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Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints

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Abstract Amplified fragment length polymorphism (AFLP) markers were employed to assess the genetic diversity amongst two large collections of *Brassica rapa* accessions. Collection A consisted of 161 *B. rapa* accessions representing different morphotypes among the cultivated *B. rapa*, including traditional and modern cultivars and breeding materials from geographical locations from all over the world and two *Brassica napus* accessions. Collection B consisted of 96 accessions, representing mainly leafy vegetable types cultivated in China. On the basis of the AFLP data obtained, we constructed phenetic trees using MEGA 2.1 software. The level of polymorphism was very high, and it was evident that the amount of genetic variation present within the groups was often comparable to the variation between the different cultivar groups. Cluster analysis revealed groups, often with low bootstrap values, which coincided with cultivar groups. The most interesting information revealed by the phenetic trees was that different morphotypes are often more related to other morphotypes from the same region (East Asia vs. Europe) than to similar morphotypes from different regions, suggesting either an independent origin and or a long and separate domestication and breeding history in both regions.

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Introduction

The *Brassica* genus comprises six crop species, each with considerable morphological variation. Through interspecific hybridizations in all possible combinations, three basic diploid plant species *Brassica rapa* (A genome, $n=10$), *B. oleracea* (C genome, $n=9$) and *B. nigra* (B genome, $n=8$) gave rise to three amphidiploid species *B. napus* (AC genome, $n=19$), *B. juncea* (AB genome, $n=18$) and *B. carinata* (BC genome, $n=17$) (U 1935). Fingerprinting using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) has generated information on the evolution of the amphidiploid species, the origins of the diploid species and the relationship between different morphotypes or cultivar groups (Mizushima 1980; Prakash and Hinata 1980; Song et al. 1988a, b, 1990; Demeke et al. 1992; Jain et al. 1994; Thormann et al. 1994; Das et al. 1999; Chen et al. 2000; Guo et al. 2002; He et al. 2002, 2003). Although sufficient proof of the origin of cultivated *B. rapa* is lacking, the most likely explanation is that the wide variation within cultivated *B. rapa* arose independently at different places in the

world from wild *B. rapa*. Only a few studies using small numbers of accessions and a limited number of RFLPs, RAPDs and AFLPs have been published (Vaughan 1977; Song et al. 1988b; Chen et al. 2000; Guo et al. 2002; He et al. 2003). The results of these studies suggest that cultivated subspecies of *B. rapa* most likely originated independently in two different centers—Europe and Asia. Turnip and turnip rape (*oleiferous* forms) are the dominating forms in the European center (Reiner et al. 1995; Gomez Campo 1999). In East Asia, leafy vegetables such as Chinese cabbage, Pak choi and Narinosa may have been domesticated first in China. China is also the center of origin of Chinese turnip rape (ssp *oleifera*) (Li 1981), which is a unique turnip rape (oil type). Other accessions of *B. rapa* most likely derived from different morphotypes in the two centers of origin and subsequently evolved separately.

B. rapa is an important vegetable crop and to a minor extent also an oil seed crop. *B. rapa* vegetables are consumed worldwide and provide a large proportion of the daily food intake in many regions of the world. It is of interest that there is a large variation in the plant organs that are consumed, which has resulted in the selection of different morphotypes depending on local preferences. Because *B. rapa* has been cultivated for many centuries in different parts of the world, the variation within the species has increased as a result of ongoing breeding. Based primarily on the organs used and secondly on their morphological appearance, a number of major cultivar groups, which have been given sub-species names in the past, can be distinguished (Diederichsen 2001).

The oil seed types (ssp. *oleifera*) fall into different subgroups based on their growth habit (spring and winter types). The Chinese turnip rape is possibly developed from Pak choi in southern China (Li 1981; Liu 1984) and shows strong branching. The separate breeding tradition in India led to the development of the Sarson types, which are very early, self-compatible and often yellow-seeded (Gomez Campo 1999).

A group of cultivars grown for their swollen stem basis are the turnip types (sp. *rapa*), which can be subdivided in vegetable and fodder turnips. This group probably represents one of the oldest groups of cultivated *B. rapa* types (Siemonsma and Piluek 1993). Manifold shapes and colors are typical characteristics of turnips, especially vegetable turnip.

A large and diverse group of *B. rapa* cultivars are cultivated for their leaves. In these leafy vegetables several subgroups can be clearly distinguished. The Chinese cabbage group (sp. *pekinensis*) is characterized by large leaves with a wrinkled surface, a pale-green color, large white midribs and heads of different shapes. Pak choi (sp. *chinensis*) does not form a head and has darker green and smooth leaves with a pronounced white midrib. Wutacai (sp. *narinosa*) forms a subgroup of Pak choi-like cultivars that differ from typical Pak choi types by their flat appearance and many dark leaves. Taicai's (or Tai tsai's) (sp. *chinensis*) are non-heading cabbage cultivars with irregularly notched leaves of different blade shapes. The tender leaves, stems and even the conical-shaped succulent taproots are edible. These types are mainly distributed throughout eastern China and are widely cultivated in the Shandong and Jiangsu provinces (Cao et al. 1997; Zhu and Zheng 2001).

Another group of cultivars also cultivated for their leaves are characterized by many, often narrow leaves that are either serrated or not serrated. These cultivars belong to the *perviridis* group, which includes neep greens from Europe, the Japanese cultivar group Komatsuna, and the *nipposinica* group, including Mizuna and Mibuna and leaf potherb mustards. The Shuicai cultivars from China resemble Mizuna or Mibuna, and the Chinese Fennie (tilling) vegetable with strong stooling leaves also belongs to the *japonica* group (Cao et al. 1997).

Another use of *B. rapa* are the stems in red purple Zicaitai (ssp. *chinensis*) from southern China. This flowering purple-stemmed Chinese cabbage has tender early inflorescences, stems and shoots which are edible.

The inflorescences of flowering cabbages, such as the Broccoletto or Cima di rapa types found in Italy, are yet another plant organ of *B. rapa* that is consumed. In China, flowering cabbages are called Caixin or Caitai. These have a growth habit similar to that of Brocoletto and probably have evolved independently. Caixin and Broceletto have a rather different taste, which also indicates their different origin (http://www.plant-names.unimelb.edu.au/Sorting/Brassica.html).

The development of AFLP technology has been useful for analyzing genetic diversity in many plant species and has considerable potential for generating a large number of polymorphic loci (Vos et al. 1995; Mackill et al. 1996; Powell et al. 1996; Koopman et al. 2001; Srivastava et al. 2001; Huang et al. 2002; Negi et al. 2004). In the investigation reported here, we used AFLP technology to analyze the relationships among 259 *B. rapa* accessions derived from different parts of the world. Special emphasis was placed on comparing European and Asian accessions, which have a long independent breeding history.

Materials and methods

Plant materials

We used the nomenclature developed by Diederichsen (2001) to describe the different cultivar groups as subspecies. In experiment A, 163 *Brassica rapa* accessions, including various morphological types and two *B. napus* species, were selected out of 230 accessions. The accessions were obtained from the Dutch Crop Genetic Resources Center (CGN) in Wageningen, the Chinese Academy of Agricultural Sciences (CAAS)-Institute for Vegetable and Flowers (IVF) and the Oil Crop Research Institute (OCRI) and from Dr. Osborn (University of

Wisconsin, Madison, Wis., USA), who provided three parental lines of mapping populations. The collection includes traditional cultivars, breeding material and modern cultivars originating from different geographical locations. All of the accessions used in the study and their origins are listed in Table 1. In an independent experiment, experiment B, 96 *B. rapa* accessions from the CAAS-IVF were studied. This experiment included mainly leafy types from different provinces or regions in China, although a few came from outside of China. These accessions represent the various morphotypes cultivated in China, and their origins are listed in Table 2.

The accessions listed in Table 1 were grown in the greenhouse and evaluated for leaf characteristics (4 weeks after sowing), flowering time, seed color and self-compatibility (see Table 4). Inflorescences were covered with plastic bags to prevent cross-pollination. Plants that set seeds on these bagged inflorescences were considered to be self-compatible.

DNA isolation and AFLP analysis

In experiment A, total DNA was extracted from lyophilized young leaves or flower buds as described by Van der Beek et al. (1992). Lyophilized plant material was ground by shaking tubes containing plant material and iron bullets in a Retsch shaker.

The AFLP procedure was performed as described by Vos et al. (1995), with minor modifications according to Bai et al. (2003). The restriction enzymes, adapters and primers used are listed in Table 3. Total genomic DNA (250 ng) was digested using two restriction enzymes, *Pst*I and *Mse*I and ligated to the adaptors. Pre-amplifications were performed in 20-µl volumes of 1× PCR buffer, 0.2 m *M* dNTPs, 30 ng Poo and Moo + C, 0.4 U *Taq* polymerase and 5 µl of a 10× diluted restriction ligation mix, using 24 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 60 s. Five-microliter aliquots of the diluted (1:20) pre-amplification product were used as templates for the selective amplification with four primer combinations (P14M51, P21M47, P13M48 and P23M50). Only *Pst*I primers were labeled with IRD-700 or IRD-800 at the 5′ end for the selective amplification. The selective amplification was carried out using the following cycling parameters: 12 cycles of 30 s at 94°C, 30 s at 65–56°C (with a 0.7°C-decrease each cycle) and 60 s at 72°C, followed by 24 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C.

Following the selective amplification, the reaction products were mixed with an equal volume of formamide-loading buffer (98% formamide, 10 m *M* EDTA pH 8.0 and 0.1% Bromo Phenol Blue). The samples were denatured for 5 min at 94°C, cooled on ice and run on a 5.5% denaturing polyacrylamide gel with a LI-COR (Lincoln, Neb.) 4200 DNA Sequencer (Myburg and Remington 2000).

In experiment B, *Eco*RI/*Mse*I were selected as the restriction enzymes, and the primer and adapter sequences are listed in Table 3. The AFLP procedure is as described for experiment A with minor modifications. The selective amplification was carried out using 12 primer combinations (E33M61, E36M47, E38M48, E32M60, E42M50, E37M60, E37M59, E32M49, E41M49, E38M62, E39M51 and E33M48).

Data analysis

In experiment A, the AFLP gel images were analyzed with the software package AFLP-QUANTAR®PRO. All AFLP bands were treated as dominant markers and scored as either present (1) or absent (0). Clearly distinguishable bands ranging from 50 bp to 500 bp were used in the data matrix and genetic analysis. Phenetic trees were constructed using MEGA 2.1 software (Kumar et al. 2001). Similarity was calculated as the proportion of AFLP markers at which the two accessions compared had the same score ($SM_{xy} = (n_{11} + n_{00})/n$; where n is the number of markers scored). The distance is 1−SM. Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA). Bootstrap values were calculated in 1,000 permutations and presented in percentages.

In experiment B, the AFLP gel images were scored by eye. Clearly distinguishable polymorphic bands ranging from 50 bp to 500 bp were scored as present (1) or absent (0). All weak and poor bands were not recorded. The data were analyzed as in experiment A.

Results

Genetic variation

In experiment A, a set of 15 accessions representing different morphotypes was screened with 16 *Eco*RI/*Mse*I and 16 *Pst*/*Mse*I primer combinations. Four pairs of *Pst*/*Mse*I primers that gave clear banding patterns with sufficient polymorphism were used to fingerprint 161 *B. rapa* and two *B. napus* accessions. The AFLP patterns between *B. rapa* accessions were very polymorphic. In total, 524 scorable amplification products ranging from 50 bp to 500 bp were generated, 476 of which were polymorphic, with an average of 119 polymorphic bands per primer combination. The level of polymorphism was more than 90%. Two *B. napus* accessions (representing an outgroup) and the *B. rapa* lines MIZ079 and PC105 displayed several monomorphic bands that contributed considerably to the polymorphism rate. If these mono-morphic bands were excluded from the analysis, the degree of polymorphism was still more than 80%.

A typical AFLP image is illustrated in Fig. 1a and shows that the Broccoletto group is clearly distinguishable by a specific set of AFLP bands. The polymorphism rates were calculated for the different cultivar groups as listed in Table 1. For the larger groups, these rates were

Table 1 List of accessions used in experiment A

Genotype[a]	Cultivar name[b]	Accession no.	Origin (country)
Chinese cabbage (ssp. *pekinensis*)			
CC-057		CGN07182	China
CC-148	Bao Tou Qing	VO2A0006	China
CC-062		CGN07189	Germany
CC-112	Bao Tou Qing	CGN15194	China
CC-160	Qing Kou Bai Cai	VO2A0044	China
CC-167	Luo Yang Large Bai Cai	VO2A0062	China
CC-147	Si Ji Qin Bao tou bai	VO2A0005	China
CC-142	Matsushima Jun Sang	CGN21732	Japan
CC-152	Huang Yang bai	VO2A0016	China
CC-048		CGN06867	Soviet Union
CC-049	Granaat	CGN07143	Netherlands
CC-153	Bao Tou Bai Cai	VO2A0020	China
CC-163	Tian jing Bai Cai	VO2A0049	China
CC-162	Luo Yang Bai	VO2A0048	China
CC-168	Luo Yang Da Bai Cai	VO2A0068	China
CC-060		CGN07185	China
CC-113	Bei jing 106	CGN15195	China
CC-093		CGN11002	China
CC-150	Yu Quan Bao Tou Qing	VO2A0012	China
CC-169	Huang Yang Bai	VO2A0069	China
CC-158	Gao Zhuang Huang Yang Bai	VO2A0034	China
CC-154	Luo Yang Da Bai Cai	VO2A0023	China
CC-155	Huang Yang Bai	VO2A0029	China
CC-166	Huang Yang Bai	VO2A0056	China
CC-156	Huang Yang Bai	VO2A0030	China
CC-071	BRA 211/69	CGN07200	Japan
CC-073	BRA 127/67	CGN07202	China
CC-125		CGN15222	Korea
CC-068		CGN07196	Bulgaria
CC-069		CGN07198	USA
CC-067		CGN07195	Japan
CC-114	Xiao Qing Kou	CGN15196	China
CC-159	Gao Zhuang Da Bai Cai	VO2A0039	China
CC-072	BRA 207/70	CGN07201	China
CC-095		CGN11005	China
CC-058		CGN07183	Czech Republic
CC-070	BRA 47/22	CGN07199	Korea
CC-165	Tian jing Bai Cai	VO2A0054	China
CC-059		CGN07184	Korea
CC-141	Kyoto Sang	CGN21731	Japan
CC-140	Kashin	CGN20771	Japan
CC-157	Huang Yang Bai	VO2A0031	China
CC-164	Tian jing Bai Cai	VO2A0053	China
CC-061		CGN07188	Yugoslavia
CC-146	Long Kang er Gao Zhuang	VO2A0001	China
CC-094		CGN11003	Japan
CC-161	Huang Yang Bai	VO2A0046	China
Pak choi (ssp. *chinensis*)			
PC-099	Chinese Bai Cai	CGN13924	China
PC-172	No 17 Bai Cai	VO2B0207	China
PC-173	Kui Shan Li Ye Bai Cai	VO2B0223	China
PC-176	Ai Jiao Hei Ye Bai Cai	VO2B0232	China
PC-107	Dwarf	CGN15184	Hong Kong
PC-175	HKG Nai Bai Cai	VO2B0226	China
PC-189	Ai Hei Ye Kui Shan Bai Cai	VO2B0715	China
PC-187	Ai Hei Ye Kui Shan Bai Cai	VO2B0695	China
PC-180	Jiang Mei Xiao Bai Cai	VO2B0612	China
PC-186	D94 Bai Cai	VO2B0694	China
PC-177	Ai Jiao Huang	VO2B0396	China
PC-171	B139 Xiao Bai Cai	VO2B0206	China
PC-195	Kuang Hei Fu Bing CC6	VO2B1299	China
PC-185	Qing Ken Bai Cai	VO2B0691	China
PC-191	Wuhan Ai Jiao Huang	VO2B0988	China
PC-193	CII	VO2B1263	China
PC-182	Nan Jiang Bai	VO2B0620	China
PC-192	Wang Yue Man	VO2B1223	China
PC-194	Qing Ken Bai Cai	VO2B1297	China

Table 1 (Contd.)

Genotype[a]	Cultivar name[b]	Accession no.	Origin (country)
PC-174	Bai Cai VS-2	VO2B0225	China
PC-178	Ai Jiao Huang You Cai	VO2B0487	China
PC-023	Si Yue Man	CGN06817	China
PC-188	Tai Wan Chi Ye Bai Cai	VO2B0697	China
PC-022		CGN06816	Netherlands
PC-076		CGN07205	China
PC-100	Cabbage Tientsin	CGN13925	China
PC-101	Tientsin; Celery,Shantung,Peking	CGN13926	China
PC-183	Ai Kuang Qing	VO2B0655	China
PC-184	Ai Jiao Bai	VO2B0656	China
Caixin (ssp. *parachinensis*)			
BRO-103	Tsja Sin; No.P1R5T5	CGN15158	Indonesia
PC-078	Choy Sam	CGN07211	Netherlands
Broccoletto (ssp. *broccoletto*)			
BRO-027	Quarantina	CGN06825	Italy
BRO-029	Norantino	CGN06828	Italy
BRO-026		CGN06824	Italy
BRO-028	Tardivo	CGN06827	Italy
BRO-025	Natalino	CGN06823	Italy
BRO-030	Sessantina	CGN06829	Italy
BRO-127	Edible Flower	CGN17278	Japan
Turnip (ssp. *rapa*)			
VT-116	Nagasaki Aka	CGN15200	Japan
VT-117	Toya	CGN15201	Japan
VT-115	Kairyou Hakata	CGN15199	Japan
VT-124	Jinengu-Kabu	CGN15221	Japan
VT-123	Terauchi-Kabu	CGN15220	Japan
VT-012	Ronde Rode Heelblad-Yurugu Red	CGN06720	Japan
VT-013	Ronde Rode Heelblad-Scarlet Ball	CGN06721	Japan
VT-007	Maiskaja	CGN06710	Soviet Union
VT-009	Ronde Rode -Tsutsui	CGN06717	Japan
FT-088	Blauwkop Heelblad-Oliekannetjes	CGN10985	Netherlands
VT-053	Teltower Kleine	CGN07167	Germany
VT-010	Platte Ronde Blauwkop Ingesneden Blad- Lila Ker	CGN06718	Hungary
VT-044	Soloveckaja	CGN06859	Soviet Union
VT-015	Bianca Lodigiana; Italiaanse Witte	CGN06724	Italy
VT-017	Platte Witte Meirapen	CGN06732	Netherlands
FT-001	Halflange Witte Blauwkop Ingesneden Blad-Barenza	CGN06669	Netherlands
FT-097	Buko; Bladraap	CGN11010	Germany
VT-018	Goudbal; Golden Ball	CGN06774	Netherlands
VT-008	Pusa Chandrina	CGN06711	India
VT-120	Platte Gele Boterknol	CGN15210	Netherlands
VT-014	Platte Witte Blauwkop Heelblad-Milan	CGN06722	Italy
VT-045	Milanskaja; Italiaanse Witte	CGN06860	Italy
VT-092	Amerikaanse Witte Roodkop Heelblad	CGN11000	Netherlands
VT-011	Platte Witte Blauwkop Ingesneden Blad-Siniaja	CGN06719	Soviet Union
FT-005	Ochsenhorner	CGN06688	Germany
VT-091	Snowball; Blanc Rond de Jersey	CGN10999	United Kingdom
VT-089	D'Auvergne Hative	CGN10995	France
FT-004	Lange Gele Bortfelder	CGN06678	Denmark
VT-006	Pusa Chandrina	CGN06709	India
VT-137		CGN20735	Uzbekistan
VT-052	Hilversumse; Marteau	CGN07166	Netherlands
VT-090	De Croissy	CGN10996	France
VT-119	Roodkop-Pfalzer	CGN15209	Netherlands
FT-047	Moskovskij	CGN06866	Soviet Union
FT-002	Grote Ronde Witte Roodkop-Norfolk; De Norfolk a Collet Rouge	CGN06673	United Kingdom
FT-003	Lange Witte Roodkop	CGN06675	Netherlands
FT-051	Krasnaja	CGN07164	Soviet Union
FT-056	Daisy; Bladraap	CGN07179	France
FT-086		CGN07223	Pakistan
Neep greens (ssp. *perviridis*)			
KOM-041		CGN06843	Japan
KOM-118	Komatsuna	CGN15202	Japan
TG-129	Vitamin Na	CGN17280	Japan
TG-131	Maruba Santo Sai	CGN17282	Japan

Table 1 (Contd.)

Genotype[a]	Cultivar name[b]	Accession no.	Origin (country)
Mizuna (ssp. *nipposinica*)			
MIZ-019	Bladmoes	CGN06790	Netherlands
MIZ-079		CGN07213	Japan
MIZ-128	Round Leaved Mibuna	CGN17279	Japan
Turnip rape (ssp. *oleifera*)			
OR-211	Yi Chang Xiao You Cai	OCRI1771	China
OR-210	Luo Tian You Bai Cai	OCRI1757	China
OR-213	Huang Po Tian You Cai	OCRI0235	China
OR-216	Xi Qiu Bai Cai	OCRI3742	China
OR-214	Chang De Nanjing Zi	OCRI1789	China
OR-212	Xing Shan You Cai	OCRI1776	China
OR-218	Gao Zhi Huang You Cai	OCRI3764	China
OR-219	Ping Ba Bai You Cai	OCRI3801	China
OR-209	Huang Gang Bai You Cai	OCRI1752	China
OR-217	Cha Yuan Bai You Cai	OCRI3752	China
SO-031		CGN06832	USA
SO-032	Pusa Kalyani	CGN06834	India
SO-034	Australian RARS	CGN06836	Bangladesh
SO-035	Somali Sarisa	CGN06837	Bangladesh
SO-037	Kalyania	CGN06839	Bangladesh
SO-038		CGN06840	Germany
SO-039	Sampad	CGN06841	Bangladesh
SO-040	Candle	CGN06842	Canada
WO-024	Svalof 0308	CGN06818	Sweden
WO-080		CGN07216	Pakistan
WO-081		CGN07217	Pakistan
WO-083		CGN07220	Pakistan
WO-084		CGN07221	Pakistan
WO-085		CGN07222	Pakistan
WO-087		CGN07226	Pakistan
WO-145	Per	KT18	USA
RC-144	Rapid cycling	FIL501	USA
Yellow Sarson (ssp. *tricolaris*)			
YS-033	Dys 1	CGN06835	Germany
YS-143	R500	FIL500	USA
Wutacai (ssp. *narinosa*)			
PC-105	BRA 77/72	CGN15171	China
B. Napus			
BN-222		OCRI0027	China
BN-226		OCRI0046	China

[a]CC, Chinese cabbage; PC, Pak choi; BRO, Broccoletto; VT, vegetable turnip; FT, fodder turnip; KOM, Komatsuna; TG, turnip green; MIZ, Mizuna; OR, Chinese turnip rape; SO, spring turnip rape; WO, winter turnip rape; RC, rapid cycling; YS, Yellow Sarson; BN, *Brassica napus*

[b]Bai, White; Cai, cabbage; Da, large; Huang, yellow; Hei, black; Kang, resistance; Tou, head; Xin, center; Xiao, small; Yang, seedling; Yuan, round; You Cai, oilseed rape

very similar: Chinese cabbage, 77%; Pak choi, 75%; winter and spring turnip rape, 77%; turnips, 82%. Two Yellow Sarson and two Mizuna accessions had remarkably similar AFLP profiles.

For experiment B, a set of 96 lines representing different morphotypes and geographical origin was screened with some *Eco*RI/*Mse*I primer combinations (48 samples are depicted in Fig. 1b). Based on the screens of experiment A and experiment B, 12 pairs of *Eco*RI/*Mse*I primers that gave clear banding patterns with sufficient polymorphism were used to fingerprint the 96 *B. rapa* accessions. In total, 332 scorable amplification products were generated, 137 of which were polymorphic, with an average of 11.5 polymorphic bands per primer combination. The level of polymorphism was 41%. The polymorphism rate for the two large groups of Chinese cabbages and Pak choi was 48% and 52%, respectively. In experiment A, the polymorphism rate was more than 70% if only Pak choi and Chinese cabbages were taken into account.

Phenetic relationships

A dendrogram was established using the AFLP fingerprints (see Fig. 2). It was evident that the amount of genetic variation present within the groups was often comparable to the variation between the different subgroups. Most accessions fell into a number of subgroups that had non-significant bootstrap values as groups, but these subgroups did represent the different morphotypes and were arranged into two main sets according to the origin of the accessions.

Table 2 List of accessions used in experiment B

Genotype[a]	Cultivar name[b]	Accession no.	Origin[c]
Chinese cabbage (ssp. *pekinensis*)			
cCC94	Huang Yang Bai	V02A0046	Si chuan
cCC98	Tai GuGu Diu	V02A1003	Tian jing
cCC120	Da Bang	V02A1489	Shang dong
cCC101	Xue Li Bai Xin Cai	V02A1096	Yun nan
cCC110	Ji Tui Bai	V02A0788	Nei meng
cCC102	Si Ji Bao Tou Qing	V02A0005	Bei jing
cCC116	Xiao Qing Kou		Shan xi
cCC117	Huang Yang Bai		Yun nan
cCC119	Caul		North Korea
cCC107	Niu Tui Bang	V02A0747	Qing hai
cCC124	Fu Shan Bao Tou	V02A1382	Hu bei
cCC121	Kao Zhuang Bai	V02A1358	Si chuan
cCC108	He Tao Wen	V02A0574	Liao ning
cCC128	Zhu Long Cai	V02A1499	Shan xi
cCC99	Xiao Shi Zi Tou	V02A0555	Jiangsu
cCC111	Xin Hua er Bao Tou	V02A0710	Nei meng
cCC118	Bleak Leaf 30 Days	V02A1564	Asia vegetables center
cCC122	Jia bai 2 hao		Hei long jiang
cCC125	Xing Cheng Xiao Cuo Cai	V02A1396	Ji lin
cCC100	He Ze Da Bao Tou		Shandong
cCC103	Zhang Zhou Zhang Pu Lei	V02A0133	Fu jian
cCC105	Xiao Qing Kou	V02A0727	Ning xia
cCC114	Gan Zhou Bai Cai	V02A1212	Jiang xi
cCC112	Da Tai Zhong Qing Ma Ye	V02A0704	Nei meng
cCC130	Xiao Gen	V02A0582	Lliao ning
cCC123	77-KR		Bengal
cCC127	Ji Nan Da Ken		Shang dong
cCC133	Xin Jiang Da Bao Tou	V02A1022	Xin jiang
cCC93	Cao Zhou Gao Zhuang	V02A0359	He nan
cCC134	Shou Guang Xiao Gen		Shandong
cCC104	Da Mao Bian	V02A0961	Shan xi
cCC109	Cheng Du Bai		Si chuan
cCC131	Cheng De Fan Xin Bai	V02A0200	He bei
cCC115	Shi Zi Tou Da Bai Cai	V02A0002	An hui
cCC132	Xiao Qing Kou		Gui zhou
cCC106	Ci Xi Huang Ya Cai		Zhe jiang
cCC129	Yao Huang Zhong Huang Ya Cai		Zhe jiang
cCC95	Wu Ping Zhai Ye Da Bai Cai	V02A0129	Fu jian
cCC96	Fen Kou Bai	V02A0172	Gui zhou
cCC64	Zao Shu Wu Hao		Hang zhou
cCC82	Chun Xia Wang Bai Cai		North Korea
cCCB70	Wan Quan		Tai wan
Pak choi (ssp. *chinensis*)			
cPC61	Jing Lv 7 Hao		Bei jing
cPC66	Si Yue Man		Nan jing
cPC67	Bi Yu		Nan jing
cPC71	Shang Hai Qing		Shang hai
cPC72	Su Zhou Qing		Su zhou
cPC75	Shang Hai Qing		Bei jing
cPC78	Jing Guan		Bei jing
cPC80	Gao Hua Qing Geng Bai Cai		Hong kong
cPC83	Kang re 605 Qing Cai		Shang hai
cPC86	Jing Guan Wang Wing Geng Cai Zhong		Shan tou
cPC88	Wu yue man		Bei jing
cPC136	Shao Yang Tiao Geng Bai	V02B1236	Hu nan
cPC139	Taicai	V02B0445	Jiangsu
cPC142	Bai Bang You Cai	V02B0544	Tian jing
cPC143	Yu Yao Xiao You Cai	V02B1278	Zhe jiang
cPC145	Lv Bian Jv Hua Xin	V02B0002	An hui
cPC154	Hou Ma You Cai	V02B0503	Shan xi
cPC155	Chun Taicai	V02B0097	Shan xi
cPC159	Wu han ai jiao huang	V02B0481	Hu bei
cPC165	Duan Hei Ye Kui Shan Bai Cai	V02B0988	Hong kong
cPC168	Piao er Cai	V02B0893	Si chuan
cPC196	Ya Li Shan Jiao Nai Bai Cai		Guang dong
cPC198	Da Tou Qing Jiang Bai Cai		Guang dong
cPC208	Hai Lv You Cai		Tian jing

Table 2 (Contd.)

Genotype[a]	Cultivar name[b]	Accession no.	Origin[c]
cPC209	Chang Geng Bai Cai		Guang zhou
cPC211	Xia Qing		Shang hai
cRC216	Rapid cycling		USA
Cai xin (ssp. *parachinensis*)			
C54	Xiang Gang Caixin		Shan tou
C58	Si Jiu Cai Xin		Bei jing
C60	Er Yue Caitai		Shang hai
C91	Si Ji Duan Ye You Qing Caitai		Guang dong
C190	Chang Sha Chi Hong Cai		Hu nan
C194	Chihua Cu Jing Te Qing Caitai		Guang dong
C195	45 Days Caixin		Guang dong
C210	48-19 Caixin		Guang dong
Zi Caitai (ssp. *chinensis* var. *purpurea* Bailey)			
C62	Zicaitai		Bei jing
Turnip (ssp. *rapa*)			
T172	Ka Ma Gu	V01C0082	Xin jiang
T173	Bai Yuan Ken	V01C0054	Si chuan
T174	Yuan Man Qing	V01C0008	He bei
T175	Man Qing	V01C0030	Shandong
T176	Hua Ye Hong Pi	V01C0036	Shan xi
T178	Yuan Xing Wu Jing	V01C0001	An hui
T179	Da Ying Pan Cai	V01C0044	Zhe jiang
T180	Ke Bu er Man Qing	V01C0020	Nei meng
T181	Ji Xian Xian Sui Man Qing	V01C0067	He nan
Wutacai (ssp. *narinosa)*			
W56	Zhong Ba Ye Wutacai		Bei jing
W87	Wutacai		Shang hai
W204	Hei You Bai Cai		Hu bei
W205	Hei Ta Cai		
Mizuna (ssp. *nipposinica*)			
S57	Bai Geng Qian Jin Jing Shui Cai		Bei jing
S84	Dong Fang Ren Sheng Cai		Bei jing
S203	Qian Jing Shui Jin Cai		Hu bei
Taicai (ssp. *chinensis* var. *tai-tsai* Lin)			
TC182	Yuan Ye Taicai	V02C0008	Shandong
TC183	Hua Ye Taicai	V02C0012	Shandong

[a]cCC, Chinese cabbage; cPC, Pak choi; C, Caixin or Caitai; T, turnip; W, Wutacai; S, Shui cai (Mizuna); TC, Taicai; cRC, rapid cycling

[b]Bai, White; Cai, cabbage; Da, large; Huang, yellow; Hei, black; Kang, resistance; Tou, head; Xin, center; Xiao, small; Yang, seedling; Yuan, round; You Cai, oilseed rape

[c]Origin refers either to a country or to province within China

In experiment A, the tree formed two main groups. Group 1 consists of accessions of Asian origin and can be subdivided in a group of Chinese cabbage cultivars (CC), one group consisting solely of Pak choi (PC1) and another group with both Pak choi and Chinese turnip rape (PC2). It also includes a small mixed group with accessions from mainly China and Japan (with two exceptions from Europe), a turnip group (T1) with accessions from Japan and a winter oilseed group (Oil1) group with accessions from Pakistan. The second group encompasses a turnip group (T2) with accessions from mainly European countries (two from India and one from USA) and the Broccoletto group (Bro) with accessions from Italy. Furthermore, two distinct subgroups are formed by two Mizuna cultivars (Miz) from Japan (Miz) and a spring oilseed and Yellow Sarson group (Oil2) with accessions from Bangladesh, USA and Germany.

The Chinese cabbage group (CC) consists of two clusters comprising solely Chinese cabbage and a less well-defined group consisting of Chinese cabbage accessions, one Pak choi type (PC101) and one fodder turnip accession (FT056). The Pak choi (PC) group is close to the CC group and is divided into PC1 and PC2. Most of the Pak choi accessions are clustered in PC1 together with two Caixin accessions (Bro103, Pc78). Bro103 is not a Broccolleto-type cultivar and should be renamed to Caixin. PC2 is a mixed cluster, containing Pak choi and Chinese turnip rape (OR) accessions. A small group of different morphotypes of oriental origin (mainly Japan and China) can be found between the PC2 and T1 groups, assuming that PC22 from the Netherlands also has an oriental origin. This latter group showed no clear structure.

The two turnip subgroups (T1 and T2) containing both vegetable and fodder turnip and the oil types originated from different geographical regions. T1 group accessions are all from Japan (except for VT007 from Russia), while T2 accessions are from the western hemisphere, namely Europe, the former Soviet

Table 3 AFLP primers used in the AFLP analyses

Primers	Sequences
M00	5′-GATGAGTCCTGAGTAA-3′
M02	M00 + C
M47	M00 + CAA
M48	M00 + CAC
M50	M00 + CAT
M51	M00 + CCA
M61	M00 + CTG
M60	M00 + CTC
M59	M00 + CTA
M49	M00 + CAG
M46	M00 + CTT
P00	5′-GACTGCGTACATGCAG-3′
P13	P00 + AG
P14	P00 + AT
P21	P00 + GG
P23	P00 + TA
E00	5′-GACTGCGTACCAATTC-3′
E33	E00 + AAG
E41	E00 + AGG
E37	E00 + ACG
E39	E00 + AGA
E42	E00 + AGT
E36	E00 + ACC
E38	E00 + ACT
E32	E00 + AAC

Union and USA (except for two accessions from India and one from Japan). In group 2, all Broccoletto accessions (Bro group) are clearly distinguishable as a separate subgroup with a high bootstrap value of 86.

In addition to the groups described above a number of less related and small outgroups could be identified.

One group consists of two Mizuna types (ssp. *nipposinica*). Another group in which high bootstrap values indicated a clear distinction is the Oil2 group, with the early yellow-seeded oil types from India and the rapid-cycling lines developed by Dr. Paul Williams (Williams and Hill 1986), probably with Yellow Sarson types in their pedigree.

Two accessions, namely a Wutacai type (PC105) and a Mizuna type (MIZ079), have a separate position based on a relatively large number of unique AFLP bands. Additionally, one Wutacai accession was collected and AFLP analysis performed with three pairs of the four primer combinations; the results indicated that this accession clusters between CC and PC1. The two *B. napus* lines were completely different from *B. rapa* species and form an outgroup with a very high bootstrap value (99).

In the analysis of experiment B with the IVF accessions, a similar pattern appeared. Different subgroups were formed, with again low bootstrap values. It was obvious that less commonly grown but morphological distinct types form no distinct subgroup but are dispersed within the main subgroups, although the Chinese cabbage groups are rather pure.

The Chinese cabbage cultivars (heading cabbage) are subdivided into two groups, CCa and CCb. The CCb group also includes a separate cluster with one Caitai accession, C190, three turnip types (T174, T172 and T176), one Taicai, TC183, and one Pak choi, cPC136. The Pak choi types are subdivided into two groups (PCa and PCb). Most of the Pak choi, Shuicai and Caixin accessions are clustered in PCa together with one Taicai accession, TC182, and one turnip accession, T173. PCb is also a mixed cluster, containing Pak choi, Wutacai and turnip accessions. One accession (cPC168) is close to T178 and actually is a turnip according to its phenotype; it should be renamed. Zicaitai C62 is not grouped into Caitai, but close to Wutacai in PCb.

There are five common accessions (CC147/cCC102, CC161/cCC94, PC189/cPC165, PC191/cPC159 and RC144/cRC216) between experiment A and B. The two Pak choi accessions (PC189/cPC165, PC191/cPC159) group similarly in both experiments; in experiment A and B they are organized into two different PC clusters. The rapid-cycling line RC144 (cRC216) forms a distinct group in experiment A, and also in experiment B it is very distinct between CCa and PCb. Common Chinese cabbage accessions group differently in both experiments. In experiment B, CC147/cCC102 is in CCb close to CC161/cCC94. In experiment A, CC147/cCC102 groups in CC, but CC161/cCC94 groups in a separate branch of a diverse little cluster between PC2 and T1.

Phenotypic variation

The *B. rapa* genus is morphologically very diverse. As illustrated above, phenetic groups follow morphological groups with respect to classification. In Table 4, ten phenotypic traits are listed for the different subgroups (CC, PC1, PC2, T1, T2, Oil1, Oil2, Bro and Miz).

Most of the variation for leaf color was found in the CC and PC groups. Chinese cabbages in CC are mostly yellow-green or light-green, while the Pak choi types in PC1 and PC2 have darker green leaves [the light-green accessions in PC2 represent most of the oilseed rapes (OR)]. The very dark-green cultivars are the Wutacai types and two Pak choi accessions, PC107 and PC175, found in PC1. Whitish petioles are characteristic for the CC and PC groups. A few accessions in these groups have light-green or green petioles.

Smooth leaves are exclusively found in the two PC groups and the MIZ group, while leaves of accessions of all other groups are wrinkled. Turnips, oil types and Mizunas all have characteristically elongated leaves.

Leaf serration is a character that was associated strongly with the UPGMA grouping in the tree. No serrated leaves or only mildly serrated ones are typical of the CC, PC and the Japanese Turnip 1 group. All oil types, the European Turnip group 2 (except VT014) and the Brocoletto's have dissected leaves. Two Mizuna lines, MIZ128 and MIZ079, have distinctly dissected leaves, while a different Mizuna type (MIZ019) has slightly serrated leaves. In experiment B, Chinese cabbage, most of Pak choi's (except cPC193, cPC154) and the Caitai and Wutacai accessions have no or mildly

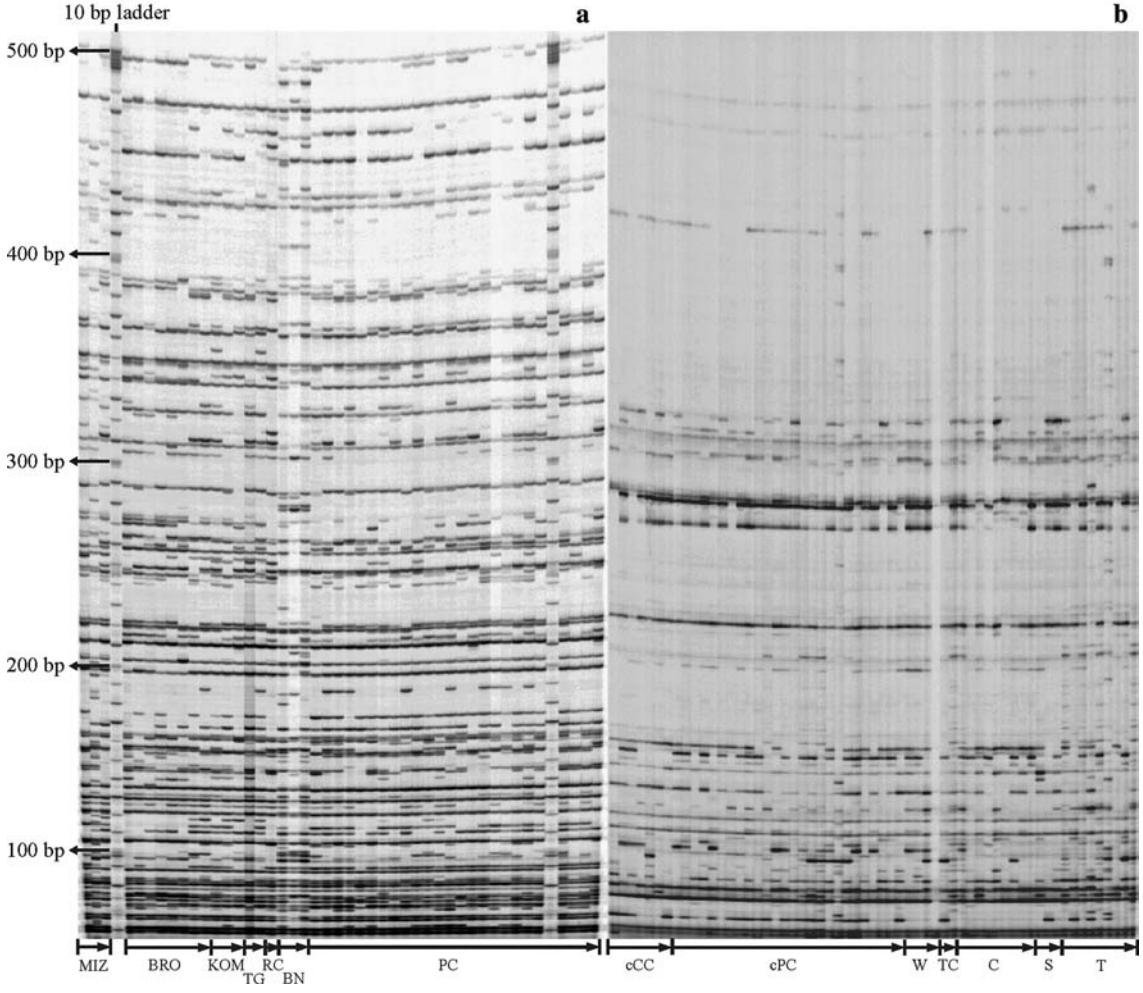

Fig. 1 An AFLP image of some *Brassica rapa* accessions using primer combination *Pst*I AG-*Mse*I CAC in experiment A and *Eco*RI AAC-*Mse*I CAG in experiment B. See Table 1 for definition of abbreviations

serrated leaves, while other Chinese types (Chinese turnips, Taicai) have dissected leaves.

The presence of trichomes (leaf hairs) is variable within all groups except in Oil1, where all genotypes have trichomes, and in the PC1 and Miz group, where hairs are absent. In PC2, the four accessions with trichomes are the Chinese oil types. All Pak choi types, and the Caixin (Bro103, PC078), Wutacai (PC105) and Mizuna accessions have no trichomes.

Yellow seeds are typical for the Yellow Sarson genotypes in the Oil2 group. Black seeds dominate in the PC groups, since especially all Chinese oil types within PC2 have dark-colored seeds.

Flowering time varies greatly among the accessions. Very early-flowering types include the Oil2 types, the Bro group and a number of PC types, including the Caixin cultivars. Late-flowering types are the Chinese turnip rape accessions in PC2 and the Oil1 types. Very late flowering types include all of the Turnip 2 accessions, which also cannot be vernalized at the seedling stage, a treatment that does accelerate flowering in the middle-to-late accessions.

The degree of self-incompatibility showed an interesting distribution. All of the PC1 and Oil 2 types are self-compatible, while most PC2, T1, Oil2, Bro, Mizuna and Komatsuna genotypes are self-incompatible. Because of their late flowering, the T2 types could not be classified for this trait. Incompatibility clearly differentiates subgroups that were found within cultivar groups with similar use or phenotype, and it separates PC1 from PC2 and Oil1 from Oil2. For experiment B, self-compatibility was not recorded.

Within the *B. rapa* species, the Broccoletto, Caixin and oil types have elongated stems or branches. Broccoletto originated from Italy and has a strong stem and short internode length (data are not shown). The edible part of this type are the small flower heads that appear when the plants are about 20 cm tall. This edible part is quite similar to that of Chinese Caixin, also called Flowering Chinese cabbage, which is also utilized in the early flower stage. Prior to flower opening, the leafy features of Caixin are similar to those of Pak choi.

Turnips also have their specific group characteristics (data not shown), which consist of a swollen hypocotyl

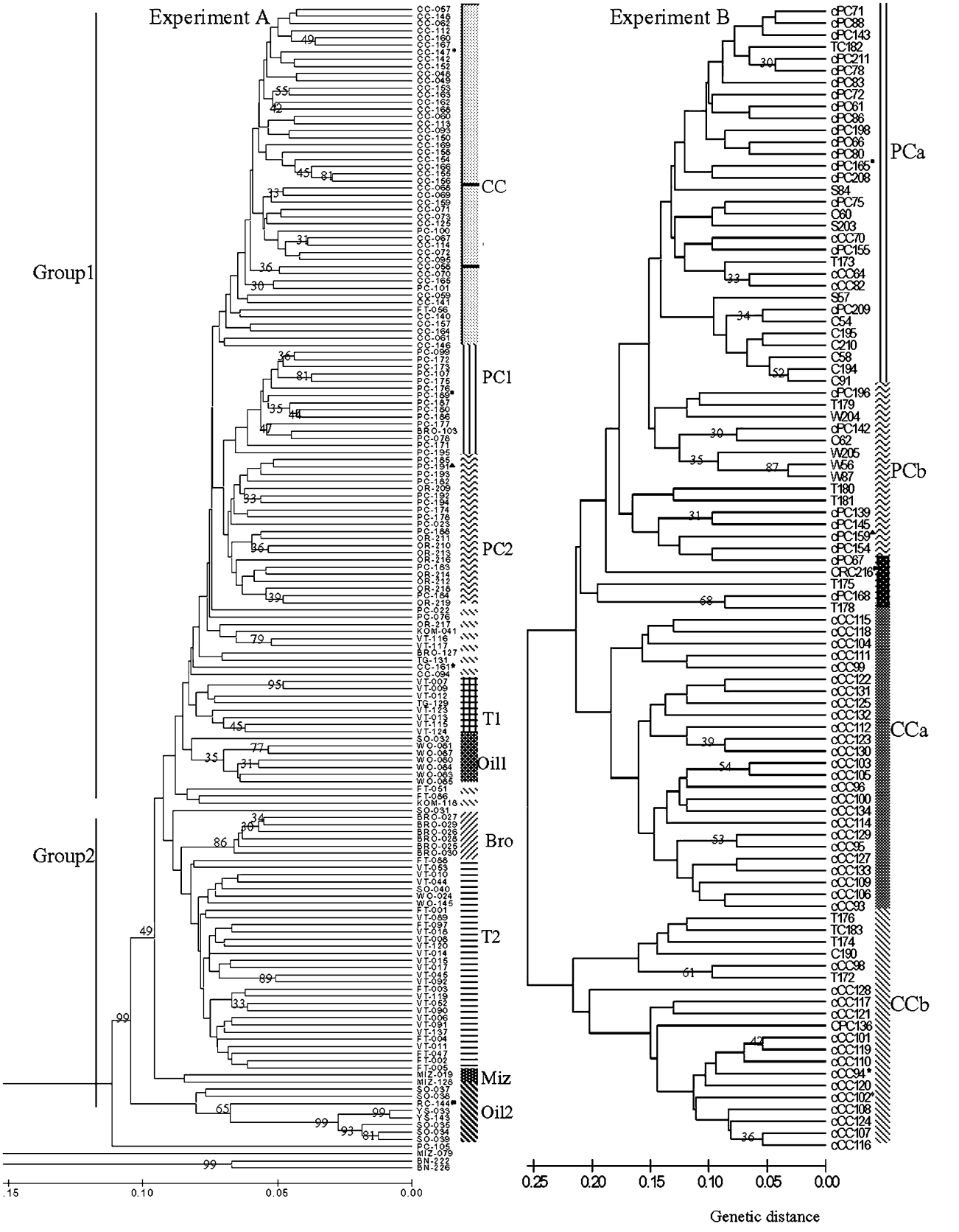

Fig. 2 UPGMA phenogram (experiments A and B) of *B. rapa* accessions based on the AFLP data obtained. *Numbers on branches* are bootstrap values (values smaller than 30 are not indicated). *Abbreviations* of the different morphotypes are as given in Tables 1 and 2. The five common accessions, CC147/cCC102, CC161/cCC94, PC189/cPC165, PC191/cPC159 and RC144/cRC216, between experiments A and B are indicated by various *symbols*

Table 4 Phenotypic characteristics for all accessions of the different morphological groups from experiment A (*nt* not tested)

Clusters		CC	PC1	PC2	T1	T2	Oil1	Oil2	Bro	Miz
Leaf surface	Smooth	1	15	11	0	0	0	0	0	2
	Wrinkled; intermediate	47	0	10	8	30	7	8	6	0
Leaf edge	Entire	1	9	15	1	0	0	0	0	0
	Slightly serrated	46	6	3	5	1	0	0	0	1
	Serrated	1	0	3	2	29	7	8	6	1
Leaf color	Yellow-green	12	0	1	0	0	0	0	0	1
	Light green	32	5	16	4	21	7	8	4	1
	Green	4	8	3	4	8	0	0	2	0
	Dark green	0	2	1	0	1	0	0	0	0
Leaf shape	Round; oval	48	15	20	7	3	0	0	6	0
	Elongate	0	0	1	1	27	7	8	0	2
Leaf firmness	Strong	0	15	21	8	24	7	6	4	0
	Intermediate; weak	48	0	0	0	6	0	2	2	2
Petiole color	White	40	13	9	0	0	0	0	0	0
	Light green; green	8	2	12	6	30	7	8	6	2
	Red	0	0	0	2	0	0	0	0	0
Trichomes	No	17	15	17	5	6	0	6	5	2
	Few	22	0	2	1	5	1	1	1	0
	Many	9	0	2	2	19	6	1	0	0
Flowering time[a]	Early	0	10	0	0	0	0	8	0	0
	Middle	0	3	2	0	0	7	0	6	0
	Late	48	2	19	8	30	0	0	0	2
Self-compatibility	Compatible	18	14	1	1	nt	0	6	0	0
	Compatible	30	1	20	7	nt	7	2	6	2
Seed color	Yellow	0	0	0	0	0	0	5	0	0
	Black	5	11	11	1	1	5	0	0	0
	Brown or pale brown	43	4	10	7	29	2	3	6	2

[a]Early flowering time, fewer than 60 days after sowing; middle flowering time, fewer than 90 days after sowing; late flowering time, more then 90 days after sowing

and a taproot. Turnips vary widely in shape and color, but as these characteristics are not associated with specific AFLP patterns they could not differentiate between groups.

Discussion

The most interesting information revealed by the dendrogram assembled in this investigation (Fig. 2) is that different morphotypes 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. The low bootstrap values for many of the groups show that most polymorphisms do not contribute to the phenotypic variation, which indicates that only a few genes are involved in causing the extreme morphologies. This may also explain why the different morphotypes could emerge independently in the different geographic regions.

Chinese turnip rapes (Chinese oil types) cluster in the PC2 group, and flowering cabbages cluster with the early-flowering PC1 group. While the clustering of Caixin with Pak choi indicates a close relationship, it is impossible to determine which type derived from which. Despite the fact that selection resulted in the use of the same organs, the two flowering cabbage groups—the Chinese Caixin types and the Italian Broccolleto types—are not at all related to each other. The Caixin types are related to the Pak choi cabbages and form a separate branch with PC1 or PCa in both experiments, whereas the Broccolleto cultivars form a clearly separate group somewhat related to European turnip (T2) and the oil types. Similarly, the Chinese oil types (Chinese turnip rape) are related to Pak choi and form a subgroup within the PC2 cluster, but they do not cluster with the oil types or turnips from different geographical origins.

Wutacai is also called flat Chinese cabbage because of its remarkable flat shape. This Chinese vegetable resembles Pak choi at the seedling stage, and its leaves are similar in structure and color as some Pak choi types, however the rosette has many more very small dark-green leaves, and the plants bolt very late. One Wutacai (PC105) accession in experiment A does not group with any of the other accessions, and it clearly deviates from the Wutacai's of experiment B that are related to the Pak choi types (PCb group). The reason why PC105 separated from PC group cannot be explained clearly, although its distinctiveness might suggest that Wutacai types have originated from several types independently due to a re-occurrence of a major mutation. Based on RFLP studies (Song et al. 1988b), one Narinosa (Wutacai) accession also seemed to fit neither group.

Turnip types that originate mainly from Japan form a variable intermediate group, which also includes some turnip greens (Bro127 from Japan resembles turnip greens more than Broccoletto) (Fig. 2a). This group of

oriental turnips is clearly different from the European fodder and vegetable turnips, and it also flowers earlier. The Chinese cabbage accession CC94, originating from Japan, does not fit in CC, but is positioned close to Japanese vegetable turnip types. This geographical distinction of the turnips can also be seen in morphological and physiological characters such as leaf shape and flowering time and might either be due to a long separation of breeding of the different turnip types or even an independent origin. Chinese turnips are located mainly in the PCb group in experiment B, and it will be interesting to see whether they are closely related to the Chinese oil types in the PC2 group.

The turnip greens characterized by many narrow leaves, which in our collection are mainly of Japanese origin, form a very diverse group that either clusters with the Japanese turnips or forms two very separate clusters. MIZ079 in particular deviates greatly from all of the other *B. rapa* accessions and is characterized by many unique AFLP bands. MIZ079 is similar to the other Mizuna types at the early seedling stage in having a large number of soft and serrated feathery leaves. However, the internodes of MIZ079 elongate quickly up to a height of about 90 cm during later development, and this line is completely self-compatible, a condition which separates it from the typical Mizuna accessions. In experiment B, Shuicai accessions that resemble Mizuna form no clearly separate cluster and group in the Pak choi cluster. This suggests that similar phenotypes were selected in both China and Japan.

When the results from experiments A and B are compared, it is remarkable that the grouping is quite similar; namely, there are two main groups each of Chinese cabbage and Pak choi, with a corresponding position for the two common Pak choi accessions and the rapid-cycling accession. Unlike the common accession CC147/cCC102, the common accession CC161/cCC94 has no corresponding position in both trees. In order to better compare the trees from both experiments, we analyzed the data of experiment A after removing all of the types that are not represented in experiment B (Oil1, Oil2, T1, T2 and Bro). This subsequent comparison between the two trees illustrated that in experiment B the two Chinese cabbage groups are much more distinct then in experiment A, while the relationship between Pak choi types is similar in both trees. It is important to mention that in experiment A, four *Pst*/*Mse*I primer combinations were used, while in experiment B 12 *Eco*RI/*Mse*I primer combinations were used. *Pst*I does not cut methylated DNA and thus avoids repetitive DNA sequences, such as the DNA located around centromeres. We do not know whether *Pst*I and *Eco*RI target different DNA regions, which would result in different polymorphism rates and consequently contribute to the higher polymorphism rate in experiment A.

In addition, distinguishable subgroups are formed by self-incompatible, dark-seeded winter oil seed types from Pakistan (Oil1) and early- flowering, yellow-seeded, self-compatible Sarson types from India and Bangladesh (Oil2), both of which are not directly related to either East Asian or European types. A previous taxonomic study of oil type *B. rapa* (sp. *oleifera*) using RAPD and AFLP fingerprints also separated the accessions into groups corresponding to seed color and self-compatibility (Das et al. 1999). The origin of the accessions was not provided, so that we cannot directly compare the studies.

The phenetic groups we found in this investigation based on AFLP data are consistent with previous proposed groups based on morphology, origin, isozymes and nuclear RFLPs (Vaughan 1977; Prakash and Hinata 1980; Song et al. 1988b). Previous studies have suggested that these two groups represent two centers of origin for *B. rapa*, each originating from distinct wild *B. rapa* populations (Song et al. 1988b). Since the two large groups in experiment A have similar genetic distances, it can be concluded that the genetic variation in both centers is of the same order of magnitude and that this might be the consequence of the number of independent domestication events, intercrossing and breeding history.

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