Evaluation of genetic diversity of *Brassica napus* germplasm from China and Europe assessed by RAPD markers

Hu Shengwu[1], J. Ovesná[2], L. Kučera[2], V. Kučera[2], M. Vyvadilová[2]

[1]Shaanxi Academy of Agricultural Sciences, Yangling, Shaanxi, China
[2]Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

ABSTRACT

The genetic diversity and the relationships among rapeseed germplasm, including a collection of 20 Chinese, 25 Czech, 2 German, 2 French, and 1 English cultivars and breeding materials were evaluated using random amplified polymorphic DNA (RAPD) markers. A total of 79 different polymorphic amplification products were obtained using10 selected decamer primers. RAPDs revealed a significant level of polymorphism among the accessions. The diversity index (DI) ranged from 1.390 to 3.491, showing a sufficient potential of selected primers to differentiate among studied genotypes. Three different metrics were used to assess genetic diversity. The best fit between *a priori* knowledge about germplasm origin and *a posteriori* grouping was found using Hamman metrics. Cluster analysis based on Hamman pairwise distance comparison divided the studied accessions into three main clusters. The first group included only accessions from China, the second group only that from Europe with the exception of Zhongshuang No. 2, a Chinese winter rape possessing European cultivars in the pedigree. The third group included accessions both from China and Europe. The results indicate the occurrence of a considerable genetic variation between Chinese and European accessions.

Keywords: *Brassica napus*; RAPD; genetic diversity; genetic variability

Oilseed rape (*Brassica napus* L.) is an important oilseed crop grown in the world. To ensure efficient rapeseed production breeders have aimed to produce highly yielding and high quality cultivars. The information on the genetic diversity in *B. napus* could help breeders and geneticists to understand the structure of *B. napus* germplasm and help them to predict which combinations would produce the best offspring. Rapeseed cultivars used in Europe are generally of very high quality, but some desirable traits are missing in European gene-pool. It was proved that Chinese lines contain some genes, which make production of hybrid seed easier without genetic manipulations.

There are various techniques available, which allow study the genetic variability of crop germplasm. Morphological traits, total seed proteins, isozymes and several types of DNA markers are well known examples. DNA-based markers provide powerful and reliable tools to reveal variations within crop germplasm and to study evolutionary relationships (Gepts 1993). Among molecular markers, random amplified polymorphic DNA (RAPD) has been employed in genetic research owing to their speed and simplicity (Welsh and McClelland 1990). RAPD analysis has been widely used in recent studies on *Brassica* crops: (1) for determining the genetic relationships between different related species (Demeke et al. 1992, Thormann et al. 1994, Ren et al. 1995), (2) for the identification of cultivars (Hu and Quiros 1991) and the percentage of hybridity (Marshall et al. 1994), (3) for the estimation of genetic relationships and diversity among crop germplasm (Kresovich et al. 1992, Hallden et al. 1994, Mailer et al. 1994, Santos et al. 1994, Divaret and Thomas 1998). Although the use of RAPD technique for the study of genetic variation has been demonstrated as suitable in many species, less attention has been actually devoted to the selection of the most suitable approaches for genetic distance coefficients calculation. We employed RAPDs to determine genetic diversity among Chinese and European gene-pool in the presented investigation, taking into account different metrics for data evaluation. Information about genetic structure of the gene-pools can be useful for rapeseed breeding.

MATERIAL AND METHODS

Plant material. The plant material for this study comprised 50 genotypes of rapeseed cultivars and breeding materials. 25 accessions proceeded from Czech Republic, 20 accessions from China, 2 accessions from Germany and France and one accession from England (Table 1).

DNA extraction. Leaves from 50 one month old greenhouse cultivated plants per accession were pooled together for DNA isolation. Total genomic DNA was extracted according to the protocol of Saghai-Maroof et al. (1984). DNAs were dissolved and stored in TE buffer (pH 8.0). DNAs concentration were estimated and standardized against known concentration of lambda/HindIII DNA digest on 0.8% agarose gel. All DNA samples were diluted to the working concentration 50ng/µl with TE buffer before use.

Table 1. List of *Brassica napus* accessions

Name of accession	Abbreviation	Breeding institute	Type	Pedigree	Quality
SL-582	CZ_SL582	SBS	breeding line	Jet Neuf × PNG 14	00
SL-584	CZ_SL584	SBS	breeding line	(BNW36 × S5125) × Arabella	00
SL-586	CZ_SL586	SBS	breeding line	BNW36 × S5125	00
SL-587	CZ_SL587	SBS	breeding line	BNW36 × S5125	00
SL-588	CZ_SL588	SBS	breeding line	BNW36 × S5125	00
SL-589	CZ_SL589	SBS	breeding line	BNW36 × S5125	00
Zorro	GE_ZORRO	NPZ Lembe	cultivar		00
Mandarin	GB_MANDA	Nickerson seeds	cultivar		00
Bristol	FR_BRIST	Cargill	cultivar		00
Capitol	FR_CAPIT	Cargill	cultivar		00
Apex	GE_APEX	Novartis seeds	cultivar		00
Slapska Stela	CZ_SLSTE	SBS	cultivar		00
Zl. jarní – 66	CZ_ZLJ66	SBS	yellow seeded breeding line	Bn 153 × SL 511/1	00
Zl. jarní – 55	CZ_ZLJ55	SBS	yellow seeded breeding line	Bn 153 × SL 511/1	00
Zl. ozimá – 4	CZ_ZLO4	SBS	yellow seeded breeding line	Bn 153 × SL 511/1	00
Zl. ozimá – 19	CZ_ZLO19	SBS	yellow seeded breeding line	Bn 153 × SL 511/1	00
Sp-92	CZ_SP92	SBS	breeding line	(Bech.3.23 × BNW36) × Arabella	00
Sp-116	CZ_SP116	SBS	breeding line	POH 285 × Malux	00
F2-30	CZ_F230	SBS	breeding line	SL 530 × AC 419	00
SP 96/1	CZ_SP961	SBS	breeding line	B001 × (BO593 × SL 37)	00
SP 98/2	CZ_SP982	SBS	breeding line	(Bech.3.23 × BNW36) × Arabella	00
SP 106/3	CZ_SP106	SBS	breeding line	S 004 × Svalöf	00
SP 112/1	CZ_SP112	SBS	breeding line	POH 285 × Malux	00
F1 289/4	CZ_SP289	SBS	breeding line	Zorro × (POH 285 × Malux)	00
DH 5/1 97	CZ_DH5	RICP	DH line	(Bech.3.23 × BNW36) × Ceres	00
DH 6/1 97	CZ_DH6	RICP	DH line	(Bech.3.23 × BNW36) × Arabella	00
DH 14/7 97	CZ_DH157	RICP	DH line	ACF 408-80	00
DH 14/9 97	CZ_DH149	RICP	DH line	ACF 408-80	00
DH 15/7 97	CZ_DH147	RICP	DH line	Jet Neuf × PNG 14	00
L161	CH_L161	SICI	breeding line		0
B1	CH_B1	SICI	breeding line	selected from D89	00
Qinyou No. 1	CH_QIN1	Shaanxi Rape seed Res. Center	cultivar	selected from Yuejinyoucai	++
L766	CH_L766	SICI	breeding line	selected from line 84-16	0
Luzhou No. 5	CH_LUZH5	Sichuan Rice Inst.	cultivar	Luzzhouzao × Tongandongzhong	++
L910	CH_L910	SICI	breeding line	selected from R1	++
L920	CH_L920	SICI	breeding line	selected from R1	++
Zhongshuang No. 2	CH_ZHON2	Chinese Oil Crops Inst.	cultivar	Start × Ganyou No. 5	00
L430	CH_L430	SICI	breeding line		0
84-16-s	CH_8416S	SICI	breeding line	selected from line 84-16	0
Yuyou No. 2	CH_YUYO2	HICI	cultivar	(7818 × Marrnoo) F2 × Qva	00
227	CH_227	HICI	breeding line		00
Qinyou No. 3	CH_QIN3	SICI	cultivar	SE8 × Midas	0
680	CH_680	SICI	breeding line		0
97-18	CH_9718	SICI	hybrid cultivar	A3 × C3	0
Niongza No. 1	CH_NIO1	Jiangsu Industrial Crops Inst.	hybrid cultivar	Niong 6A × R1	00
Za 5900	CH_Z5900	Huazhong Agric. Univ.	hybrid cultivar	1141A × 5900	00
Youyan No. 7	CH_YOU7	Guizhou Oilcrops Inst.	hybrid cultivar	224A × 1536-119	0
Baozayou No. 13	CH_BAO13	Baoji Agric. Res. Inst.	hybrid cultivar	89A × 90-595C	0
Qinyou No. 2	CH_QIN2	Shaanxi Rape Seed Res. Centre	hybrid cultivar	Shaan 2A × Ken C1	++
A1	CH_A1	SICI	breeding line	Shaan 2A × B1	00

Quality: ++ refer to high erucic acid and high glucosinolate content, 0 low erucic acid, 00 low erucic acid and glucosinolate content; DH – doubled haploid, SBS – Slapy Breeding Station, HICI – Henan Industrial Crops Institute, RICP – Research Institute of Crop Production, Prague, SICI – Shaanxi Industrial Crops Institute

Table 2. Primers used for generating RAPDs in *Brassica napus* accessions

Serial number	Primer's name	Sequence (5'–3')	Fragment Numbers*	Fragment size range (bp)
1	ABN-02	ACCAGGGGCA	10	344–2036
2	ABN-06	GAGACGCACA	5	300–1250
3	ABN-13	AGCGTCACTC	10	400–1600
4	ABN-18	GCTGAGGTCA	7	300–1500
5	AB2-01	CCCAAGGTCC	4	500–2000
6	AB2-05	TCAGGGAGGT	11	500–1300
7	AB2-09	CTTCACCCGA	15	500–1200
8	AB2-06	AAGACCCATC	4	750–1500
9	AB2-10	CACCAGGTGA	6	800–2500
10	AB2-20	AACGGTGACC	7	300–1600

* No. of polymorphic fragments with different sizes produced by individual primers

DNA amplification. The total reaction volume for DNA amplification was 25 µl. Reaction mixtures contained 1× reaction buffer supplied with DyNAzymeTM DNA polymerase, (10mM Tris-HCl, pH 8.8, 1.5mM $MgCl_2$, 50mM KCl and 0.1% Triton X-100), 200µM each of dATP, dGTP, dCTP and dTTP (Promega), 0.2µM primer, 1.0 U DyNaZyme TM DNA polymerase (Finnzymes, Finland) and approximately 50 ng of genomic DNA. DNA amplification was performed using MJ Research thermocycler, Model PTC-200 (Watertown, MA) programmed to 1 cycle of 1 min at 94.0°C, 1 min of 37°C and 1 min 30 s of 72.0°C, followed by 36 cycles of 1 min at 92.0°C, 1 min at 37°C and 1 min 30 s at 72.0°C following by the final extension 10 min at 72.0°C. After amplification, PCR products were separated by electrophoresis in 1.5% agarose gel with 1× TAE buffer, stained with ethidium bromide and photographed under the UV light. The reproducibility of the amplification products was tested twice for each primer.

Data analysis. For each accession, a binary matrix reflecting specific RAPD band presence (1) or absence (0) was generated. Pair-wise distances between the accessions based on (a) Jaccard similarity metrics (Jaccard 1907), (b) Dice distance metrics (Nei and Li 1979) and (c) Hamman metrics (Spath 1980) were calculated with the use of RAPDALG program (The RAPDistance Package, Armstrong et al. 1994). UPGMA-clustering was conducted using the statistical software package STATISTICA (StatSoft, Inc.) or alternatively consensus trees were constructed using Phylip software package Felsenstein (1989). Diversity indexes (DI) were calculated according to Dahleen (1997) based on Shannon's information statistics.

RESULTS

Primer survey

Forty decamer primers (kit AB2 and kit ABN Advanced Biosystem, England) were initially screened for their ability to produce polymorphic patterns using 10 accessions: 5 from China, 5 from Europe. Ten decamer primers, which gave reproducible and distinct polymorphic amplification products, were selected for evaluation of diversity across all the accessions. Each of the selected 10 primers varied greatly in the ability to produce different polymorphic fragments ranging from 4 to 15 per reaction. On average 7.9 different polymorphic bands per reaction were record-

Table 3. Numbers of polymorphic fragments produced by individual primers and their diversity indexes as calculated according to Dahleen (1997)

Primer's name	Fragment numbers*	DI	% max DI	DI/fragment
AB2-01	4	2.199	63.0%	0.550
AB2-06	4	1.390	39.8%	0.348
ABN-18	7	3.020	86.5%	0.431
AB2-20	7	3.044	87.2%	0.435
ABN-02	10	3.453	98.9%	0.345
ABN-06	5	2.152	61.6%	0.430
AB2-05	11	3.491	100.0%	0.317
AB2-09	15	3.482	99.7%	0.232
ABN-13	10	3.135	89.8%	0.314
AB2-10	6	2.377	68.1%	0.396

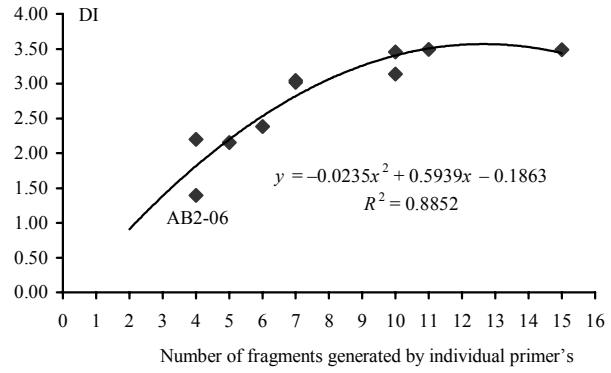

Figure 1. Comparison of information contribution of individual primers in dependence on the number of generated RAPD polymorphic fragments

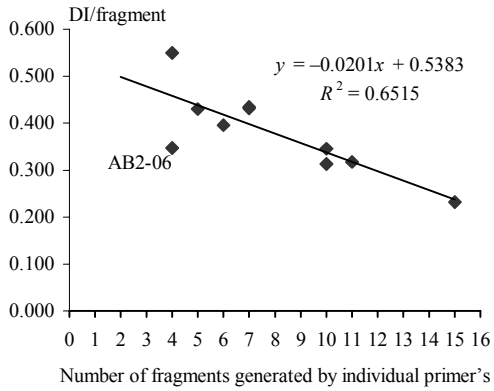

Figure 2. Information contribution of individual polymorphic fragments in dependence on the number of fragments generated by RAPD primer

ed (Table 2). A total of 79 different polymorphic amplification products were obtained using 10 selected arbitrary primers across 50 accessions.

Data analysis

Diversity indexes of the individual primers (Table 3) varied from 1.390 for primer AB2-06 to 3.491 for primer AB2-05, with and average mean 2.774. The more polymorphic fragments are amplified by individual primer, the more information about diversity could be obtained, even if contribution of individual polymorphic fragments is decreasing (Figures 1 and 2). However, primer AB2-06 does not fit perfectly in the calculated dependence. There were two products amplified by the primer AB2-06 repeatedly detectable across all the accessions except two genotypes. Such products might contribute to the differentiation among accessions in a slightly different way.

Three different metrics were used to process RAPD data and calculate the pair-wise distances. Genetic similarities were estimated to be between 0.095 (L910 versus L920) and 0.895 (Baozayou No. 13 versus Apex), when Jaccard metrics was employed. Values 0.050 (L910 versus L920) and 0. 810 (Baozayou No. 13 versus Apex) were calculated using Dice metrics and 0.051 (L910 versus L920) and 0. 646 (Baozayou No. 13 versus Apex) for Hamman metrics. Calculated distances were used later to generate dendrograms (STATISTICA package) and consensus trees (Phylip). The same clustering was generated using Jaccard and Dice distance metrics, only relative genetic distances differed. However, dendrogram generated from Hamman metric grouped accessions in slightly different way (Figures 3 and 4). The Hamman metrics based clustering is the most in accordance with the geographic origin and pedigree information. Phylip consensus trees grouped accession into similar but not identical groups. Also here the results based on Hamman metrics were in better accordance with *a priori* knowledge of germplasm origin (Figures 5 and 6), but unlike STATISTICA package did not cluster yellow-seeded accessions together.

The suitability of individual approaches for data processing can be further demonstrated on the groups of Czech breeding lines listed as SL-582 to SL-589. Lines possessing progenitor BNW36 × S5152 are clustered more or less into the same group using both STATISTICA software and Phylip software. Cultivar Zorro, originated in Germany was included tightly into this group using Phylip software and bootstrapping. Bootstrapping values were more significant when Hamman calculations for pair-wise distances were employed. On the other hand STATISTICA software reflects better our *a priori* knowledge of the plant material origin – all SL lines and cultivar Zorro are also grouped into one cluster, but it is apparent from dendrogram (Figure 4), that genetic similarity is not so high between the group of SL lines and cv. Zorro.

Three main clusters were observed when Hamman metrics and STATISTICA software was employed (Figure 4). The first group included only 16 rape accessions from China. The second group contains rape accessions both winter and spring type from Europe with the exception of Zhongshuang No. 2, a Chinese rape accession with double zero quality. According to the pedigree information, Zhongshuang No. 2 was developed from a cross between Start (a Poland winter type oilseed rape) and Ganyou No. 5. In the third main group, 4 Chinese accessions and 2 European accessions were grouped together. According to the pedigree information, Qinyou No. 1 was selected from the offspring of Yuejinyoucai, a winter type developed from an Italian genotype imported into China in 1950's. Other 3 Chinese accessions were developed from the crosses between European cultivars and Chinese cultivars.

Similar results were obtained using principal component analysis (Figure 7). PC1 accounted for 33% of the total variation and separates Chinese accessions containing no European progenitors in the pedigree from the others. PC2 accounted for 14% of the variation and to-

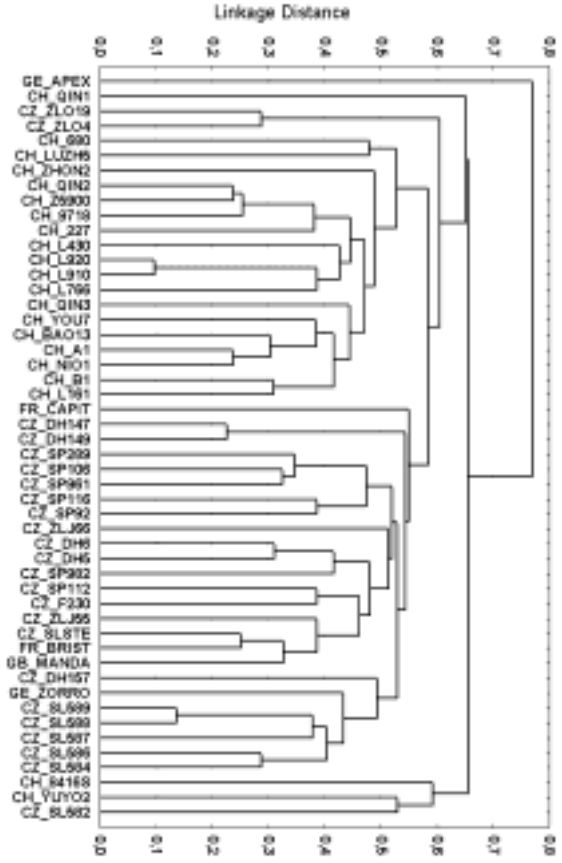

Figure 3. Dendrogram produced using UPGMA cluster analysis based on similarity matrix demonstrating the association among 50 rapeseed accessions; similarity values were calculated according to Jaccard (1907) based on 79 different RAPD polymorphic fragments

gether with PC1 grouped: (a) genetic resources of solely Chinese origin, (b) European materials and (c) germplasm of exotic origin (e.g. products of crosses between European and Chinese lines or progenies possessing in the pedigree products of wide hybridization of *B. napus* and *B. rapa*). Newly developed Chinese hybrid cultivars are also positioned slightly apart from other Chinese cultivars with lower quality.

DISCUSSION

A collection of germplasm was studied using RAPDs to show a degree of diversity among genotypes. The information can be used in breeding programmes. It has been proved that RAPD can be suitable and efficient tool for genetic characterization of many plant species including oilseed rape (Hu et al. 1999). It was possible to expect that European and Chinese gene-pools have different basis. Nevertheless, only one quarter of primers amplified sufficiently polymorphic products. On the other hand, using only 10 decamer primers we were able to differentiate among all 50 accessions, even between DH lines of the same origin. It was shown that RAPD could be efficiently used for rapeseed genotype profiling without necessity to employ high throughput, sophisticated and costly equipment, which is necessary e.g. for AFLP analysis (Lombard et al. 2000).

Genetic distance measures can be calculated using different approaches. Based on RAPD data, different metrics have been used to cluster the genotypes of *Brassica napus* up to now. Mailer et al. (1994) used Nei and Li (1979) similarity to group 23 cultivars. Li and Wu (1997) used Ward's cluster method to group 40 oilseed rape. However, there are a few reports investigating comparison of different metrics for examination of the variability in this crop. Peltier (1995) and Divaret and Thomas (1998) showed that suitability of different metrics for accession clustering varied. For closer related genotypes within outcrossed species Jaccard and Dices coefficient were found to be more suitable, whereas for more distant genotypes e.g. Sokal and Michener metrics was recommended. Three different metrics were used for grouping the accessions in this investigation. Our *a priori* knowledge of the accessions based on geographic origin and pedi-

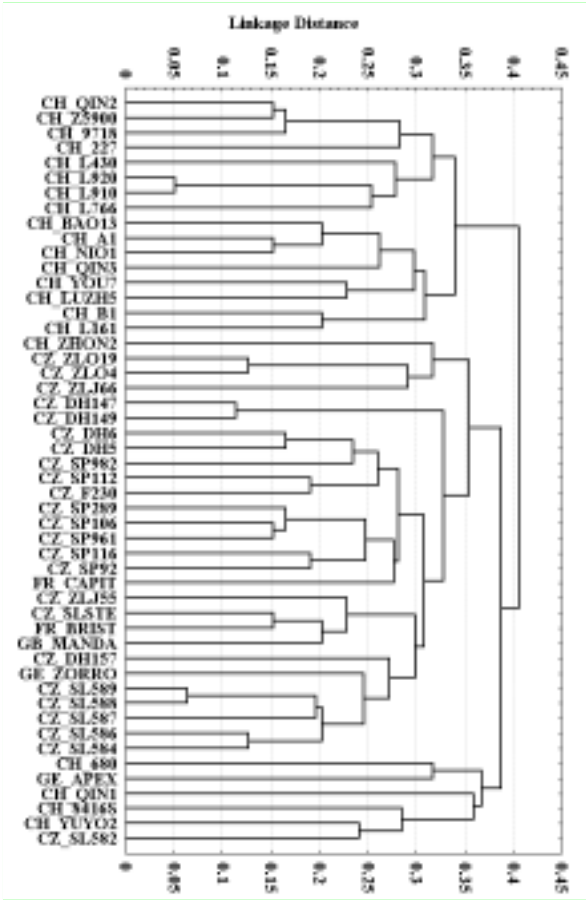

Figure 4. Dendrogram produced using UPGMA cluster analysis based on similarity matrix demonstrating the association among 50 rapeseed accessions; similarity values were calculated according to Hamman (Spath 1980) based on 79 different RAPD polymorphic fragments

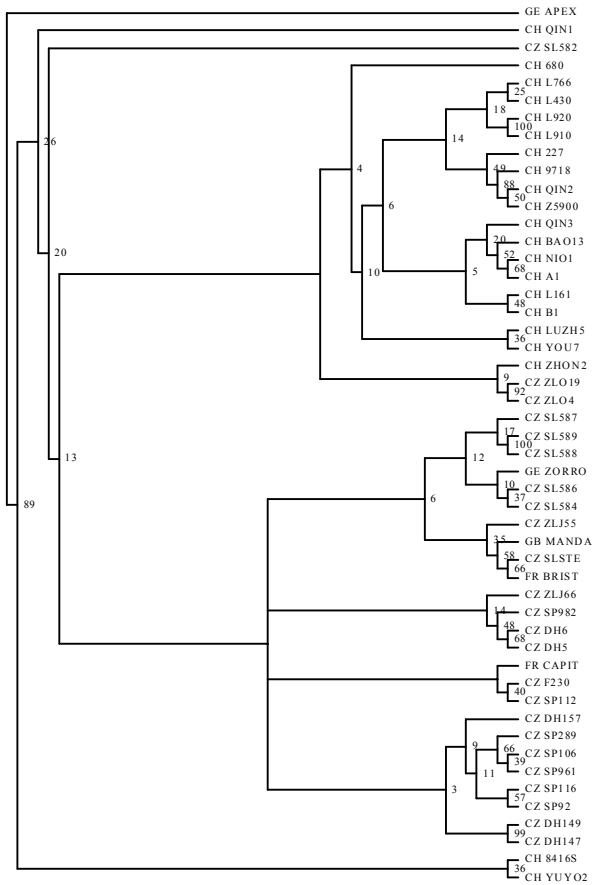

Figure 5. Consensus tree for 50 rapeseed accessions obtained from RAPD data set after bootstrapping generated by SeqBoot with the use of Jaccard metrics; the number of he forks indicate the number of times the groups consisting of the individual accessions which are to the right of that fork occurred among the trees out of 100 trees

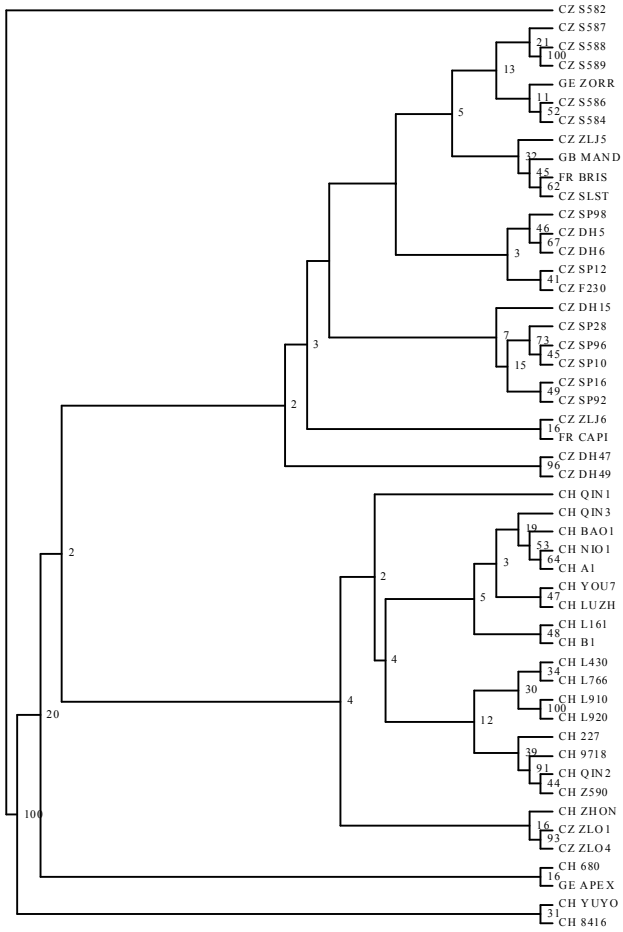

Figure 6. Consensus tree for 50 rapeseed accessions obtained from RAPD data set after bootstrapping generated by SeqBoot with the use of Hamman metrics; the number of the forks indicate the number of times the groups consisting of the individual accessions which are to the right of that fork occurred among the trees out of 100 trees

gree information was compared to the obtained trees. The best fit between *a priori* classification and *a posteriori* grouping of accessions investigated in this work was obtained using Hamman metrics and STATISTICA program. Jaccard's and Dice's metrics give more importance to the presence of identical signals in pair-wise comparison of two accessions than to the missing ones. Hamman metrics stress mainly the significance of differential signals between the pair of genotypes and the same relevance is attached to signals missing or presenting in two accessions. This might explain the better suitability of Hamman metrics for more distant gene pool evaluation. However, all approaches clearly separated accession of European and Chinese origin.

In our study, the cluster analyses separated the accessions into three main groups. The relationships between spring and winter accessions from Europe were shown to be closer than that between Chinese accessions and European accessions. This is not fully consistent with the findings of Song and Osborn (1992) and Diers and Osborn (1994), who separated most of the winter, spring and rutabaga *B. napus* accessions into different groups using RFLP markers, and indicated that the genetic backgrounds of spring and winter oilseed rape accessions are distinct. In fact, Czech breeders, whose material was mostly evaluated, have used crosses between the winter and spring accessions in cultivar development. Thus some of lines have both winter and spring materials in their pedigrees. Such an approach may explain the results presented in this study.

Two pairs of sister lines were also included to determine the similarity between them. Lines 910 and 920 had only 4 fragments differences and were the most related materials among studied accessions using all the three metrics. Lines 776 and 84-16-s showed 37 fragment differences. The parental material of the latter lines was very distant, which may explain the high dissimilarity among these two sister lines. Such information is precious for planning of crosses to wider or to maintain genetic variation in dependence on the breeding aim.

It can be concluded, from the above analyses, that the grouping of accessions based on the cluster analysis was

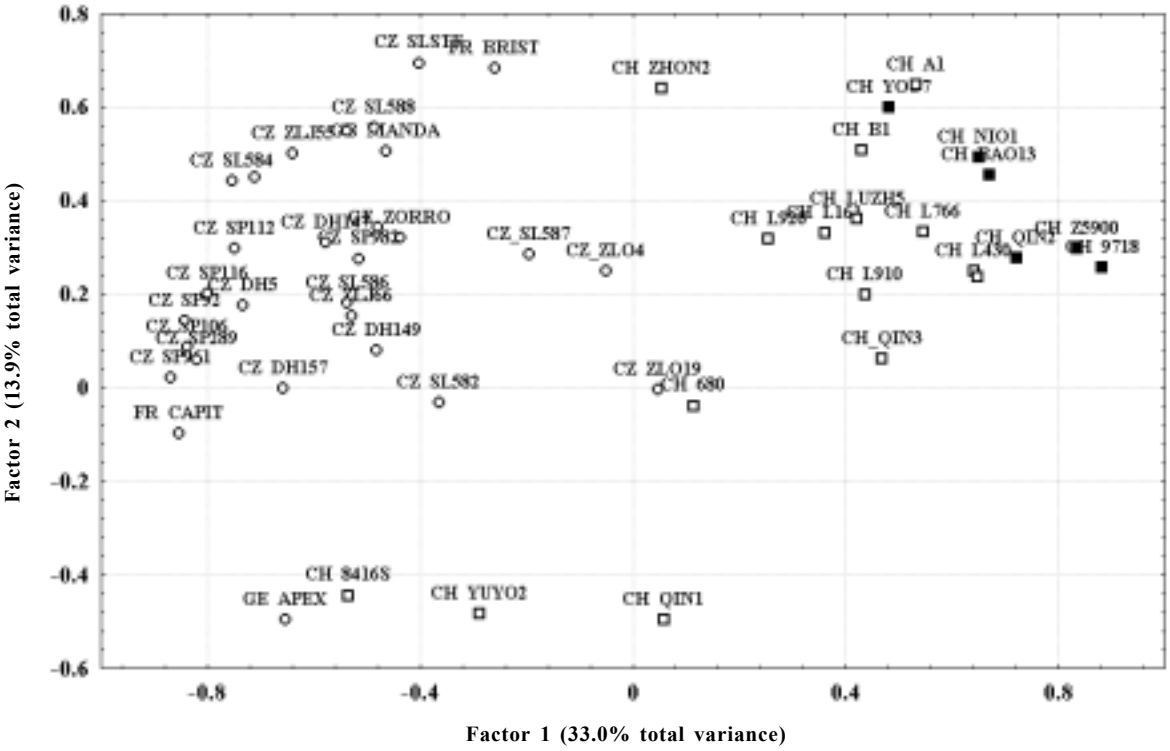

Figure 7. Biplot of first two principal components extracted from the RAPD analysis of 50 rapeseed accessions (Hamman metrics)

○ Czech and European accessions □ Chinese accessions except hybrid cultivars ■ Chinese hybrid cultivars

generally consistent with known pedigree information and geographic origin. The European accessions were distinguished from Chinese groups. Similar results were also obtained by Diers and Osborn (1994), Meng et al. (1996) by RFLP markers, and by Li and Wu (1997) by RAPD markers, and by Zhao and Becker (1998) by isozymes. The study demonstrated that the RAPD is a simple and fast technique to compare the genetic relationships and the patterns of variation among accessions of this crop. In view of the genetic differences, the European rapeseed would be important germplasm resources for enriching the genetic background of Chinese rapeseed, and vice versa.

REFERENCES

Armstrong J.S., Gibbs A.J., Pealcall R., Weiller G. (1994): The RAPDistance Package ftp:1/life.anu.edu.au/pub/software/RAPDistance or http/life.anu.edu.au/molecular/software/rapd.htmt.

Dahleen L.S. (1997): Mapped clone sequences detecting difference among 28 North American barley cultivars. Crop Sci., *37*: 952–957.

Demeke T., Adams R.P., Chibbar R. (1992): Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. Theor. Appl. Genet., *84*: 990–994.

Diers B.W., Osborn T.C. (1994): Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. Theor. Appl. Genet., *88*: 662–668.

Divaret I., Thomas G. (1998): Use of RAPD markers to analyze the genetic variability of a collection of *Brassica oleracea* L. In: Thomas G., Monteiro A.A. (eds.): Proc. Int. Symp. Brassicas. Acta Hort., *459*: 255–262.

Felsenstein J. (1989): PHYLIP – Phylogeny Inference Package (Version 3.2). Cladistics, *5*: 164–166.

Gepts P. (1993): The use of molecular and biochemical markers in crop evolution studies. In: Hecht M.K. (ed.): Evolutionary biology. Plenum Press, New York, *27*: 51–54.

Hallden C., Nilsson N.O., Rading I.M., Sall T. (1994): Evaluation of RFLP and RAPD markers in a comparison of *Brassica napus* breeding lines. Theor. Appl. Genet., *88*: 123–128.

Hu J., Li G., Struss D., Quiros C.F. (1999): SCAR and RAPD markers associated with 18-carbon fatty acids in rapeseed, *Brassica napus*. Plant Breed., *118*: 145–150.

Hu J., Quiros C.F. (1991): Identification of broccoli and cauliflower cultivars with RAPD markers. Plant. Cell. Rep., *10*: 505–511.

Jaccard P. (1907): Etude comparative de la distribution florale dans une portion des Alpes et des Jura. Bull. Soc. Vandoise Sci. Nat., *44*: 223–270.

Kresovich S., Williams J.G.K., McFerson J.R., Routman E.J., Schaal B.A. (1992): Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random am-

plified polymorphic DNA assay. Theor. Appl. Genet., *85*: 190–196.

Li W.N., Wu R.X. (1997): RAPD molecular markers and genetic diversity among 40 cultivars of *Brassica napus* in China. Chin. Biodiv., *5*: 246–250.

Lombard V., Baril C.P., Dubreuil P., Blouet F., Zhang D. (2000): Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: consequences for varietal registration. Crop Sci., *40*: 1417–1425.

Mailer R.J., Scarth R., Fristensky B. (1994): Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theor. Appl. Genet., *87*: 697–704.

Marshall P., Marchand M.C., Lisieczko Z., Landry B.S. (1994): A simple method to estimate the percentage of hybridity in canola (*Brassica napus*) F1 hybrids. Theor. Appl. Genet., *89*: 853–858.

Meng J., Sharpe A., Bowman G. (1996): Genetic diversity of *Brassica napus* detected with RFLP markers. Acta Genet. Sin., *23*: 293–306.

Nei M., Li W.H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci. USA, *76*: 5269–5273.

Peltier D. (1995): Utilisation des RAPD pour 1a construction de phenogrammes et de phylogrammes chez petunia. In: Beruille A., Tersas M. (eds.): Techniques et utilisations des marqueurs moleculaires. INRA: 187–202.

Ren J.P., Mc Ferson J.R., Li R.G., Kresovich S., Lamboy W.F. (1995): Identities and relationships among Chinese vegetable *Brassicas* as determined by random amplified polymorphic DNA markers. J. Amer. Soc. Hort. Sci., *120*: 548–555.

Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A., Allard R.W. (1984): Ribosomal DNA spacer-lenght polymorphism in barley. Mendelian inheritance, chromosomal location and population dynamics. Proc. Nat. Acad. Sci., *81*: 8014–8018.

Santos J.B. dos, Nienhuis J., Skroch P., Tivang J., Slocum M.K. (1994): Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor. Appl. Genet., *87*: 909–915.

Song K., Osborn T.C. (1992): Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome, *35*: 992–1001.

Spath H. (1980): Cluster analysis algorithms. Ellis Horwood, Chichester, England.

Thormann C.E., Ferreira M.E., Camargo C.E.A., Tivang J.G., Osbron T.C. (1994): Comparison of RFLP and RAPD markers to estimating genetic relationships within and among Cruciferrous species. Theor. Appl. Genet., *88*: 913–980.

Welsh J., McClelland M. (1990): Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res., *18*: 7213–7218.

Zhao J.Y., Becker H.C. (1998): Genetic variation in Chinese and European oilseed rape (*B. napus*) and turnip rape (*B. campestris*) analysed with isozymes. Acta Agron. Sin., *24*: 213–220.

Received on October 3, 2002

ABSTRAKT

Hodnocení genetické diverzity genetických zdrojů *Brassica napus* z Číny a Evropy pomocí RAPD markerů

Genetická diverzita a vztahy mezi genovými zdroji řepky, které zahrnují kolekci šlechtitelských materiálů a odrůd z Číny (20 položek), České republiky (25), SRN (2), Francie (2) a Velké Británie (1), byly vyhodnoceny pomocí RAPD markerů. RAPD odhalila významný stupeň polymorfismu mezi jednotlivými analyzovanými zdroji. Deset RAPD primerů amplifikovalo 79 odlišných polymorfních produktů. Hodnota indexu diverzity (DI) se pohybovala v rozmezí 1.390–3.491, což jsou hodnoty vyhovující pro rozlišení jednotlivých zdrojů. Pro odhad genetické diverzity souboru byly použity tři odlišné metriky. Nejlepší shoda mezi předběžnými znalostmi o původu hodnocených genových zdrojů a jejich konečným shlukováním byla nalezena při použití metriky podle Hammana. Shluková analýza založená na metrice podle Hammana odlišila tři skupiny. První skupina zahrnuje čínské zdroje, druhá skupina se skládá pouze z evropských zdrojů s výjimkou odrůdy Zhongshuang No. 2, což je čínská odrůda s evropskými odrůdami ve svém rodokmenu. Třetí skupina zahrnovala odrůdy pocházející jak z Číny, tak z Evropy. Výsledky naznačují významnou míru genetické variability mezi evropskými a čínskými genovými zdroji.

Klíčová slova: *Brassica napus*; RAPD; genetická diverzita; genetická variabilita

Corresponding author:

RNDr. Jaroslava Ovesná, CSc., Výzkumný ústav rostlinné výroby, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 424, fax: + 420 233 022 286, e-mail: ovesna@vurv.cz