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ANALYSIS OF THE A GENOME GENETIC DIVERSITY AMONG *BRASSICA NAPUS, B. RAPA* AND *B. JUNCEA* ACCESSIONS USING SPECIFIC SIMPLE SEQUENCE REPEAT MARKERS

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Abstract

This investigation was aimed at evaluating the genetic diversity of 127 accessions among *Brassica napus, B. rapa,* and *B. juncea* by using 15 pairs of the A genome specific simple sequence repeat primers. These 127 accessions could be clearly separated into three groups by cluster analysis, principal component analysis, and population structure analysis separately, and the results analyzed by the three methods were very similar. Group I comprised of mainly *B. napus* accessions and the most of *B. juncea* accessions formed Group II, Group III included nearly all of the *B. rapa* accessions. The result showed that 36.86% of the variance was due to significant differences among populations of species, indicated that abundance genetic diversity existed among the A genome of *B. napus, B. rapa,* and *B. juncea* accessions. *B. napus, B. rapa*, and *B. juncea* have the abundant genetic diversity in the A genome, and some elite genes can be used to broaden the genetic base of them, especially for *B. napus,* in future rapeseed breeding program.

Key words: Genetic diversity; Simple sequence repeat; A genome; *Brassica* cultivars.

Introduction

Brassica rapa (AA, 2n = 20), *B. nigra* (BB, 2n = 16) and *B. oleracea* (CC, 2n = 18) are three *Brassica*diploid species, then *B. napus* (AACC, 2n = 38), *B. Juncea* (AABB, 2n = 36), and *B. carinata* (BBCC, 2n = 34) were obtained from spontaneous interspecific crosses among these three diploids, and all these six *Brassica* species form the U's triangle (U N, 1935).

B. napus, B. rapa, and *B. juncea* are three important *Brassica* cultivars in China and they all have the A genome. *B. rapa* and *B. juncea* were cultivated in China for centuries which have established diversity (Liu, 2000). *B. napus* was the youngest among the three species, and may have 500 years cultivation history (Downey & Robbelen, 1989; Gomez-Campo & Prakash, 1999). *B. napus* was first introduced to China from the 1930s to 1940sfrom Europe and Japan, separately (Liu, 2000). After decades of development, *B. napus* became the most important oil crop in China (Wang, 2010). Chinese rapeseed breeders developed most of the rapeseed cultivars through pedigree breeding method and interspecific hybridization between the introducing foreign *B. napus* accessions and the indigenous *B. rapa* varieties (Liu, 2000).The introgression of Chinese *B. rapa* could significantly diversify the genetic basis of the rapeseed and play an important role in the evolution of Chinese rapeseed (Qian *et al.,* 2006).

Accumulated evidence has shown that each of the three basic *Brassica* genomes (A, B, and C) has undergone profound changes in different species (Nishio, 2000; Pires *et al.,* 2004), and has led to the concept of the "subgenome" (Li *et al.,* 2004). Significant intersubgenomicheterosis was observed in hybrids between traditional *B. napus* and the new type *B. napus* (Qian *et al.,* 2005; Li *et al.,* 2006; Xiao *et al.,* 2010; Zou *et al.,* 2010). The later were produced by the partial introgression of subgenomic components from different species such as *B. rapa* (Qian *et al.,* 2005) and *B. carinata* (Li *et al.,* 2006) into *B. napus*. Lu *et al.* (2006) utilized the yellow-seeded *B. juncea* as the gene donor to cross with the double-low *B. napus* cultivar and developed novel yellow-seeded *B. napus* lines with high oil content and double-low or low erucic quality.

The A genome of both *B. juncea* and *B. napus* was derived from *B. rapa*, however, few studies were conducted to evaluate the A genome diversity of these three species. Though the A genome of *B. rapa* and *B. napus* are mostly functionally conserved, some genomic rearrangements have occurred in the A genome between the two species, which indicate that genetic diversity of the A genome existed between *B. napus* and *B. Rapa* (Suwabe *et al.,* 2008; Jiang *et al.,* 2011). Availability of simple sequence repeat (SSR) primers specific for the A genome linkage groups in *Brassica* (Xu *et al.,* 2010; Bus *et al.,* 2011), made it possible to detect genetic diversity among *B. napus, B. rapa,* and *B. juncea* accessions for this genome. In this study, 127 *Brassica* accessions which corresponding to *B. napus, B. juncea,* and *B. rapa* were evaluated by the A genome specific SSR molecular markers, in order to analyze genetic diversity of the A genome from three species and provide useful information in the future rapeseed breeding programs.

Materials and Methods

Plant materials: The plant materials used in the present study included 127 elite accessions, of which 68 accessions were *B. napus*, 46 accessions were *B. rapa,* and 13 accessions were *B. juncea*. Thirty-four of the 68 *B. napus* accessions were from China, 10 from Canada, seven from Czech Republic, five from the United States, three from Germany, two each from Japan, Sweden, Australia, and Poland, and one from France (Table 1). All of these accessions were planted in the experimental station of Northwest A&F University at Yangling, Shaanxi, PR China on 18 Sept. 2013.

Table 1. *Brassica* accessions used in the present investigation.

Code	Accession name	Origin	Type	Species type
1	Wesbell	Australia	Line	*B. napus*
2	Wesbery-1	Australia	Line	*B. napus*
3	Ac Excel	Canada	Line	*B. napus*
4	Defender A	Canada	Line	*B. napus*
5	Defender B	Canada	Line	*B. napus*
6	E0 Excel	Canada	Line	*B. napus*
7	Heateol	Canada	Line	*B. napus*
8	Holly	Canada	Line	*B. napus*
9	RF04	Canada	Line	*B. napus*
10	Tribute	Canada	Line	*B. napus*
11	Belinda	Canada	OP	*B. napus*
12	Celebra	Canada	Op	*B. napus*
13	161	China	Line	*B. napus*
14	220	China	Line	*B. napus*
15	227	China	Line	*B. napus*
16	6206	China	Line	*B. napus*
17	82089	China	Line	*B. napus*
18	8C	China	Line	*B. napus*
19	C105	China	Line	*B. napus*
20	C3	China	Line	*B. napus*
21	D89	China	Line	*B. napus*
22	Danza C1	China	Line	*B. napus*
23	Pol A	China	Line	*B. napus*
24	Pol B	China	Line	*B. napus*
25	Y6	China	Line	*B. napus*
26	Y7	China	Line	*B. napus*
27	Ganza 1F	China	Line	*B. napus*
28	Huayehui	China	Line	*B. napus*
29	Jinyou no.7	China	Line	*B. napus*
30	Mianhui no.1	China	Line	*B. napus*
31	Qin 7F	China	Line	*B. napus*
32	Qinyou no.3	China	Line	*B. napus*
33	Shaan 2A	China	Line	*B. napus*
34	Shaan 2B	China	Line	*B. napus*
35	Shaan 2C	China	Line	*B. napus*
36	Zhongshuang no.10	China	Op	*B. napus*
37	Zhongshuang no.2	China	Op	*B. napus*
38	Zhongshuang no.4	China	Op	*B. napus*
39	Zhongshuang no.5	China	Op	*B. napus*
40	Zhongshuang no.6	China	Op	*B. napus*
41	Zhongshuang no.7	China	Op	*B. napus*
42	Zhongshuang no.9	China	Op	*B. napus*
43	Zhongyou 821-1	China	Op	*B. napus*
44	Zhongyou 821-2	China	Op	*B. napus*
45	Chuanyou no.18	China	Op	*B. napus*
46	Chuanyou no.20	China	Op	*B. napus*
47	Cando	Czech	Op	*B. napus*
48	Catonic	Czech	Op	*B. napus*
49	Ozima repka odila	Czech	Line	*B. napus*
50	Ozima repka oaza	Czech	Line	*B. napus*
51	SP-116	Czech	Line	*B. napus*
52	Baros	Czech	Op	*B. napus*
53	Lisolde	Czech	Op	*B. napus*
54	Tapidor	France	Op	*B. napus*
55	Expander	Germany	Op	*B. napus*
56	Rwsiu	Germany	Op	*B. napus*
57	Sollux	Germany	Op	*B. napus*
58	Nonglin no.36	Japan	Op	*B. napus*
59	Nonglin no.41	Japan	Op	*B. napus*
60	Bronowski	Poland	Line	*B. napus*
61	Libra	Poland	Op	*B. napus*
62	Casino	Sweden	Op	*B. napus*
63	WW1291	Sweden	Line	*B. napus*

Table 1. (Cont'd.).

Code	Accession name	Origin	Type	Species type
64	KS3073	USA	Line	*B. napus*
65	KS3077	USA	Line	*B. napus*
66	KS3132	USA	Line	*B. napus*
67	KS3248	USA	Line	*B. napus*
68	Plainsman	USA	Line	*B. napus*
69	Parkland	Canada	Op	*B. rapa*
70	Yayou no.1	China	Op	*B. rapa*
71	Tobin-1	Canada	Op	*B. rapa*
72	Tobin-2	Canada	Op	*B. rapa*
73	Youcai D1	China	Op	*B. rapa*
74	Chinese Cabbage Hybrid	China	Hybrid	*B. rapa*
75	Shanghaiqing (Yufeng)	China	Op	*B. rapa*
76	Baiye Tacai	China	Op	*B. rapa*
77	Huainan Huangxincai	China	Op	*B. rapa*
78	Shanghaiqing (Yongan)	China	Op	*B. rapa*
79	Heiyou Baicai	China	Op	*B. rapa*
80	Xialv Mingxing	China	Op	*B. rapa*
81	Rekang 50	China	Op	*B. rapa*
82	Shanghai Jimaocai	China	Op	*B. rapa*
83	Siji Xiaobaicai	China	Op	*B. rapa*
84	Yuanzhong Heiyoucai	China	Op	*B. rapa*
85	Tianyou no.2	China	Op	*B. rapa*
86	Tianyou no.8	China	Op	*B. rapa*
87	Haoyou no.11	China	Op	*B. rapa*
88	703	China	Op	*B. rapa*
89	200	China	Op	*B. rapa*
90	257	China	Op	*B. rapa*
91	Longyou no.6	China	Op	*B. rapa*
92	Longyou no.9	China	Op	*B. rapa*
93	Longyou no.8	China	Op	*B. rapa*
94	Binxian Yimen Youcai	China	Op	*B. rapa*
95	Binxian Beiji Youcai	China	Op	*B. rapa*
96	Binxian Xinmin Youcai	China	Op	*B. rapa*
97	Longquan Heiyoucai	China	Op	*B. rapa*
98	Jingning Heizi	China	Op	*B. rapa*
99	Baiyu	China	Op	*B. rapa*
100	Huangze Youcai	China	Op	*B. rapa*
101	Baishui Youcai	China	Op	*B. rapa*
102	Fenyang Youcai	China	Op	*B. rapa*
103	Linqi Youcai	China	Op	*B. rapa*
104	Xinjiangxian Youcai	China	Op	*B. rapa*
105	Yongshou Huaipinglinchang	China	Op	*B. rapa*
106	Linyou	China	Op	*B. rapa*
107	Linyou Tongshuwan	China	Op	*B. rapa*
108	Lingyou Cuimunanba	China	Op	*B. rapa*
109	Lingyou Cuimubanpo	China	Op	*B. rapa*
110	Yellow Sarson	Indian	line	*B. rapa*
111	Dongyoucai no.1	China	OP	*B. rapa*
112	Tianxuan no.8	China	OP	*B. rapa*
113	Tianyou Xinxuan	China	line	*B. rapa*
114	Gaoke Yinzhong 4	China	OP	*B. rapa*
115	2598-1	China	line	*B. juncea*
116	2598-2	China	line	*B. juncea*
117	Fanqi Xiaohuang	China	line	*B. juncea*
118	Laifeng Mawei	China	line	*B. juncea*
119	Qinghai Yejie	China	line	*B. juncea*
120	Shaan Jie	China	line	*B. juncea*
121	Weiyuan Dahuangjie-1	China	line	*B. juncea*
122	Weiyuan Dahuangjie-2	China	line	*B. juncea*
123	Weiyuan Youcai	China	line	*B. juncea*
124	Wenxi Youcai	China	line	*B. juncea*
125	Xinjiang no.2	China	line	*B. juncea*
126	Yuanjie-1	China	line	*B. juncea*
127	Huairen Youcai	China	line	*B. juncea*

DNA extraction and simple sequence repeat analysis: Fifteen three-leaf stage plantlets were randomly chosen from each accession for total genomic DNA isolation using the Cetyltrimethyl ammonium bromide (CTAB)method (Murray and Thompson, 1980).The DNA pellet was dissolved in 50 µL TE buffer (1mM ethylene-diaminetetraacetic acid (EDTA) and 10 mMTris-HCl, pH 8.0). All DNA samples were tested on 0.8% agarose gel for their concentration and quality and stored at -20°C.

One hundred and ninety-two SSR primers used in the A genomic diversity analysis in *Brassica* were synthesized by Sangon Biotechnology Company (Shanghai, China) (Bus *et al.*, 2011; Xu *et al.*, 2010), and these primers were widely distributed on 10 total chromosomes of the A genome. Polymerase chain reactions (PCR) were performed in a total volume of 10 µL containing about 50 ng of genomic DNA, 2 mM $MgCl_2$, 0.1 µM of each primer, 150 µMdNTP, and 1x Taq buffer. The PCR program (PTC-200, Bio-RAD, Hercules, CA) was as follows: 5 min pre-denature at 94°C; 0.5 min denature at 94°C, 1 min annealing at 56°C, and 0.75 min extension at 72°C for 40 cycles; and 5 min incubation at 72°C. The PCR products were separated by 8% polyacrylamide gel electrophoresis (w/v) gel in 1x Tris-borate-EDTA (TBE) and visualized by silver staining.

Data analysis: Each locus of each accession was recorded as presence (1) or absence (0) in the SSR analysis. Polymorphism information content (PIC) was calculated by the formula:

$$PIC=1-\sum_{j=1}^{n} P_{ij}^{\ 2}$$

where P_{ij} is the frequency of the *j*th microsatellite allele of the *i*th marker locus and *n* is the total number of allele. The data were analysed using the qualitative routine to generate simple matching coefficients (SMC), calculated as SMC= *a/(n-d)*, where *a* is the number of bands in common between two accessions, *n* is the number of bands in the matrix, and *d* is the number of bands absent in both accessions (Sokal & Michener, 1958). SMC was used to construct a dendrogram by the unweighted pair-group method with arithmetic mean (UPGMA) and the sequential, hierarchical and nested clustering (SHAN) routine in the NTSYS program (Rohlf, 2001). Principal component analysis (PCA) was performed with the same program using the Decenter and Eigen procedures.

The 0, 1 matrix of SSR markers was also used for population structure analysis by Structure version 2.3.3 (Falush *et al.*, 2003, 2007; Pritchard *et al.*, 2000) as described previously (Li *et al.*, 2012). For the analysis of molecular variance (AMOVA), all accessions were classified into three groups based on their genetic background. The components of variance attributable to different genetic background and among individuals within genetic background were estimated from the genetic distance matrix, as specified in the AMOVA procedure in ARLEQUIN version 3.1 (Schneider *et al.*, 2000). 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 Fst values, a value of F statistic analogs computed from AMOVA, were used to compare genetic distances between any two groups.

Results

Marker polymorphism: Fifteen SSR primers (Table 2) were selected from 192 SSR primers because they were more polymorphic and produced stronger fragments. These 15 SSR primers were used to amplify the whole 127 accessions. A total of 58 polymorphic fragments were detected by these SSR primers. The number of detected alleles per primer pair ranged from 2 to 7 with a mean of 3.87 (Table 2). Two primers, BrgMS383 and BrgMS318, produced the largest number of polymorphic alleles (7 alleles) followed by BrgMS165 (5 alleles) and BrgMS1774 (5 alleles). Primer pairs BrgMS635, BrgMS135, and BrgMS571 produced the lowest number of polymorphic allele (2 alleles for each primer combination). The PIC value ranged from 0.173 for BrgMS571 to 0.831 for BrgMS318 with a mean of 0.567.

Table 2. The set of A genome polymorphic SSR primers.

Name	Chr	Forward primer	Reverse primer	Locus	PIC value
BrgMS635	A1	GTGTTTCTCTTCAACGCCTTTT	CACAAAGAATCCCCACAGATTT	2	0.374
BrgMS37	A1	CTGCCTTTGGATCGTCTTCTAT	TACGAGGTTCGGTTTTCTTCAT	4	0.688
BRAS078	A1	ATTGGGTTCTGACCTTTTCTC	CTTTTCCTCATCGCTACCAC	3	0.579
BrgMS382	A3	TCATCTCCCCTCACTTTCTCAT	ATGATCTGTTGTTGTCGGTTTG	3	0.589
Na12-E02	A3	TTGAAGTAGTTGGAGTAATTGGAGG	CAGCAGCCACAACCTTACG	4	0.686
BrgMS135	A4	GCATCACCCCTAGTTAATCGAA	AAGAAGGGAGAAACCTGAAACC	2	0.347
BrgMS165	A5	TCTATGTAATCGTCGTCGCAGT	GCTCTTTCTCAGTCCCTCTTGA	5	0.652
BnGMS662	A5	CGATCGAATTGCACTGTACT	ATGCACAGAGCTGAAGAAAT	3	0.545
BrgMS1757	A5	ATCGTCTCCACCACCTTATCC	ATCGGTAATTGAAATCGAGAGG	3	0.424
BrgMS1774	A5	GCAAGTTACAAGCTACCCCTTT	AAGCGGAGGAGGTTAATGTAGA	5	0.665
BrgMS318	A9	AACGAAAGACTCGACAGAAAGG	GTGAAGGTCAGGCGAATTTAAG	7	0.831
BrgMS571	A9	TCCCCACCCAGATGAGAGTAT	GAAAGGTCAAGAAGGTGCTGTT	2	0.173
BrgMS287	A10	TGGGTCTCAGTTTCCATTTTCT	TGCTTGTGAATCTTTGTGTGTG	4	0.609
BrgMS383	A10	TCGGGCAGATAAAGTAATCCAT	AGAAACCCCTTCACAACAATGA	7	0.797
BrGMS4514	A10	CTTTCACAACTCACCAGTGCAT	TGTTGTTCCATGTCACACCTTT	3	0.553

Cluster and principal component analysis: A dendrogram was generated using the UPGMA method based on SSR data (Fig. 1). All 127 *Brassica* accessions fell into three major clusters. Cluster I consisted of 67 *B. napus* accessions. Cluster II contained 11 *B. juncea* accessions and one *B. napus* accession 'No.5' from Canada. Cluster III contained all 46 *B.rapa* accessions and two *B. juncea* accessions, 'No.116' and 'No.123'.

The principal component analysis result was similar to the cluster analysis (Fig. 2). The first two principal components accounted for 22.59% and 10.13% of the total variation, respectively. Based on the first two components, the 127 accessions could be clearly separated into three groups. Group I composed of 66 *B. napus* accessions and one *B.rapa*accession 'No.69'. Eleven *B. juncea* accessions formed Group II. Group III included 45 *B. rapa* accessions, two *B. napus* accessions 'No.21' and'No.51', and two *B. juncea* accessions 'No.116' and'No.123'.

Population structure analysis: Three groups were formed when Structure version 2.3.3 was used to analyze the population structure of the total 127 accessions (Fig. 3). Group I contained 65 *B. napus* accessions and one *B. rapa* accession 'No.69'. Group II contained 12 *B. juncea* accessions, one *B. napus* accession 'No.5', and two *B. rapa* accessions 'No.97 and No.100'. Group III contained 43 *B. rapa* accessions, two *B. napus* accessions 'No.21 and No.51', and one *B. juncea* accession 'No.116'.

Analysis of molecular variance: All accessions were classified into three groups based on their species for AMOVA. The result indicated that 36.86% of the variance was due to differences among populations of species and 63.14% was due to difference within species (Table 3). The pairwise Fst values of three species ranged from 0.3491 to 0.4192, which are all significant (Table 4). The highest pairwise Fst value was between *B. juncea* and *B. napus* accessions, which indicated they have the farthest relationship.

Discussion

Rapeseed breeders are more and more concerned about the narrowing of the genetic base of germplasm. *B. napus*, *B. rapa*, and *B. juncea* are three important *Brassica* oil crops in China and they all have the A genome. Both *B. rapa* and *B. juncea* are considered as good resources to widen the genetic base of *B. napus* and the elite genes can be exchanged through interspecific crosses of *B. napus* and *B. rapa*, and *B. juncea*, such as disease and herbicide resistance (Somers *et al.*, 2002; Qian *et al.*, 2005, 2006; Garg *et al.*, 2010; Liu *et al.*, 2010; Li *et al.*, 2013). In this study, 127 *Brassica* accessions which corresponding to *B. napus*, *B. juncea*, and *B. rapa* were evaluated by the A genome SSR markers with the aim of investigating genetic diversity of the A genome of these three species. Three major groups that corresponding to the three *Brassica* species were generated in the current analysis, which obtained by cluster analysis, principal component analysis, and population structure analysis, respectively. The result indicated that 36.86% of the variance was due to significant differences among populations of species. The highest Fst value (0.4192) of three *Brassica* species was found between *B. napus* and *B. juncea*, and lowest Fst value (0.3491) was found between *B. napus* and *B. rapa*, which indicated that abundance genetic diversity existed among the A genome of *B. napus*, *B. rapa*, and *B. juncea* accessions. Thus, the genetic variation existing among the A genome of *B. rapa* and *B. juncea*, remains largely unexplored in genetic improvement of *B. napus*.

In total 192 SSR primers, 15 A genome specific SSR primers were selected to evaluate the genetic diversity of 127 accessions of three *Brassica* species. In this investigation, the *B. rapa* accessions from Canada have the greatest genetic distance from *B. rapa* accessions of Asia (Fig. 1), which indicated the selected primers could be used to analyse the A genome genetic diversity because *B. rapa* accessions only have the A genome.

The plant materials used in the present study only included 13 *B. juncea* accessions, which came from different geographical regions of China, such as Shaanxi, Qinghai, and Xinjiang provinces. However, the A genome of *B. juncea* show more diversity with the A genome of *B. napus*, than the A genome of *B. rapa* by the structure analysis. A set of 95 accessions of *B. juncea* representing oil and vegetable mustards from China, France, India, Pakistan, and Japan were assessed by Wu *et al.* (2009), they reported that the accessions from different regions of China showed abundant diversity. They suggested that the A genome of *B. juncea* will play important role in the future *B. napus* breeding.

In our results, 127 accessions were divided into three groups, which correspond well with three *Brassica* species separately with several exceptions. These results occurred probably because the crosses were happened among *B. napus*, *B. rapa*, and *B. juncea* accessions, which made the gene exchange in the A genome (Qian *et al.*, 2005; Garg *et al.*, 2010), and these outliers may be valuable for the rapeseed genetic improvement.

In early days, *B. rapa* and *B. juncea* were the important traditional oilseed crops in China before the introduction of *B. napus* from Europe and Japan (Liu, 2000), and the materials used in this study are representative accessions in China, which could be used to cross with the *B. napus* in the breeding program. All accessions were divided into three groups which are corresponding to *B. napus*, *B. rapa*, and *B. juncea* separately. *B. rapa* and *B. juncea* could be important germplasm resources for enriching the genetic background of *B. napus*, which will be very valuable for future rapeseed breeding program.

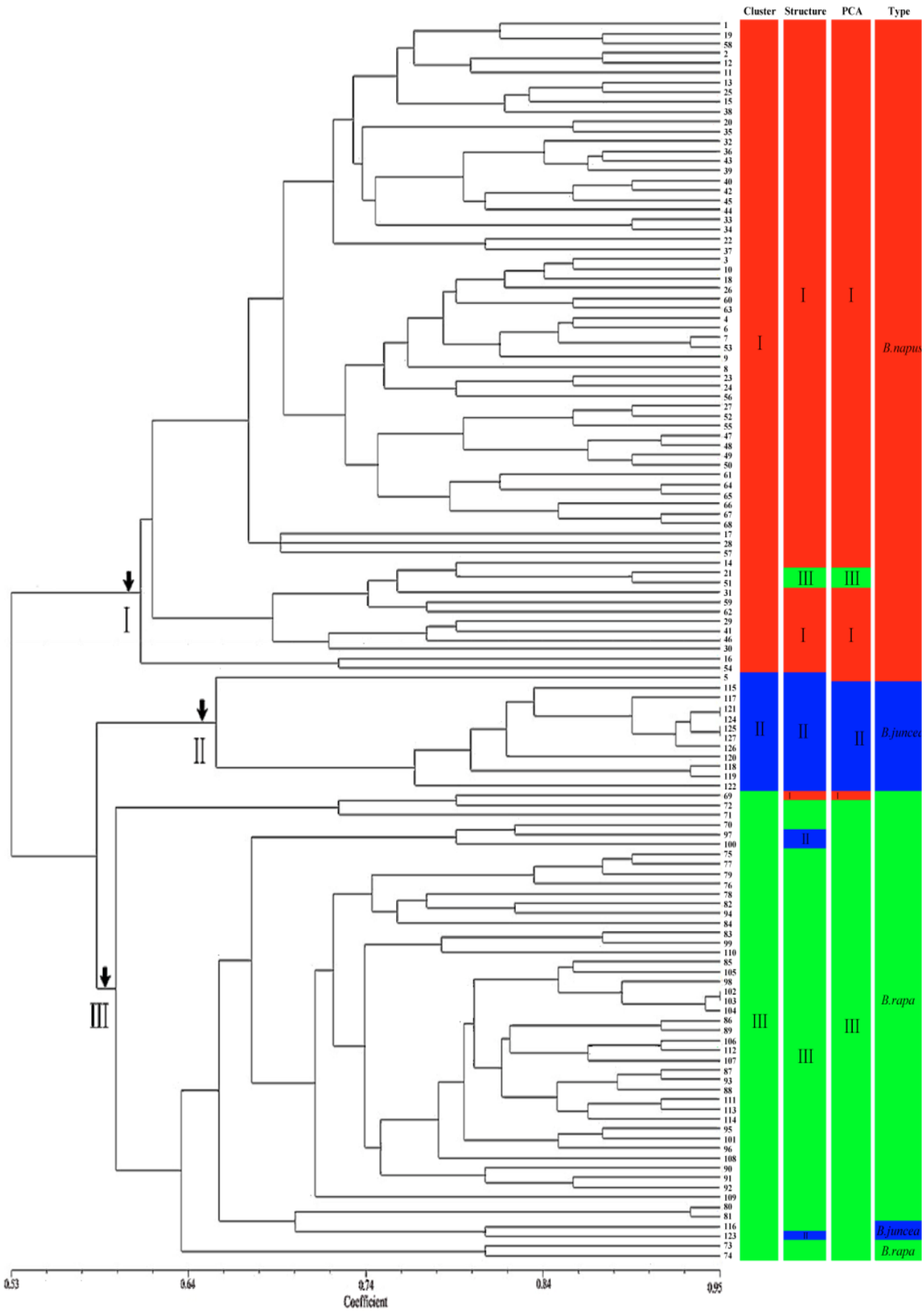

Fig. 1. Clustering of 127 rapeseed accessions by unweighted pair-group method with arithmetic mean method with Dice index. Brief results of population structure analysis and principal components analysis also showed in the right part in comparison with the cluster analysis.

Table 3. Analysis of molecular variance of accessions from three *Brassica* species.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	2	380.9053	5.0103	36.86 %
Within populations	124	1064.0869	8.5813	63.14 %
Total	126	1444.9921	13.5916	100 %

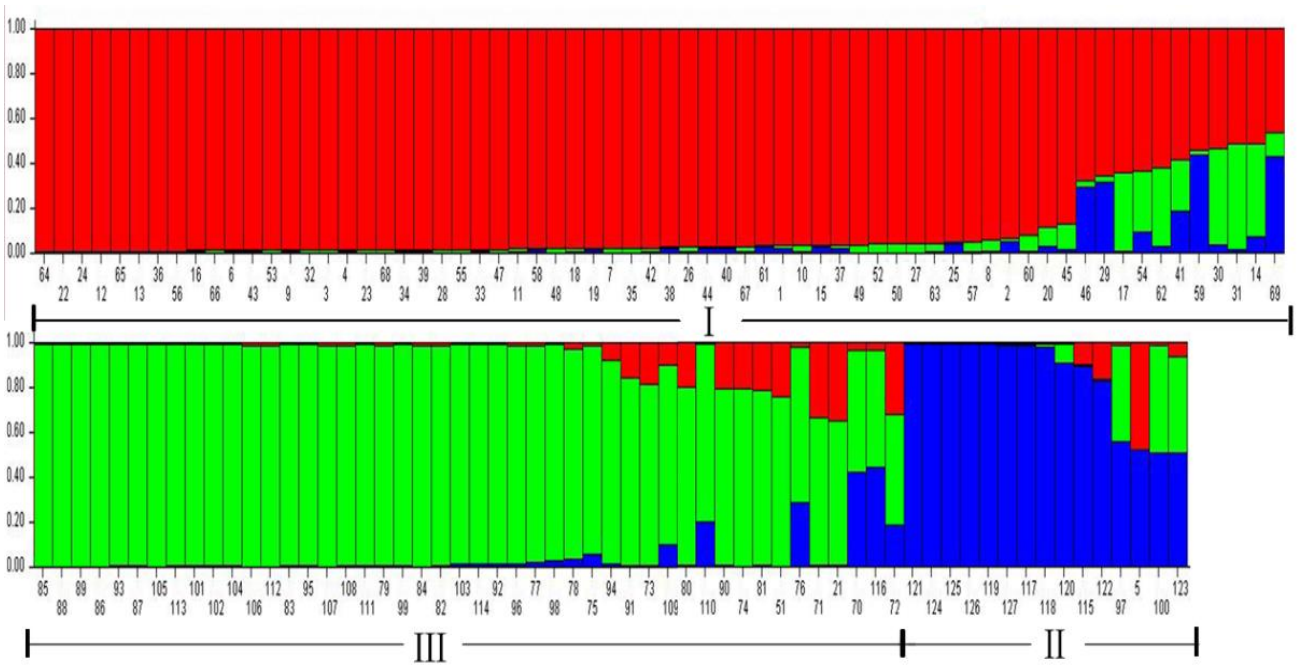

Fig. 3. Population structure of the tested *Brassica* accessions suggested by structure analysis (K = 3). Three colors represent three inferred groups (I – III). Each bar represents each accession. The estimated genetic fraction of each accession of each inferred group was indicated in different colors. The numbers under each bar is the same accession numbers in Table 1.

Table 4. Population pairwise Fsts (values of *F* statistic analogs) between different *Brassica* species.

	B. napus	*B. rapa*	*B .juncea*
B. napus	0		
B. rapa	0.3491	0	
B. juncea	0.4192	0.3645	0

Significance level = 0.01

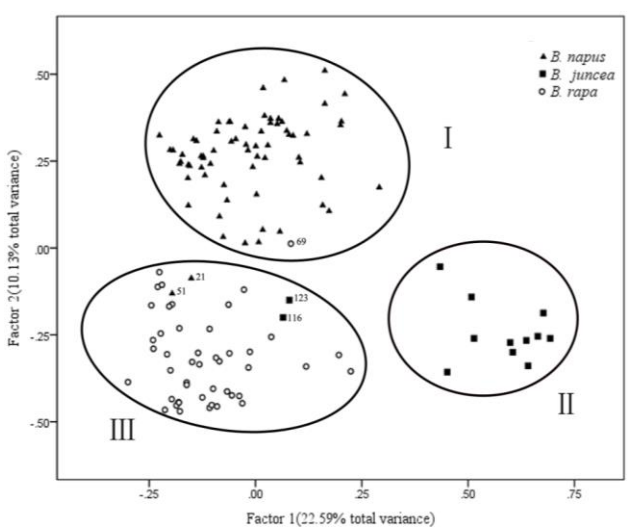

Fig. 2. Biplot of the first two major principal components extracted from simple sequence repeat data.

Conclusion

In this investigation, the A genome genetic diversity was evaluated among 127 accessionsof *B. napus*, *B. rapa*, and *B. juncea* by using the specific SSR primers. Generally, these accessions were clearly separated into three groups, which corresponding to the three *Brassica* species, by cluster analysis, principal component analysis, and population structure analysis. The results showed that abundance genetic diversity existed among the A genome of *B. napus*, *B. rapa*, and *B. juncea* accessions, and some elite genes can be used to broaden the genetic base of them, especially for *B. napus*, in future rapeseed breeding program.

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