Genetic Diversity Assessment in Winter Oilseed Rape (*Brassica napus* L.) Collection Using AFLP, ISSR and SSR Markers

Lenka HAVLÍČKOVÁ[1], Eva JOZOVÁ[1], Andrea RYCHLÁ[2], Miroslav KLÍMA[3], Vratislav KUČERA[3] and Vladislav ČURN[1]

[1]Biotechnological Centre, Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic; [2]Research Institute of Oilseed Crops, Opava, Czech Republic; [3]Crop Research Institute, Prague-Ruzyně, Czech Republic

Abstract

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The genetic diversity of 94 accessions of winter oilseed rape (*Brassica napus* L.), representing past and contemporary material utilized in the Czech breeding programmes, was determined using microsatellites (SSRs), ISSRs and AFLPs. All three kinds of markers differed in the range of observed polymorphism and differentiated clearly each accession. Altogether 89 SSR, 1003 AFLP and 53 ISSR markers were evaluated. Their average rates of polymorphic bands were 100%, 53.9% and 90.6%, respectively, indicating high genetic diversity among the accessions. The greatest genetic distance was found by ISSRs (62.3%) whereas significantly lower distances of 49.4% in SSRs and 35.5% in AFLPs were observed. The genetic similarity matrix clearly distinguished all accessions. A set of the most distinct varieties was established. The analysis of the genetic pattern of the accessions indicated two groups comprising most of the modern Czech breeding materials, revealing a distinct shift in breeding. Surprisingly, molecular analyses did not support breeders' views about a narrow genetic base of the Czech breeding materials. The choice of appropriate technology for different aspects of germplasm evaluation is also discussed.

Keywords: *Brassica napus*; genetic distances; genetic diversity; molecular markers

The genetic base of oilseed rape (*Brassica napus*) is quite narrow due to its limited geographic range and intensive breeding (Girke *et al*. 2012). Research on *Brassica* germplasm and evaluation of its genetic diversity could accelerate the efficient use of genetic variation through establishing a breeding programme (Stokes *et al.* 2010; Harper *et al.* 2012). Heterosis in hybrids is based on genetic completion between divergent parents, so the information on genetic diversity could help breeders better understand the genetic structure of germplasm and to predict which cross combinations would produce good F_1 hybrids (Yu *et al.* 2007). Breeders currently choose components for hybrid combinations based on desirable characteristics without any information about their affinity, although the genetic distance is a prerequisite for heterosis to a certain extent.

Recently, numerous markers for description of genetic resources have been developed such as isozymes, storage proteins or DNA based markers (Čurn 1995; Zhao & Becker 1998; Schlötterer 2004). At present, molecular methods have become essential parts of most studies on genetic diversity. Molecular methods are very useful for estimating features such as gene flow, genetic drift and degree of outbreeding, while other marker systems may be very useful for studying adaptive variation (Rao & Hodgkin 2002). Several methods such as

RFLP (Diers & Osborn 1994), RAPD (Shiran *et al.* 2006), AFLP (Yu *et al*. 2007), SSR (Hasan *et al.* 2006), cpSSR (Zamani-Nour *et al.* 2013), RAMP (Wei *et al.* 2005), SNP (Hayward *et al.* 2012) were used for characterization and evaluation of *B. napus* collections. These methods could improve strategies for germplasm conservation and increase the utilization of plant genetic resources. It is important, however, to understand that different markers have distinct properties, will reflect various aspects of genetic diversity and therefore can give different results (Nesbitt *et al.* 1995; Karp & Edwards 1997). Not many studies have combined more than two molecular techniques to evaluate the pattern of genetic diversity of oilseed rape (OSR). For this reason, comparative studies of different marker systems are a necessity to determine the relative merits of the various approaches in order to allow researchers to make an appropriate choice of methodology (Rao & Hodgkin 2002).

The aim of this study was to examine the genetic diversity among the selected accessions of winter oilseed rape by molecular markers. Comprehensive examination of the characteristics of this diversity at a molecular level subsequently provides a set of the most diverse genotypes for use in line and hybrid breeding programmes. Furthermore, this investigation could also provide a direct comparison of three molecular marker systems for the assessment of oilseed rape genetic diversity.

MATERIAL AND METHODS

Plant material. The core collection of 94 *B. napus* genotypes, assembled by breeders under the Czech Rape Association, including selected landraces, modern and older cultivars from different geographical regions and current breeding materials with different qualitative traits, was used for molecular analyses. Seeds of all *B. napus* genotypes were obtained from Crop Research Institute (CRI), Prague and from Research Institute of Oilseed Crops, Opava. The investigated accessions and their origins are listed in Table 1.

DNA isolation and ISSR, SSR and AFLP analyses. Total DNA was isolated from 100 mg of samples of lyophilized cotyledons collected from 36 representative plants per genotype. DNA was extracted using the CTAB extraction protocol (Doyle & Doyle 1987). After initial testing of a wide range of 30 ISSR primers, three primers generating the stable and repeatable pattern of markers were selected for ISSR analyses:

Table 1. List of *Brassica napus* accessions selected for molecular analyses

No.	Accession name	Type	Country of origin	Year of first registration
1	Adriana	C	FR/UK/DE	2006
2	Aglona	C	CZ	1993
3	Aplaus	C	CZ	2007
4	Arot	C	DE	2010
5	Asgard	C	DE	2006
6	Atlantic	C	FR	2005
7	Bellevue	C	DE	2007
8	Benefit	C	CZ	2009
9	Cadeli	C	US	2007
10	Californium	C	FR	2002
11	Catana	C	US/UK/FR	2006
12	Compakt	C	DE	2008
13	Contact	C	FR, DE	2000
14	Da Vinci	C	DE	2009
15	Dangal	C	UA	
16	Dar Laniv	C	UA	
17	Digger	C	DE	2000
18	DK Cabernet	C	US	2007
19	DK Casper	C	US	2009
20	ES Alegria	C	FR	2008
21	ES Astrid	C	FR	2003
22	ES Venus	C	FR	2009
23	Express	C	DE/UK	1999
24	Falcon	C	DE	1993
25	Goya	C	DE	2007
26	Chagall	C	DE	2008
27	Idol	C	FR	1995
28	Iwan	C	DE	2007
29	King 10	C	DE	2008
30	Komando	C	FR/DE	2006
31	Labrador	C	FR	2005
32	Ladoga	C	FR	2004
33	Loreley	C	DE	2008
34	Manitoba	C	FR, DE	2005
35	Mickey	C	AT	2009
36	Mira	C2	CS	1978
37	Mirage	C	DE	2007
38	Navajo	C	UK	1999
39	NK Diamond	C	FR	2008
40	NK Fair	C	FR	2004
41	NK Morse	C	FR	2000
42	NK Nemax	C	FR	2007
43	NK Passion	C	FR	2005

Table 1 to be continued

No.	Accession name	Type	Country of origin	Year of first registration
44	Odila	C	CZ	1997
45	Oksana	C	CZ	2007
46	Omaha	C	UK	2000
47	Omikron	C	CZ	1995
48	Oponent	C	CZ	2006
49	Opus	C	CZ	2007
50	Remy	C	DE	2007
51	Robust	C	DE	2005
52	Sherlock	C	DE	2009
53	Silesia	C2	CS	1983
54	Siska	C	DE	2005
55	Slapská	C1	CS	1945
56	Slapská Stela	C	CZ	1996
57	Solida	C2	CS	1986
58	Sonáta	C	CS	1990
59	Sveta	C	UA	
60	Totem	C	FR	2008
61	Třebíčská	C1	CS	1941
62	Vittek	C	DE	2009
63	Winner	C	DE	2001
64	Wisent	C	DE	2010
65	Zhongshuang No. 9	C	CN	
66	3196/1n	BF	CZ	
67	3258/1n	BF	CZ	
68	3338/i	BF	CZ	
69	4924/4	BF	CZ	
70	C 542	B	CZ	
71	C 547	B	CZ	
72	C 567	B	CZ	
73	ČŽL 20	BY	CN	
74	ČŽL 24	BY	CN	
75	DH 4729/09	B	CZ	
76	DH 4736/09	B	CZ	
77	OP 4947/07	B	CZ	
78	SG-C 2269	B	CZ	
79	SG-C 768	B	CZ	
80	SL 737	B	CZ	
81	Rf 4069/2	CMSO	CZ**	
82	Rf 4108/3	CMSO	CZ**	
83	A115 CMS line	CMSO	CZ**	
84	Rf 3358	CMSO	CZ**	
85	Rf 3372	CMSO	CZ**	
86	Rf 3388	CMSO	CZ**	

Table 1 to be continued

No.	Accession name	Type	Country of origin	Year of first registration
87	OP 1 (mCMSS for S3)	CMSS	CZ	
88	S2 -CMS	CMSS	CZ*	
89	S3 -CMS	CMSS	CZ*	
90	S7/1/2 (Rf for CMSS S2; S3)	CMSS	CZ*	
91	AIK 128/1 (Tandem 6/85)	SI	CZ	
92	AIK 20/9 (WRG 15)	SI	CZ	
93	AIK 21/55 (Liropa)	SI	CZ	
94	AIK 22/17 (Tandem 1/85)	SI	CZ	

Country codes – see ISO 3166-1; CZ* – Chinese donors of CMS backcrossed to Czech lines/cultivars; CZ** – French donors of CMS backcrossed to Czech lines/cultivars; L – landrace; C – cultivar: C1 – old cultivars and landraces, C2 – "0" type, C – "00" type cultivars; B – breeding material – oil yield; BF – breeding material – changed content of fatty acids; BY – yellow-seeded breeding material; CMSO – CMS *Ogu*-INRA genetic resources; CMSS – CMS *Shaan* 2A genetic resources (mCMSS – maintainer; Rf – restorer); SI – self-incompatible genetic resources

UBC 812 (5'-GA)$_8$A-3'; UBC840 (5'-GA)$_8$YT-3' and UBC 845 (5'-CT)$_8$RG-3'. PCR amplification reactions were carried out in the total volume of 10 µl containing 1× PPP Master Mix (Top-Bio), 12.5 pmol of primer, 1× BSA and 50 ng template DNA. Amplifications were performed using the following programme: pre-denaturation for 2 min at 95°C, 40 cycles of 20 s at 93°C, 1 min 52°C and 20 s at 72°C, finally, 6 min at 72°C. PCR products were dissolved by electrophoresis on 2% agarose gel in 1× TBE buffer using the following programme: 20 min at 40 V followed by 280 min at 80 V and visualized by EtBr staining. All analyses were performed in pairs, and only samples with the same pattern of ISSR markers were scored. Altogether 19 SSR markers were selected according to variability (PIC value between 0.5 and 0.8, number of alleles between 5 and 10) with high stability and low stutter and genome position (one each chromosome to cover all chromosomes present in *B. napus*). PCR amplification, detection and analysis of microsatellites were performed according to Plieske and Struss (2001). AFLP analysis was carried out as described

by Vos *et al.* (1995) with three primer combinations: *Eco*RI+ACG/*Mse*+AGT, *Eco*RI+ACG/*Mse*+ACC and *Eco*RI+ACG/*Mse*+ATT. PCR products were analysed using capillary electrophoresis on an ABI PRISM 3130xl sequencer (Applied Biosystems, Foster City, USA) and data were analysed by GeneMapper software.

Band scoring and data analysis. Molecular data were analysed using a digital image analysis and fingerprint patterns from each of the three marker types (ISSR, SSR and AFLP) were transformed into a binary character matrix with 1 for the presence or 0 for the absence of a band at a particular position in a lane. Genetic distance matrices were generated using Nei and Li (1979) metrics. For all three molecular markers the Weighted Arithmetic Mean of genetic distances (WAM) was calculated on the basis of frequency values in the appropriate class of genetic diversity. In this study 94 accessions were analysed, thus, a lower half distance matrix consisting of 4371 elements = values of genetic distance between two particular accessions [((94 × 94) – 94)/2 = 4371], and these values were divided into classes ranging each 5 percent and displayed in the form of histogram for each marker used. Cluster analysis (UPGMA) and principal coordinates analysis (PCO) were also performed. These analyses were calculated using MVSP 3.1 (Kovach Computing Services, Anglesey, U.K.) and DARwin 5.0.158 (CIRAD, Montpellier, F) software packages. Genetic structure was calculated using Structure 2.3.4 software package (Pritchard *et al.* 2000). To determine the most likely number of clusters we followed the approach of Evanno *et al.* (2005) and Kolář *et al.* (2012).

RESULTS

Microsatellites, AFLP and ISSR generated 89, 1003 and 53 bands, which were 100, 53.94 and 90.57% polymorphic, respectively. From the obtained patterns, it was possible to identify all analysed accessions even when closely related breeding lines, undefined breeding materials or doubled haploid (DH) lines were included in the molecular analyses. Clear recognition of all analysed accessions is demonstrated from outputs of PCO (Figure 1) and cluster – UPGMA analyses (Figure 2). Although these results allow the unambiguous identification of genetic resources via particular and quite uneven distribution, no clustering depending on the origin (breeding or geographical) was evident. Nevertheless, one exception was detected, regarding the group of specific accession CMS *Ogu*-INRA genetic resources: there is a close relationship between mother CMS line and Rf lines that should be taken into account when choosing suitable diverse components to improve these materials.

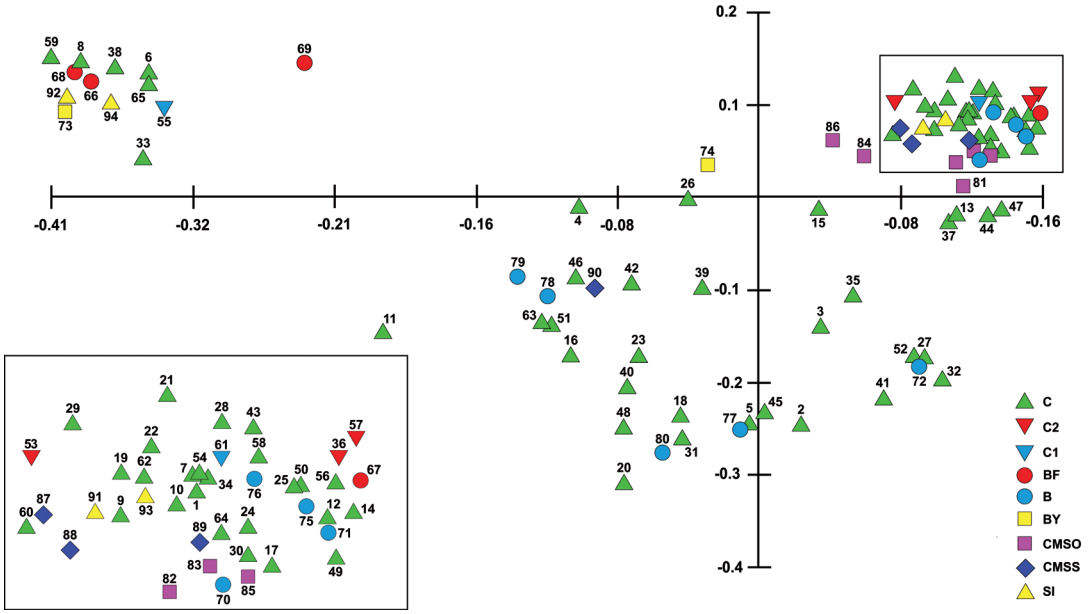


Figure 1. Principal coordinates analysis (PCO) of SSR, ISSR and AFLP markers in 94 *Brassica napus* accessions
C – cultivar; C1, C2, BF – breeding material – changed content of fatty acids; B – breeding material – yield of oil; BY – yellow-seeded breeding material; CMSO – CMS *Ogu*-INRA genetic resources; CMSS – CMS *Shaan* 2A genetic resources; SI – self-incompatible genetic resources; dense group of samples in the right upper corner is shown in detail window; for explanation of symbols used for different types of plant material see Table 1

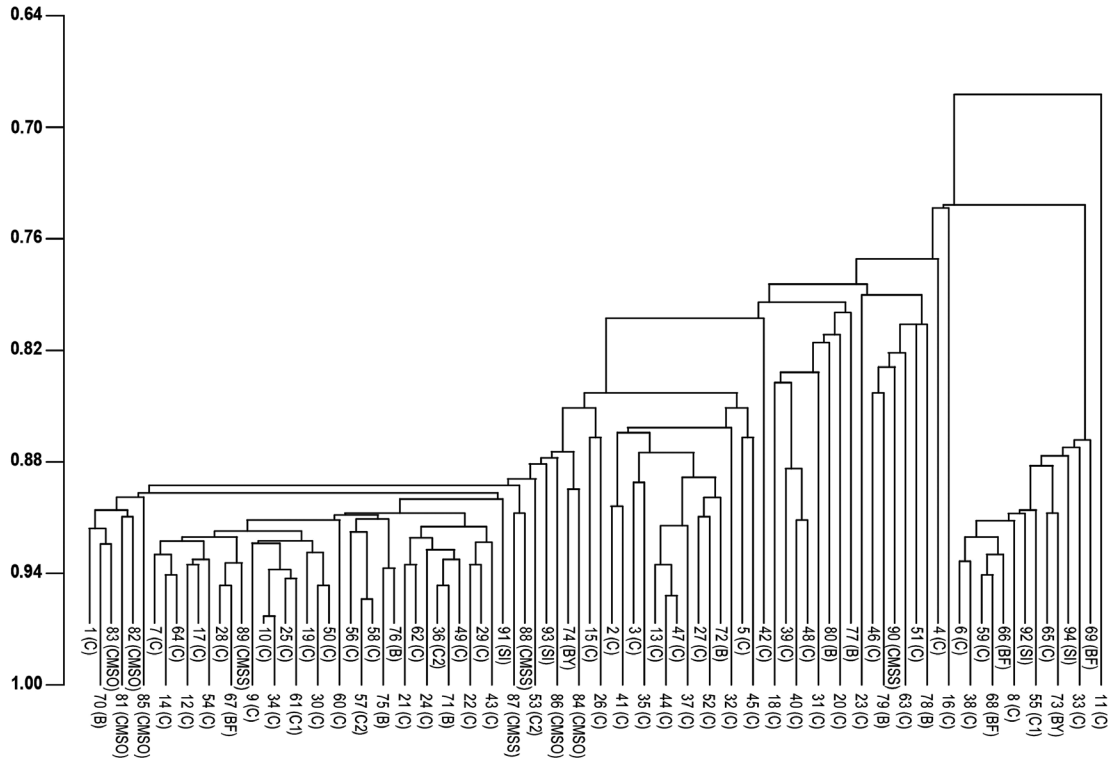


Figure 2. Cluster analysis (UPGMA dendrogram) of SSR, ISSR and AFLP markers in 94 *Brassica napus* accessions

Three molecular marker systems used in this study differed also in the extent of detected diversity. Particular genetic distances between two specific items can be obtained from a distance matrix (not shown, available on the website: http://biocentrum.zf.jcu.cz/projekty.php) and character and distribution of genetic diversity for all three markers are given in Figure 3. For ISSR represented markers with the highest detectable extent of genetic variation; the lowest genetic distances (0.038) were determined between five pairs of accessions (10-43, 21-69, 49-67, 66-67 and 75-76), the highest distance (0.623) between 81 (CMSO) and 87 (mCMSS for S3) with French and Czech origin, respectively. In SSR the lowest genetic distances (0.022) were recorded in closely related modern German cultivars (13-26) and breeding materials (75-78), the highest distance (0.494) was observed between old Czech cultivar Silesia (53) and Chinese cultivar Zhongshuang No. 9 (65). AFLP manifested generally a higher level of genetic similarity; the lowest genetic distance (0.037) was between French cultivars Californium and Manitoba (10, 34), the highest distance (0.355) between US cultivar Catana and French NK Morse (11, 41), respectively.

All three molecular markers differed not only in the extent of detectable genetic diversity but also in the ability to detect the genetic diversity among individual accessions. This parameter was expressed as WAM and reflects the frequency and size of the genetic distance. The highest genetic distances between *B. napus* accessions were recorded using ISSR markers, where WAM among all 94 accessions was 25.71%. SSR markers reached average WAM (22.51%) and the highest similarity and dense clustering of samples were recorded in AFLP (WAM = 18.68%).

Results and outputs from the STRUCTURE software are given in Figure 4. Classification of accessions according to Q1/Q2 values (Figure 4D) led to the formation of three blocks: (a) the first block included accessions with Q1 value in the range of 0.003–0.013 with prevalence of Czech breeding materials or genotypes which were widely used in recent Czech breeding programmes; (b) the second block formed a transition zone; and (c) the third block included accessions with Q1 values in the range of 0.991–0.998 (Q2 = 0.009–0.002). The second and third blocks included accessions without clearly visible character of classification. Similar results are presented in Figure 4C, when classification is done according to the year of registration (release) of a particular cultivar. More informative are results presented in Figure 4B, where the data are classified by the country of origin and year of registration (release) of a particular cultivar. A similar pattern of genetic

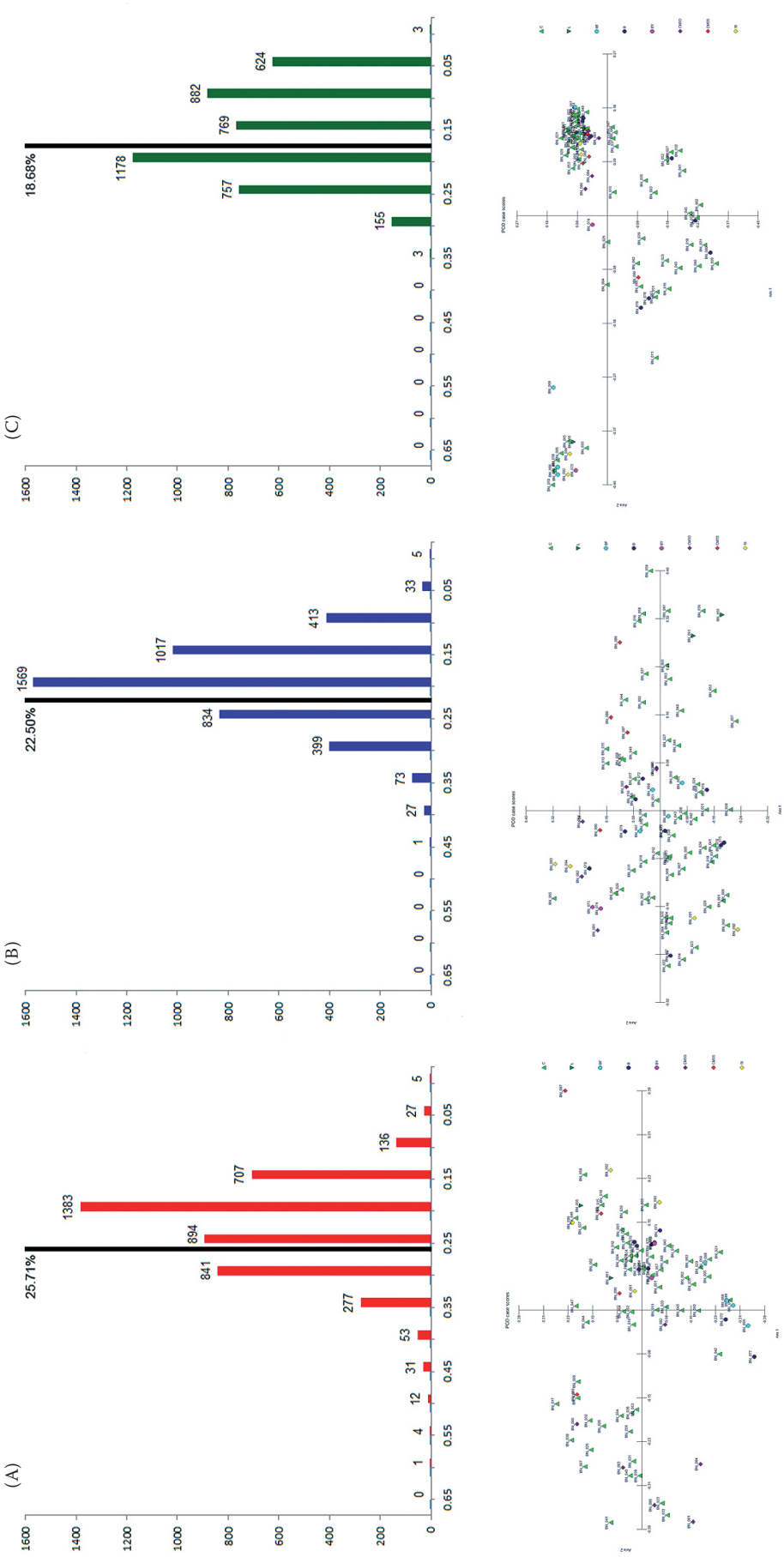
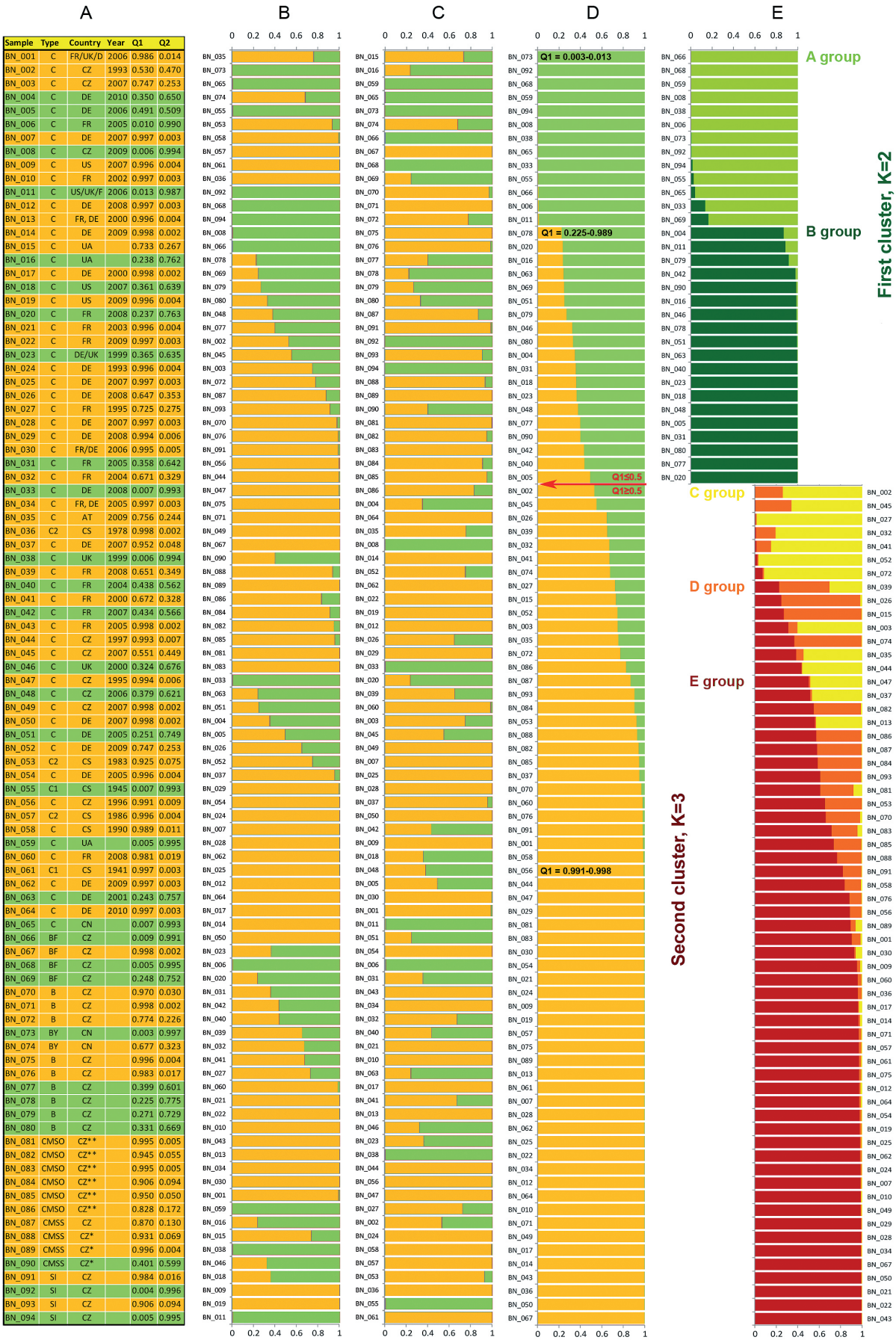


Figure 3. Comparison of the extent and distribution of genetic diversity in all three analysed marker systems
A – ISSR; B – SSR; C – AFLP; *x*-axis – classes of genetic diversity (range 0–1, corresponding to 0–100% of genetic diversity); *y*-axis – number of occurrences in a particular class (94 accessions generated a lower distance half matrix of 4371 elements, unit distances on the main diagonal are not included in the total number of elements); black line – position of WAM; clustering of samples based on PCA analysis is shown schematically below the histogram representing the distribution of genetic diversity

A

Sample	Type	Country	Year	Q1	Q2
BN_001	C	FR/UK/D	2006	0.986	0.014
BN_002	C	CZ	1993	0.530	0.470
BN_003	C	CZ	2007	0.747	0.253
BN_004	C	DE	2010	0.350	0.650
BN_005	C	DE	2006	0.491	0.509
BN_006	C	FR	2005	0.010	0.990
BN_007	C	DE	2007	0.997	0.003
BN_008	C	CZ	2009	0.006	0.994
BN_009	C	US	2007	0.996	0.004
BN_010	C	FR	2002	0.997	0.003
BN_011	C	US/UK/F	2006	0.013	0.987
BN_012	C	DE	2008	0.997	0.003
BN_013	C	FR, DE	2000	0.996	0.004
BN_014	C	DE	2009	0.998	0.002
BN_015	C	UA		0.733	0.267
BN_016	C	UA		0.238	0.762
BN_017	C	DE	2000	0.998	0.002
BN_018	C	US	2007	0.361	0.639
BN_019	C	US	2009	0.996	0.004
BN_020	C	FR	2008	0.237	0.763
BN_021	C	FR	2003	0.996	0.004
BN_022	C	FR	2009	0.997	0.003
BN_023	C	DE/UK	1999	0.365	0.635
BN_024	C	DE	1993	0.996	0.004
BN_025	C	DE	2007	0.997	0.003
BN_026	C	DE	2008	0.647	0.353
BN_027	C	FR	1995	0.725	0.275
BN_028	C	DE	2007	0.997	0.003
BN_029	C	DE	2008	0.994	0.006
BN_030	C	FR/DE	2006	0.995	0.005
BN_031	C	FR	2005	0.358	0.642
BN_032	C	FR	2004	0.671	0.329
BN_033	C	DE	2008	0.007	0.993
BN_034	C	FR, DE	2005	0.997	0.003
BN_035	C	AT	2009	0.756	0.244
BN_036	C2	CS	1978	0.998	0.002
BN_037	C	DE	2007	0.952	0.048
BN_038	C	UK	1999	0.006	0.994
BN_039	C	FR	2008	0.651	0.349
BN_040	C	FR	2004	0.438	0.562
BN_041	C	FR	2000	0.672	0.328
BN_042	C	FR	2007	0.434	0.566
BN_043	C	FR	2005	0.998	0.002
BN_044	C	CZ	1997	0.993	0.007
BN_045	C	CZ	2007	0.551	0.449
BN_046	C	UK	2000	0.324	0.676
BN_047	C	CZ	1995	0.994	0.006
BN_048	C	CZ	2006	0.379	0.621
BN_049	C	CZ	2007	0.998	0.002
BN_050	C	DE	2007	0.998	0.002
BN_051	C	DE	2005	0.251	0.749
BN_052	C	DE	2009	0.747	0.253
BN_053	C2	CS	1983	0.925	0.075
BN_054	C	DE	2005	0.996	0.004
BN_055	C1	CS	1945	0.007	0.993
BN_056	C	CZ	1996	0.991	0.009
BN_057	C2	CS	1986	0.996	0.004
BN_058	C	CS	1990	0.989	0.011
BN_059	C	UA		0.005	0.995
BN_060	C	FR	2008	0.981	0.019
BN_061	C1	CS	1941	0.997	0.003
BN_062	C	DE	2009	0.997	0.003
BN_063	C	DE	2001	0.243	0.757
BN_064	C	DE	2010	0.997	0.003
BN_065	C	CN		0.007	0.993
BN_066	BF	CZ		0.009	0.991
BN_067	BF	CZ		0.998	0.002
BN_068	BF	CZ		0.005	0.995
BN_069	BF	CZ		0.248	0.752
BN_070	B	CZ		0.970	0.030
BN_071	B	CZ		0.998	0.002
BN_072	B	CZ		0.774	0.226
BN_073	BY	CN		0.003	0.997
BN_074	BY	CN		0.677	0.323
BN_075	B	CZ		0.996	0.004
BN_076	B	CZ		0.983	0.017
BN_077	B	CZ		0.399	0.601
BN_078	B	CZ		0.225	0.775
BN_079	B	CZ		0.271	0.729
BN_080	B	CZ		0.331	0.669
BN_081	CMSO	CZ**		0.995	0.005
BN_082	CMSO	CZ**		0.945	0.055
BN_083	CMSO	CZ**		0.995	0.005
BN_084	CMSO	CZ**		0.906	0.094
BN_085	CMSO	CZ**		0.950	0.050
BN_086	CMSO	CZ**		0.828	0.172
BN_087	CMSS	CZ		0.870	0.130
BN_088	CMSS	CZ*		0.931	0.069
BN_089	CMSS	CZ*		0.996	0.004
BN_090	CMSS	CZ*		0.401	0.599
BN_091	SI	CZ		0.984	0.016
BN_092	SI	CZ		0.004	0.996
BN_093	SI	CZ		0.906	0.094
BN_094	SI	CZ		0.005	0.995



structure is found in accessions of Czech, German as well as French or Chinese origin. Detailed assessment of the genetic structure of the analysed OSR cultivars and breeding materials based on the analysis of the two individual clusters (divided by value Q1 ≤ 0.5 and Q1 ≥ 0.5) identified material based on a set of allele frequencies within these clusters into 5 groups in total (Figure 4E). The analysis of the first cluster divides in detail the population structure into two sub-clusters, where most representatives of group A belong to the Czech material used mainly in CRI, while group B contains predominately European cultivars released in the period 1999–2010. The second cluster was further divided into three groups, where the main group E mostly contains Czech cultivars and materials for SI and CMS, German and French cultivars. Groups D and C include assorted cultivars of the world and of different ages.

DISCUSSION

Due to its relatively narrow genetic base, oilseed rape improvement is increasingly reliant on evaluation of its genetic diversity, better management of breeding population (Cowling *et al.* 2012) and utilization of existing genetic diversity in order to establish a promising breeding programme (Lombard *et al.* 2000; Asghari *et al.* 2011). In oilseed rape, unlike several other important crops, we cannot use sources of genetic variation from natural populations (Prakash & Hinata 1980) and therefore techniques and approaches recognizing the extent of genetic variability are very beneficial. Molecular markers used in this study have also been used as effective tools to provide molecular data and evaluate genetic relationships in other studies although they did not include such diverse genetic resources (Sobotka *et al.* 2004; Li *et al.* 2011; Abdelmigid 2012). The importance and benefit of this study lies in the direct comparison of all accessions tested by three molecular techniques and their genetic diversity evaluation for the purposes of combinational crossing of distant genotypes in line and hybrid breeding.

Molecular analyses of 94 *B. napus* accessions performed in this study did not surprisingly confirm assumptions about the narrow genetic base of rapeseed core collection (Girke *et al.* 2012). Such an assumption is correct only if restricted sets of oilseed rape are analysed (e.g. materials from a particular breeding programme). Results of molecular analyses also revealed high genetic distances between modern cultivars and breeding materials of special purposes. Similar results were presented on the panel of Australian germplasm by Cowling (2007). All three molecular markers, distinct in their nature and performance, differed in the extent and ability to detect diversity. Some comparative analyses suggest that AFLPs are the most suitable markers, with the most monomorphic fragments (Russell *et al.* 1997; Pejic *et al.* 1998), but high discriminatory power (Behera *et al.* 2008) due to a high number of bands in a single amplification (Pejic *et al.* 1998). However, based on easier technology used, it seems that the most successful technique is ISSR which combines most advantages of SSR and AFLP technologies (Reddy *et al.* 2002) and provides the best information about genotypes in terms of their genetic distances. SSR, ISSR and AFLP provide polymorphic information based on DNA repeat variation and DNA sequence polymorphisms and all markers have been proved as suitable for distinguishing between genotypes that are genetically very similar (McGregor *et al.* 2000; Sarwat *et al.* 2008). Therefore, the selection of a suitable marker is more dependent on the price and technologies available for target users.

For an overall assessment of genetic variability and the degree of diversity of rapeseed genetic resources in the analysed collection in addition to standard descriptive characteristics (type of marker, polymorphic bands, marker analysis using UPGMA and PCO approaches) other two procedures were applied. To evaluate the extent of detectable genetic diversity between individual accessions the parameter WAM was exploited. The STRUCTURE software package for assessment of multi-locus genotype data was used for evaluation of the genetic structure in rapeseed accessions. Results from both these approaches show the more valuable interpretation of the results, allow direct comparison and selection marker system and also highlight another way to process the genetic diversity data. Assessment of

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Figure 4. Results of the analysis of genetic structure of accessions from the rape core collection using the Structure software package (Admixture Model, Allele Frequencies Correlated, K = 2, Length of Burnin Period and MCMC: 100 000); 4A – list of analysed accessions, their characteristics, Q1 and Q2 values (outputs of Structure analysis) and sorting according to Q1 value (4D – lowest to highest); 4B and 4C – sorting according to the country of origin and year of registration (release), respectively; 4E detailed analysis of cluster 1 (Q1 ≤ 0.5) and cluster 2 (Q1 ≥ 0.5) from the main cluster 4D that divided the core collection into 5 subgroups

the population genetic structure is commonly used in ecological and evolutionary studies (Kolář *et al.* 2012) but not for evaluation of genetic resources. It may lead (as shown in our example) to clarification of cultivar grouping and also to recommendations for expanding the genetic background in breeding programmes. For instance, the position of Czech CMS breeding materials in group E together with French materials can be explained by the fact that these materials are based on the French donor of *Ogu*-INRA type of male sterility. In comparison with results of Bus *et al.* (2011) we obtained a less detailed resolution of oilseed rape accessions (i.e. K = 2) and therefore we decided to separately evaluate populations belonging to two main clusters for the purpose of getting a more detailed resolution. This can be explained using only winter oilseed rape germplasm. The inclusion of a swede, winter OSR, semi-winter OSR, spring OSR, spring fodder and vegetable led to the formation of a less compact and more resolvable cluster (Bus *et al.* 2011).

Oilseed rape breeders may especially benefit from the precise identification of all genetic resources, using all three molecular marker systems. It was verified that ISSRs using the proposed protocol, are stable, reliable and inexpensive markers with great sensitivity to reveal genetic diversity. Detection of genetic distances between particular accessions from the core collection facilitates selection of parental components on the basis of the highest genetic diversity. Czech breeders under the Czech Rape Association apply this system of selection of parental components at present. In these cases, results of molecular analyses may help to reveal the real diversity of genetic resources of oilseed rape. Thus, they can be useful for accurate selection of parental components in hybridization programmes and can increase the efficiency of both line and hybrid breeding.

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Corresponding author:

Ing. Lenka Havlíčková, Ph.D., Jihočeská univerzita v Českých Budějovicích, Zemědělská fakulta, katedra speciální produkce rostlinné, Studentská 13, 370 05 České Budějovice, Czech Republic; e-mail: lenk@post.cz