accordance with cluster analysis and PCA results.

Analysis of molecular variance – On the basis of *Brassica* species and their geographical origin, all the 65 accessions used in this study were classified into three groups, exotic *B. rapa*, Chinese *B. rapa,* and other *Brassicas*, for analysis of molecular variance (AMOVA). The AMOVA results indicated that 26.10% of the total variation was due to differences among populations and 73.90% variation was due to difference within populations (Table 3). The pairwise F_{st} values of three groups were all significant (Table 4).

Discussion

B. rapa is both an economically and agriculturally important crop with wide geographical ranges (Prakash and Hinata 1980; Liu 1984). Various studies have been conducted to explore the genetic diversity in *B. rapa* (McGrath and Quiros 1992; Zhao et al. 2005; Takuno et al. 2007; Warwick et al. 2008; Fu and Gugel 2009; Annisa and Cowling 2013). The previous studies suggested that the classical Old World is the center of origin of *B. rapa*, with centers of diversity in East Asia along with ancient trade routes in Asia, and recent migration to the New World (Song et al. 1990; Zhao et al. 2005; Guo et al. 2014). In the present investigation, with the combination of SSR and SRAP data, the 65 *Brassica* accessions tested were divided into five major clusters based on the results of cluster, PCA, and structure analysis. Eleven other *Brassica* accessions were separated from *B. rapa* accessions and formed three clusters (Cluster III, Cluster IV, and Cluster V). The majority of the tested *B. rapa* accessions appeared into two main clusters (Cluster I and Cluster II). Cluster I contained 11 of 12 exotic accessions, with only one exotic accession No. 42, yellow sarson with Indian origin not revealed in this cluster. Cluster II contained 38 *B. rapa* accessions from China. According to our expectations, exotic and indigenous Chinese *B. rapa* used in this study were clearly distinguished from each other. It can be concluded that genetic relationships among accessions had the tendency to associate with their geographical origins. Our results are in accordance with previous studies on that the cluster of *B. rapa* was aligned with geographical origin (Zhao et al. 2005, 2007; Takuno et al. 2007; Warwick et al. 2008; Annisa and Cowling 2013). We supported that Europe and East Asia are two centers of diversity of *B. rapa* (Song et al. 1990; Zhao et al. 2005; Guo et al. 2014). *Brassica* accessions also gathered based on their own species. However, two *B. rapa* accessions (No. 37, 41) from China appeared out of its own group and joined *B. napus* group, unexpectedly. These two accessions were collected in the border area between *B. napus* and *B. rapa* cultivated region in Shaanxi, China, and *B. rapa* is readily crossed with *B. napus* to form viable progenies, perhaps these two accessions were the natural hybrid of *B. napus* and *B. rapa*, and their genetic makeup were more close to *B. napus.*

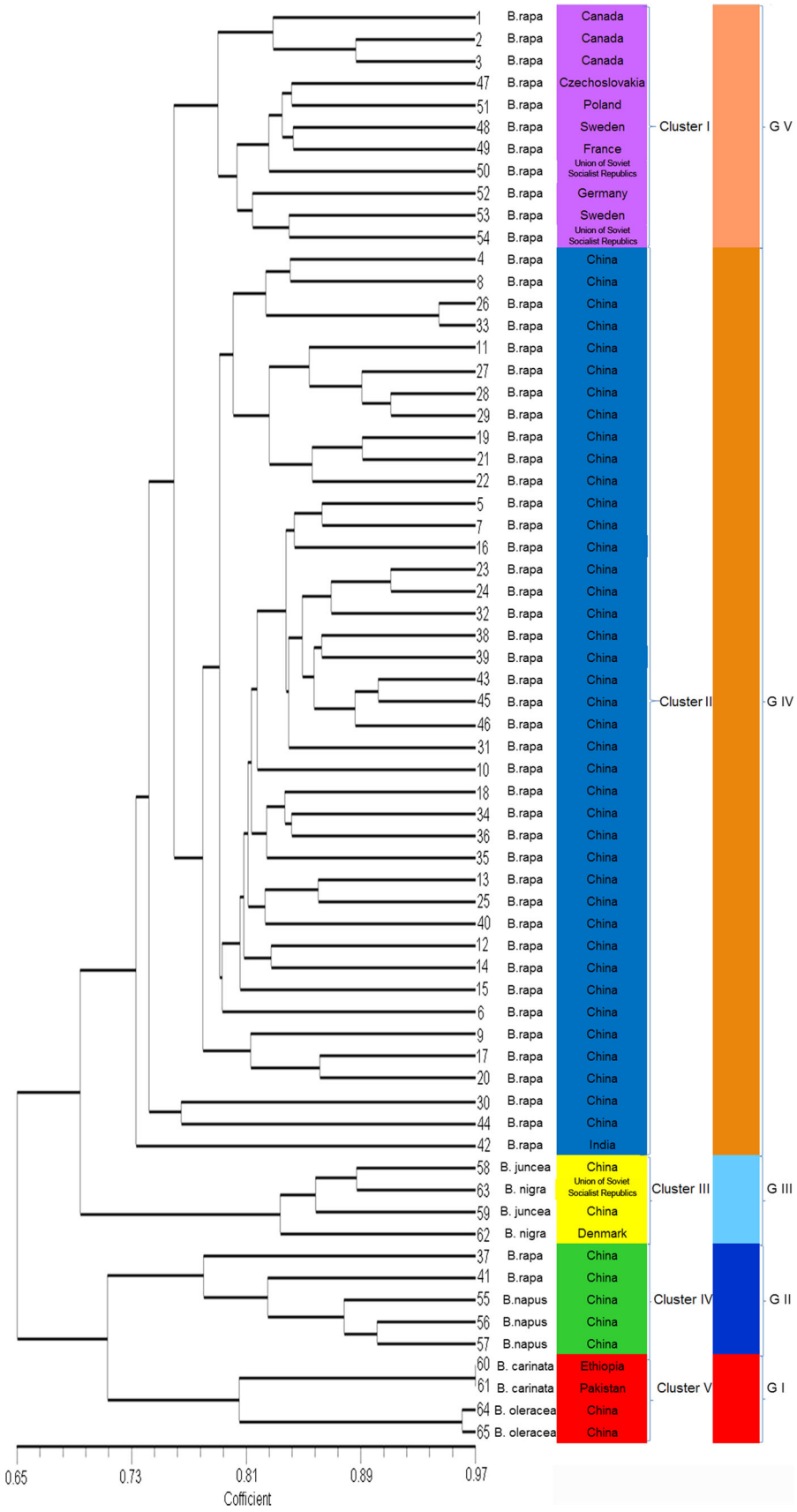

Fig. 1 Clustering of 65 *Brassica* accessions by unweighted pair-group method with arithmetic mean method with SMC index. Brief results of population structure analysis also showed in the right part in comparison with the cluster analysis

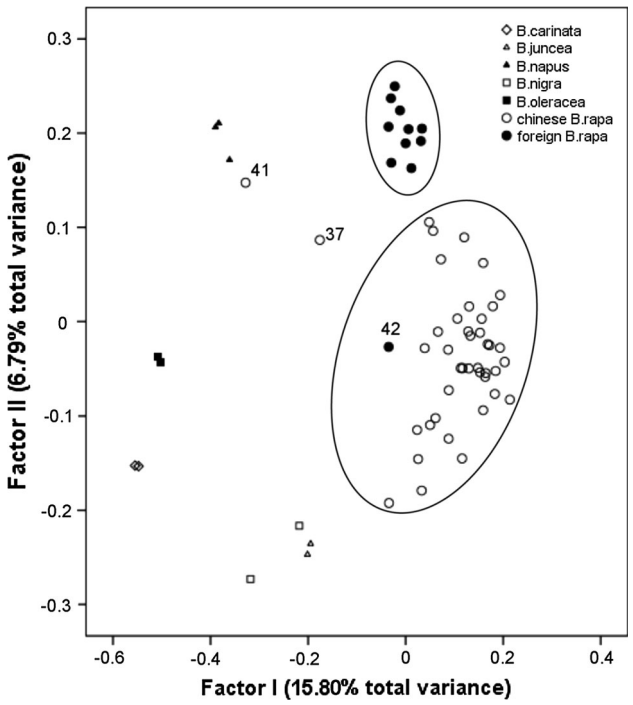

Fig. 2 Biplot of the first two major principal components extracted from SRAP and SSR data

Table 4 Population pairwise F_{st} (values of F statistic analogs)

	Chinese *B. rapa*	Exotic *B. rapa*
Exotic *B. rapa*	0.1633	
Other *Brassica*	0.3231	0.3126

Significance level = 0.05

To measure the source and percentage of variation in *B. rapa*, we divided the tested *Brassica* accessions into three groups: domestic *B. rapa,* exotic *B. rapa*, and other *Brassica* in AMOVA analysis. The results of AMOVA showed that 73.90% of the total genetic variation was found within the groups, and 26.10% among groups, which is in accordance with the previous results (Takuno et al. 2007; Ofori et al. 2008; Zhao et al. 2009; Pino del Caprio et al. 2011; Annisa and Cowling 2013) and is probably due to high rates of cross-pollination of the species. Although the variance among groups is relative low compared with genetic variance within groups, it confirmed that certain genetic variation exists between exotic and Chinese *B. rapa* tested in the present study. Therefore, exchange of the genes between exotic and Chinese *B. rapa* by hybridization

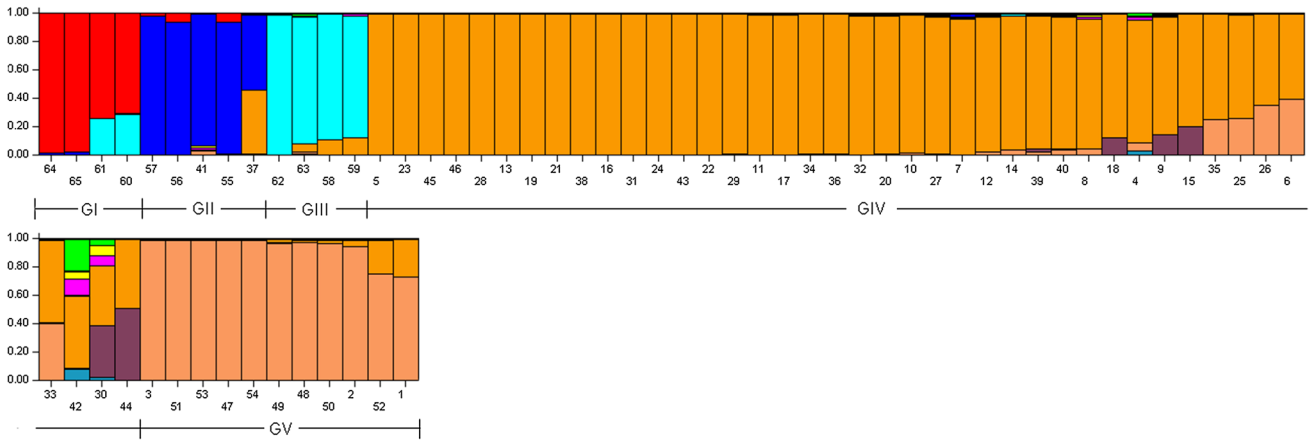

Fig. 3 Population structure of the tested *Brassica* accessions suggested by structure analysis ($K = 5$). The dominant *five colors* represent five inferred groups (GI-GV). Each bar represents each accession. The estimated genetic fraction of each accession of each inferred group was indicated in *different colors*. The numbers under each *bar* is the same accession numbers in Table 1. (Color figure online)

Table 3 Analysis of molecular variance of *Brassica* accessions from different geographical origin

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among populations	2	553.59	14.38	26.10
Within populations	62	2524.47	40.72	73.90
Total	64	3078.06	55.10	

Fixation Index F_{st}: 0.2610

Significance tests (1023 permutations)

will be more beneficial for *Brassica* species in future breeding programs.

The Chinese *B. rapa* accessions displayed in the cluster II could be divided into some subgroups. Three *B. rapa* subsp. *pekinensis* and eight *B. rapa* subsp. *chinensis* accessions were appeared separately within Cluster II; this was not in agreement with the previous results (He et al. 2002; Zhao et al. 2005). They reported separation of leafy *B. rapa* from oilseed *B. rapa*. Our results supported the view that the oilseed *B. rapa* was selected from root or leafy domesticated types in the Old World and East Asia (Reiner et al. 1995; Gómez-Campo and Prakash 1999). Since the number of exotic accession is limited, we cannot reach a conclusive remark about the relationship of the exotic *B. rapa* in the present study. However, there existed considerable genetic difference between Chinese and exotic *B. rapa* tested in the present study as shown by 26.10% genetic variation between them revealed by AMOVA analysis. In the present study, three accessions (No. 30, 42, and 44) of *B. rapa* were found to be genetically distinct from other *B. rapa*, and among them, No. 42 was yellow sarson from India. Annisa and Cowling (2013) reported that the 74 Indian oilseed *B. rapa* in their experiment formed a unique cluster, which is distinguished from the European oilseed *B. rapa*. Hence, it would be desirable to put these genotypes in hybridization program to broaden the genetic diversity of the *B. napus* and to further improve the breeding work of the latter.

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