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Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed

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Abstract In spite of its short history of being an oil crop in China, the Chinese semi-winter rapeseed (*Brassica napus* L., $2n = 38$, AACC) has been improved rapidly by intentional introgression of genomic components from Chinese *B. rapa* ($2n = 20$, AA). As a result, the Chinese semi-winter rapeseed has diversified genetically from the spring and winter rapeseed grown in the other regions such as Europe and North America. The objectives of this study were to investigate the roles of the introgression of the genomic components from the Chinese *B. rapa* in widening the genetic diversity of rapeseed and to verify the role of this introgression in the evolution of the Chinese rapeseed. Ten lines of the new type of rapeseed, which were produced by introgression of Chinese *B. rapa* to Chinese normal rapeseed, were compared for genetic diversity using amplified fragment length polymorphism (AFLP) with three groups of 35 lines of the normal rapeseed, including 9 semi-winter rapeseed lines from China, 9 winter rapeseed lines from Europe and 17 spring rapeseed lines from Northern Europe, Canada and Australia. Analysis of 799 polymorphic fragments revealed that within the groups, the new type rapeseed had the highest genetic diversity, followed by the semi-winter normal rapeseed from China. Spring and winter rapeseed had the lowest genetic diversity. Among the groups, the new type rapeseed group had the largest average genetic distance to the other three groups. Principal component analysis and cluster analysis, however, could not separate the new type rapeseed group from Chinese normal rapeseed group. Our data suggested that 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. The use of new genetic variation for the exploitation of heterosis in *Brassica* hybrid breeding is discussed

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Introduction

Rapeseed (*Brassica napus* L., AACC) is an important oilseed crop in the world. Based on the differences in growth habit, it is mainly classified into three ecotype groups: winter rapeseed in Europe, which can only turn from vegetative to reproductive growth after a long period of low temperatures (vernalization); semi-winter rapeseed from China, which can flower after a short period of vernalization; and spring rapeseed in Northern Europe, Canada and Australia, which can reproduce without vernalization.

In China, rapeseed is a main oilseed crop, which accounts for about 85% acreage of oilseed *Brassica* (Fu 2000). The history of rapeseed production, however, is rather short. It was introduced from Europe in the 1930–1940s either directly or via Japan. Due to better production potential and superior disease resistance, it has meanwhile replaced the traditional oilseed crop, *B. rapa* (AA), which has been cultivated for more than

6,000 years, with young stem and leaves as vegetables, and seeds for oil production. Meanwhile, Chinese rapeseed cultivars have been selected which are well adapted to local environments, mainly by introgressions from Chinese *B. rapa*. In this study, rapeseed with high proportion of alleles from the genome of Chinese *B. rapa*, derived from interspecies hybridizations, will be referred to as the "new type" rapeseed whereas those not directly derived from interspecies hybridizations will be referred to as "normal" rapeseed.

The grouping into the three ecotype groups has been confirmed with the help of isoenzyme and DNA markers (Becker et al. 1995; Dier and Osborn 1994; Ma et al. 2000; Meng et al. 1997). We speculated that the introgression of Chinese *B. rapa* mainly contributed to the large genetic distances between Chinese rapeseed and the spring and winter rapeseed groups and to the evolution of Chinese rapeseed. In order to verify this speculation, this study aims to detect the genetic changes after the introgression of Chinese *B. rapa* by comparing new type rapeseed (Qian et al. 2005; Li et al. 2005) with normal rapeseed for genetic distance using amplified fragment length polymorphism (AFLP). The creation of the new genetic diversity for the exploitation of *Brassica* subgenomic heterosis will be discussed.

Materials and methods

Plant materials

Four groups of 45 cultivars were chosen from the main rapeseed growing regions in the world (Table 1), These include (1) ten new type rapeseed lines, six lines derived from Chinese *B. napus* × Chinese *B. rapa* and (Chinese *B. napus* × Chinese *B. rapa*) × Chinese *B. rapa* (Qian et al. 2005), four new type rapeseed lines derived from Chinese *B. napus* × (*B. carinata* × Chinese *B. rapa*) (Li et al. 2005), (2) nine semi-winter rapeseed lines from China, four parental *B. napus* of new type rapeseed and five elite inbred lines, (3) nine winter rapeseed lines from Europe, and (4) 17 spring rapeseed lines from Northern Europe, Canada and Australia. The new type of rapeseed lines were chosen because they possessed a high proportion of alleles from the genome of *B. rapa* and showed good agricultural performance in central China. The other lines were selected due to outstanding yielding capacities under growing conditions in the respective part of the world or because they were parents of superior hybrid varieties. Their geographical origins and growth habits are listed in Table 1.

Table 1 List of 45 accessions from different rapeseed growing regions used in this study

Code	Accession	Origin	Growth habit
1	Female of hybrid Haza 6	China	Semi-winter
2	Male of hybrid Haza 6	China	Semi-winter
3	Zhongyou 821	China	Semi-winter
4	Female of hybrid Haza 4	China	Semi-winter
5	Male of hybrid Haza 4	China	Semi-winter
6[a]	HAU 01	China	Semi-winter
7[a]	HAU 02	China	Semi-winter
8[a]	HAU 03	China	Semi-winter
9[b]	HAU 04	China	Semi-winter
10[b]	HAU 05	China	Semi-winter
11[b]	HAU 06	China	Semi-winter
12[b]	HAU 07	China	Semi-winter
13[c]	HAU 08	China	Semi-winter
14[c]	HAU 09	China	Semi-winter
15[c]	HAU 10	China	Semi-winter
16[d]	HAU 11	China	Semi-winter
17[d]	HAU 12	China	Semi-winter
18[d]	HAU 13	China	Semi-winter
19[d]	HAU 14	China	Semi-winter
20	NPZ 01	France	Winter
21	Bristol	France	Winter
22	NPZ 02	Great Britain	Winter
23	NPZ 03	Germany	Winter
24	NPZ 04	Germany	Winter
25	MSL 01	Germany	Winter
26	Express	Germany	Winter
27	MSL 02	Germany	Winter
28	MSL 03	Germany	Winter
29	Lisonne	Germany	Spring
30	NPZ 05	Denmark	Spring
31	NPZ 06	Denmark	Spring
32	Haydn	Denmark	Spring
33	MSL 04	Germany	Spring
34	MSL 05	Germany	Spring
35	MSL 06	Canada	Spring
36	MSL 07	Canada	Spring
37	Profit	Canada	Spring
38	Excel	Canada	Spring
39	NPZ 07	Canada	Spring
40	NPZ 08	Canada	Spring
41	MSL 08	Australia	Spring
42	MSL 09	Australia	Spring
43	Barossa	Australia	Spring
44	Mystic	Australia	Spring
45	NPZ 09	Australia	Spring

[a]New type *B. napus* derived from (*B. napus* × *B. rapa*) × *B. rapa* (Qian et al. 2005)
[b]New type *B. napus* derived from *B. napus* × *B. rapa* (Qian et al. 2005)
[c]New type *B. napus* derived from *B. napus* × (*B. carinata* × *B. rapa*) (Li et al. 2005)
[d]Parental *B. napus* of new type rapeseed

DNA extraction and AFLP analysis

Approximately 20–40 plants from each accession were grown in the greenhouse. Young leaves were collected from 1 month-old seedlings, and pooled for each accession. The genomic DNA was isolated using the CTAB method according to Saghai-Maroof et al. (1984). DNA was restricted with *Pst* I and *Mse* I. After adapter ligation PCR was run with 29 primer combinations essentially as described by Vos et al. (1995). The DNA fragments were separated on a Li-Cor model 4000 Sequencer.

Data analysis

The polymorphic bands were scored among accessions with 1 or 0 for the presence and absence of an AFLP

band, respectively. The genetic distances (GD) between accessions X and Y were calculated using the formula from Nei and Li (1979):

$$GD_{xy} = 1 - 2N_{xy}/(N_x + N_y);$$

where N_{xy} is the number of common bands shared by accession X and Y, and N_x and N_y is the total number of bands in accession X and Y, respectively. The data from the GD matrix among 45 accessions were subjected to cluster analysis using the unweighted pair group method and arithmetic averages (UPGMA) and principal component analysis (PCA) from the NTSYS-PC program (Rohlf 1997). Confidence values of each node of cluster dendrogram were performed by 500 bootstrap resamplings over loci using TFPGA ver. 1.3 (Miller 1997).

An analysis of molecular variance (AMOVA) was performed using the ARLEQUIN software (Schneider et al. 2000) to test the genetic structure within or among groups, sorted according to the geographical origin such as Europe, China, Canada and Australia, and growth habit such as winter type, semi-winter type and spring type (Table 1).

Results

Seven hundred and ninetynine polymorphic AFLP bands, detected among 45 accessions amplified from 29 primer combinations, were employed to analyze the genetic variances among accessions with different geographical regions and growth habits. The results of AMOVA are shown in Table 2. Highly significant variances were found among and within geographical origin groups and growth habit groups ($P \leq 0.001$). The proportion of variation accounted for was 20.17% by geographical origin and 24.48% by growth habit, indicating that the growth habit is more important for the genetic variances between accessions than the geographical origin.

The importance of the growth habit character in genetic diversification of rapeseed could also be supported by cluster analysis, which was performed based on the data of genetic distances between accessions, ranging from 0.04 to 0.60, with an average of 0.36. The rapeseed lines could be clustered into three main groups (Fig. 1), the spring rapeseed group, the winter rapeseed group and the semi-winter rapeseed group from China. This result is in accordance with the differences between accessions in terms of growth habit. The bootstrap values above 50 are listed above the respective branches in Fig. 1. The highest bootstrapping values were found in the winter rapeseed group.

Considering the genetic diversity among the three groups, there are large differences between the Chinese rapeseed group and the spring and winter rapeseed groups, and small genetic differences between spring and winter rapeseed. Considering the genetic diversity within groups, no pronounced clustering of European and Canadian spring rapeseed accessions was found. A similar tendency was found in the winter rapeseed lines, which did not show clustering in accordance with the geographical origin. The Chinese lines, however, exhibited a rather high diversity. For example, three new type rapeseed lines (HAU 08, HAU 09 and HAU 10) together with their parental *B. napus* (HAU 11), were far distant from the other accessions and groups (Fig. 1). This finding of rather high diversity in Chinese rapeseed is supported by the PCA in Fig. 2, where the total variation explained by first and second principal components were 25.18 and 13.21%, respectively.

Both methods, clustering and PCA analysis failed to separate the Chinese breeding lines into two subgroups, the new type rapeseed (N group) and the Chinese normal rapeseed (C group). In order to show the genetic changes after introgression from Chinese *B. rapa*, two subgroups of Chinese rapeseed were compared with the spring rapeseed (S group) and winter rapeseed (W group). The average genetic distances among and within groups and subgroups are listed in Table 3. The highest average distance was detected between the new type rapeseed and spring rapeseed (N/S), followed by N/N. The lowest diversity was found within the winter rapeseed group (W/W). The average of genetic distances between both subgroups of Chinese rapeseed and European winter rapeseed were lower when compared to spring rapeseed (N/W < N/S; C/W < C/S). However, N/W was significantly higher than C/W, and N/S was significantly higher than C/S for the average genetic diversity ($P \leq 0.01$).

Since there was no accession with introgressions only from *B. carinata*, the sole contribution of *B. carinata* for increasing the genetic diversity could not be determined.

Table 2 Analysis of molecular variance (AMOVA) of the 45 accessions with different geographical origin and growth habit

Group/source	*df*	Variance component	Variation accounted for (%)
Geographical origin group			
Among geographical origin groups	3	26.39*	20.17
Within geographical origin groups	41	104.43*	79.83
Total	44	130.82	
Growth habit group			
Among growth habit groups	2	32.85*	24.48
Within growth habit groups	42	101.33*	75.52
Total	44	134.18	

* Significant at $P = 0.001$

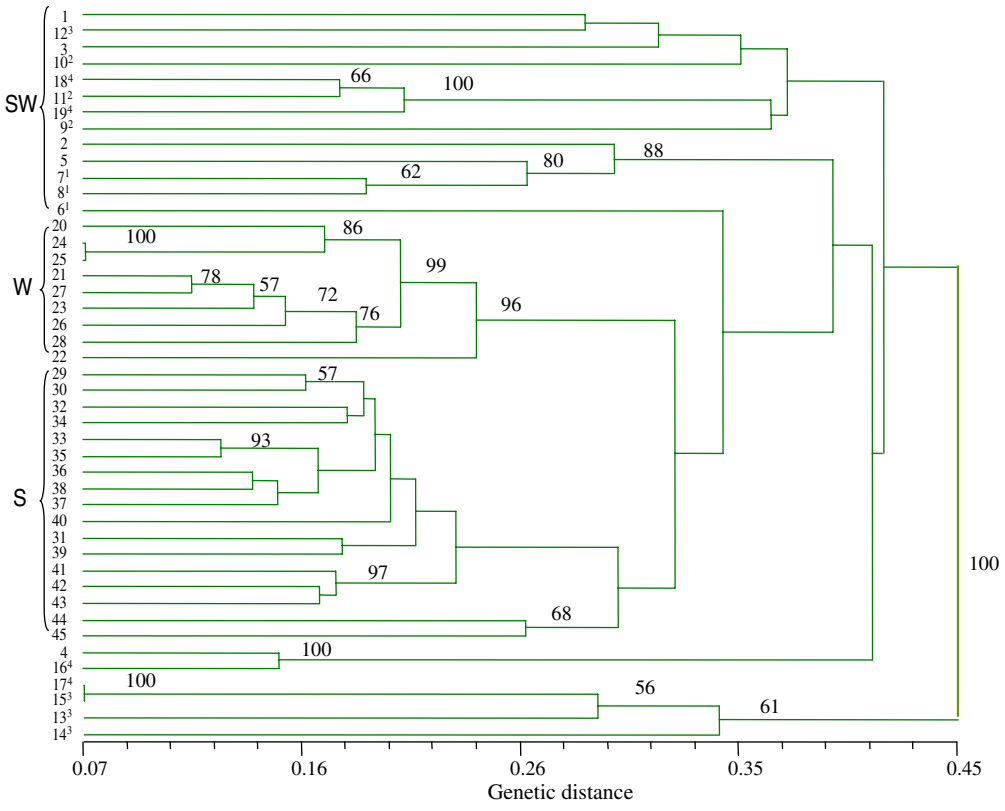

Fig. 1 Cluster analysis of Nei's matrix distances among 45 accessions revealed by 799 AFLP markers. The code of accessions was shown in Table 1. The groups were characterized based on the growth habit of accessions within the group (*S* spring rapeseed group; *SW* semi-winter rapeseed group; *W* winter rapeseed group). The Bootstrap values above 50 were present on the branches, calculated with 500 replications

In contrast, the positive effect of *B. rapa* introgressions in this respect was obvious by comparing the new type rapeseed with their parental *B. napus*. This is exemplified by the comparison between the new type HAU 2 and its parent HAU 12 with spring and winter rapeseed. The average genetic distances between HAU 2 and spring and winter rapeseed are 0.43 ± 0.01 and 0.44 ± 0.02, respectively, whereas the average genetic distances between HAU 12 and spring and winter rapeseed are 0.37 ± 0.02 and 0.38 ± 0.02, respectively.

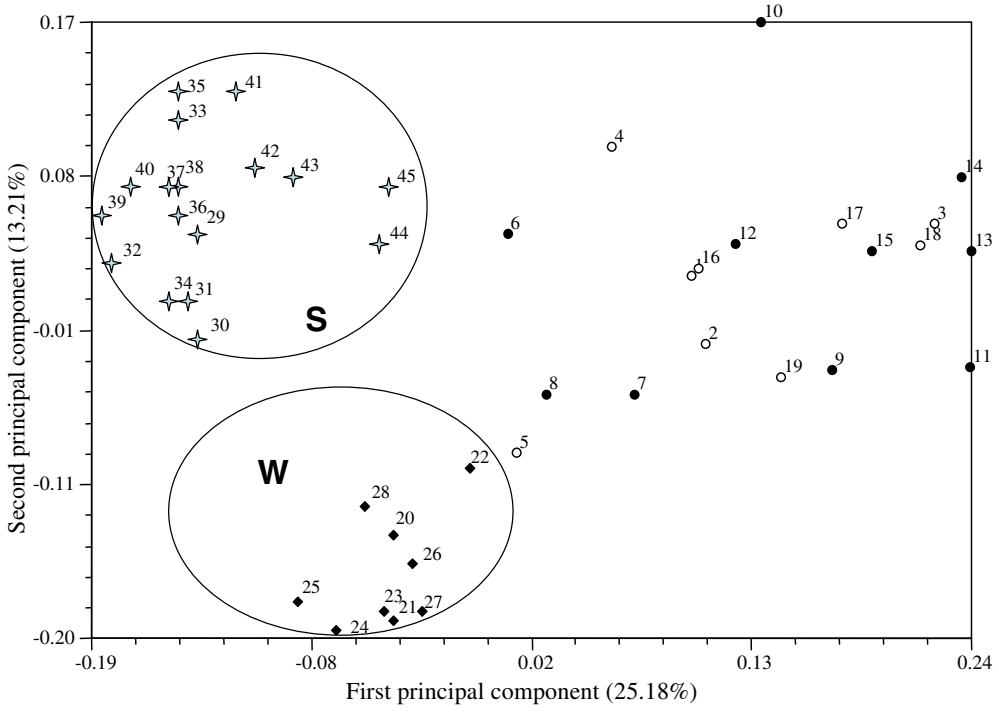

Fig. 2 Associations among 45 accessions revealed by a principle component analysis. The spring type, winter type, new type and Chinese normal rapeseed were plotted by *solid stars, solid squares, solid circles* and *open circles*, respectively

Table 3 Average genetic distance within and among new type rapeseed (N group), Chinese normal rapeseed (C group), spring rapeseed (S group) and winter rapeseed (W group) calculated using Nei's method (Nei and Li 1979)

Group	Cultivar count	Average genetic distance (standard deviation)			
		N group	C group	W group	S group
N group	10	0.427 (0.079)			
C group	9	0.394 (0.081)	0.364 (0.074)		
W group	9	0.424 (0.056)	0.388 (0.040)	0.193 (0.042)	
S group	17	0.430 (0.064)	0.402 (0.042)	0.323 (0.036)	0.226 (0.051)

Discussion

Brassica napus was domesticated only about 400–500 years ago (Gómez-Campo 1999), for this reason the germplasm was rather narrow compared with its parental species *B. rapa* and *B. oleracea* (CC). Recently, efforts have been made to widen the germplasm of rapeseed by introgressions from its parental species (Chen and Heneen, 1989; Engqvist and Becker 1994; Olssen, 1960; Qian et al. 2005; Seyis et al. 2003; Udall et al. 2004). Our results suggest that introgressions into *B. napus* from Chinese *B. rapa* significantly increased the genetic diversity of this species, and that those introgressions played an important role in the Chinese rapeseed evolution in respect of the proceed of rapeseed domestication in China. There are three arguments to support those findings. First, the 'A' genome from *B. napus* was derived from European *B. rapa* because *B. napus* originated from a spontaneous hybridization between *B. rapa* and *B. oleracea* in Europe (U N1935). Second, Chinese *B. rapa* differs from European *B. rapa*. It is known that there are two independent centres of origin for *B. rapa*, East Asia and Europe. Accessions derived from these centres are clearly different at the morphological (Liu 2000; Sun 1946), isoenzyme and DNA levels (Denford and Vaughan 1977; Qian et al. 2003; Song et al. 1988; Zhao et al. 2005; Zhao and Becker 1998). Third, the alleles from Asian *B. rapa* have been introgressed frequently into rapeseed in Asia because crossings between *B. napus* and *B. rapa* are quite easy and euploid rapeseed can readily be identified from the offspring. More than 50% of the cultivars released in China and Japan were derived from *B. napus* × *B. rapa* crossings (Liu 1985; Shiga 1970). It should also be mentioned that genetic variation within the 'C' genome can be increased by introgressions from *B. carinata*. In our study, a number of new type rapeseed with introgressions from *B. carinata* were clearly distant from the other accessions.

The genetic diversity resulting from 'A' genome introgressions can be exploited in rapeseed breeding worldwide. One strategy is to widen the rapeseed germplasm pool by introgressions from Asian oilseed rape. Since we found that the genetic diversity within spring and winter rapeseed is lower than within Chinese rapeseed, it is suggested to increase the genetic variation by crossing spring and winter rapeseed with Chinese *B. napus* or Chinese *B. rapa*. Udall et al. (2004) found that the seed yield of spring canola could be improved by introducing favorable alleles from Chinese material. The other strategy is to exploit subgenome heterosis between different subgenome pools. Qian et al. (2005) found strong heterosis in hybrids between new type rapeseed and normal type rapeseed. Presently, this subgenomic heterosis is being tested by growing hybrids between new type and spring and winter rapeseed in the main rapeseed growing regions in the world.

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