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INTER SPECIES TESTING OF BRASSICA MICROSATELLITES AVAILABLE IN PUBLIC DOMAIN AND THEIR POTENTIAL UTILIZATION FOR COMPARATIVE GENOMICS IN CRUCIFERAE

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Abstract

Many of the microsatellites available in the public domain have been used successfully or tested on species other than that from which they were originally isolated. In this study we report on the distribution of more SSR loci within Brassicaceae species. A selection of available primer pairs was screened against a panel of *Brassica rapa, B. carinata, B. juncea, B. napus* and *B. nigra* lines. PCR conditions were kept uniform to facilitate high throughput genotyping. Initial screening comprised of separation of PCR products on 3.5% metaphor agarose gels and visualization by staining with Ethidium bromide. The PCR products were assessed on the basis of polymorphism and number of fragments amplified. Those with a small number of fragments (1-4) that showed polymorphism either within or between species were then screened again using a larger panel of lines. All the primer pairs that could be used successfully in *B. rapa* were examined in other *Brassica* and Cruciferous species. More than 90% of the primer pairs successfully amplified the corresponding microsatellite regions in the *Brassica* species tested. Based on these results, we concluded that the selected microsatellites have a high potential for the development of DNA markers that could contribute to the genetic analysis of *Brassica* and other Cruciferae.

Introduction

Microsatellites also designated as simple sequence repeats are composed of short nucleotide sequences repeated in tandem. They occur throughout the genome of eukaryotes with distinct characteristics in each species (Tautz & Renz, 1984; Dib *et al*., 1996; Dietrich *et al*., 1996). They show a high polymorphism and co-dominant inheritance, and can be easily detected by PCR-based methods. These characteristics have prompted their use as molecular markers for genetic studies of various plants, including genetic mapping, marker assisted selection and population analysis (Kresovich *et al*., 1995; Broun & Tanksley, 1996).

Microsatellites of *Brassica* have been studied, especially in *B. napus*, as indicated at the *Brassica* Microsatellite Information Exchange (http//www.brassica.info/ssr/SSRinfo.htm). Because *B. napus* is an amphidiploid species that originated from spontaneous hybridization between *B. rapa* and *B. oleracea*. The development of microsatellite markers in diploid species is also necessary for comprehensive studies on *Brassica* genetics.

Comparative genetic analysis has been a common strategy for investigating genetic relationships and chromosome collinearity among Cruciferous species (Lagercrantz & Lydiate, 1996; Ryder *et al*., 2001; Babula *et al*., 2003). Some researchers have revealed the presence of conserved regions for gene content and gene order between *Brassica* species (Scheffler *et al*., 1997; Quiros *et al*., 2001; Parkin *et al*., 2002).

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In *Brassica* genetics, RFLPs and RAPDs have been the main techniques used for various genetic analyses such as genome mapping (Somers *et al*., 2001) and construction of linkage maps (Song *et al*., 1991; Landry *et al*., 1992). However they are associated with limitations for comparative genomics among related species. RFLPs are commonly used in comparative genomics (Lagercrantz, 1998; Kole *et al*., 2002), although the data obtained from RFLP analysis are some times difficult to interpret because of signals derived from cross-hybridization with additional loci. This may disturb the detailed comparison of corresponding chromosomal regions between species. For this reason a DNA marker that can be transferred to related species with a high accuracy would be required for comparative analysis in *Brassica* genetics. In this paper we report on the polymorphisms and applicability of selected microsatellites to Cruciferous species. Our results indicated that microsatellites as DNA markers are not only a powerful tool for practical breeding but may also contribute to the understanding of genetics and to phylogenetic studies in *Brassica*.

Materials and Methods

Plant materials and extraction of genomic DNA: All the plants used in this study are listed in Table 1. Genomic DNA was isolated from fresh young leaves of a single plant from each line or accession by the CTAB method (Murray & Thompson, 1980).

PCR amplification of microsatellite loci: The microsatellites tested in this study were selected from the collection available in the public domain (Lowe *et al*., 2002, 2004; www.brassica.info/ssr/SSRinfo.htm) and are listed in Table 2. PCR was performed in a volume of 20 µL containing 20 ng of DNA template, 20 pmol of each primer, 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, 1X PCR reaction buffer [10 mM Tris HCl (pH 8.3), 50 mM KCl, Fermentas] and 1 unit of Taq DNA polymerase (Fermentas). Amplifications were performed using a standard amplification cycle in a GeneAmp PCR System 9700 thermal cycler and SSR polymorphisms were separated on 3.5% metaphor (Bio Whittaker Mol. Appl.). Electrophoresis was performed at 100 V for 5-6 hr in 1X TBE buffer (Sambrook *et al*., 1989). The 300 mL gel contained 25 µL EtBr (10mg/mL) and the results were visualized under UV light with Gel Doc 2000, and pictures were digitally recorded with Quantity One software (Bio-Rad Lab., Hercules, CA). The microsatellite loci successfully amplified in *B. rapa* were further tested in *B. nigra*, *B. carinata*, *B. napus and B. juncea*.

Data analysis: The fragments amplified by microsatellite primers were scored as present (1) or absent (0), as described for example by Alamerew *et al*., (2004) for analysis of polyploid wheat. The genotype data was converted to a similarity matrix using the Dice similarity index (Dice, 1945), described as follows by Nei & Li (1979):

$$S = 2Nab\ (2Nab + Na + Nb)$$

where Nab is the number of bands shared by genotypes a and b in each pair wise comparison and Na and Nb are the numbers of bands present in the respective genotypes. The SAHN module of NTSYSpc (version 2.01, Exeter Software, Setauket, NY, USA) was used to generate a Dendrogram based on the UPGMA algorithm (un-weighted pair group method with arithmetic average).

Table 1. List of *Brassica* accessions surveyed with SSRs during present study.

Sr. #	Accession number	Collection/Donor organization	Collection / Donor No.	Origin	Location
1.	23638(Bc)	PGRC	CN 40216	Canada	107 Science Place, Saskatoon
2.	23640(Bc)	PGRC	CN 101619	Canada	107 Science Place, Saskatoon
3.	23641(Bc)	PGRC	CN 101621	Canada	107 Science Place, Saskatoon
4.	23644(Bc)	PGRC	CN 101626	Canada	107 Science Place, Saskatoon
5.	23648(Bc)	PGRC	CN 101630-A	Canada	107 Science Place, Saskatoon
6.	23651(Bc)	PGRC	CN 101632	Canada	107 Science Place, Saskatoon
7.	23652(Bc)	PGRC	CN 101633	Canada	107 Science Place, Saskatoon
8.	23655(Bc)	PGRC	CN 101654	Canada	107 Science Place, Saskatoon
9.	23656(Bc)	PGRC	CN 101661	Canada	107 Science Place, Saskatoon
10.	23658(Bc)	PGRC	CN 101698-A	Canada	107 Science Place, Saskatoon
11.	22850(Bc)	BARI, Chakwal	Chakwal-raya	Pakistan	Chakwal
12.	22863(Bc)	AARI, Faisalabad	Peela-raya	Pakistan	Faisalabad
13.	1521(Bj)	ARS-USDA	PI-426296	USA	NA
14.	1547(Bj)	ARS-USDA	PI-426327	USA	NA
15.	1575(Bj)	ARS-USDA	PI-426359	USA	NA
16.	1578(Bj)	ARS-USDA	PI-426363	USA	NA
17.	1605(Bj)	ARS-USDA	PI-426394	USA	NA
18.	1618(Bj)	ARS-USDA	PI-426408	USA	NA
19.	1639(Bj)	PARC/JICA	003109(01)	Pakistan	70 Km E to Bahawalpur
20.	1674(Bj)	CRP/Oilseed	Local Sarson	Pakistan	NARC, Islamabad
21.	1676(Bj)	CRP/Oilseed	Dacca Raya	Pakistan	NARC, Islamabad
22.	1677(Bj)	CRP/Oilseed	ELIGHT	Pakistan	NARC, Islamabad
23.	1689(Bj)	PARC/IBPGR	001537(01)	Pakistan	15 Km W to Jamesabad
24.	1694(Bj)	PARC/JICA	003098(01)	Pakistan	15 Km S to Chowk Azam

Table 1. (Cont'd.).

Sr. #	Accession number	Collection/Donor organization	Collection / Donor No.	Origin	Location
25.	1702(Bj)	PARC/JICA	003156(01)	Pakistan	16 Km NE to Bannu
26.	1715(Bj)	CRP/Fodder & Forages	103	Pakistan	NARC, Islamabad
27.	1721(Bj)	CRP/Fodder & Forages	F-184	Pakistan	NARC, Islamabad
28.	1751(Bj)	ARS-USDA	PI-426422	USA	NA
29.	1762(Bj)	PARC/JICA	003090(03)	Pakistan	23 Km N Talagang
30.	19500(Bj)	CRP/Oilseed	UCD-8/1	Pakistan	NARC, Islamabad
31.	19508(Bj)	CRP/Oilseed	Mustard-B	Pakistan	NARC, Islamabad
32.	19511(Bj)	CRP/Oilseed	Sultan Raya	Pakistan	NARC, Islamabad
33.	19493(Bj)	CRP/Oilseed	BARD-1	Pakistan	NARC, Islamabad
34.	19510(Bj)	ORS, Khanpur	Khanpur-raya	Pakistan	Rahim Yar Khan
35.	1690(Bn)	PARC/IBPGR	001540(04)	Pakistan	Farmland
36.	1691(Bn)	PARC/KUJ	002265(07)	Pakistan	Grain Market
37.	1693(Bn)	PARC/JICA	003097(03)	Pakistan	10 Km S to Chowk Muhajar
38.	1695(Bn)	PARC/JICA	003107(01)	Pakistan	30 Km W to Hasilpur
39.	1696(Bn)	PARC/JICA	003117(01)	Pakistan	05 Km E to Alipur
40.	1697(Bn)	PARC/JICA	003122(02)	Pakistan	13 Km NE to R.Y. Khan
41.	1698(Bn)	PARC/JICA	003134(02)	Pakistan	11 Km W to Fazilpur
42.	1699(Bn)	PARC/JICA	003143(01)	Pakistan	33 Km N to D.G. Khan
43.	1700(Bn)	PARC/JICA	003149(02)	Pakistan	20 Km W to D.I. Khan
44.	1701(Bn)	PARC/JICA	003152(03)	Pakistan	17 Km N to D.I. Khan
45.	1705(Bn)	PARC/PGRI	003377(07)	Pakistan	Market
46.	1706(Bn)	PARC/PGRI	003381(04)	Pakistan	Mandi
47.	1720(Bn)	CRP/Fodder & Forages	F-175	Pakistan	NARC, Islamabad
48.	16104(Bn)	PARC/PGRI	004213(01)	Pakistan	Farm store

Table 1. (Cont'd.).

Sr. #	Accession number	Collection/Donor organization	Collection / Donor No.	Origin	Location
49.	16105(Bn)	PARC/PGRI	004214(06)	Pakistan	Punch-Kotli road
50.	22851(Bn)	BARI, Chakwal	Chakwal Sarson	Pakistan	Talagang - Chakwal road
51.	22853(Bn)	ARI, D.I.Khan	Takwara	Pakistan	Ratta Kulachi
52.	22855(Bn)	NIFA, Peshawar	Abasin-95	Pakistan	On Peshawar G.T. road.
53.	23632(Bn)	ARI, Mingora	Pak Cheen-89	Pakistan	NA
54.	23636(Bn)	CRP/Oilseed	19-H	Pakistan	NA
55.	23637(Bn)	CRP/Oilseed	Rainbow	Australia	NA
56.	23633(Bn)	CRP/Oilseed	Shiralee	Australia	NARC, Islamabad
57.	23634(Bn)	CRP/Oilseed	Dunkeld	Australia	NARC, Islamabad
58.	1602(Br)	ARS-USDA	PI-426390	USA	NA
59.	1626(Br)	PARC/JICA	003092(02)	Pakistan	32 Km SW to Talagang
60.	1662(Br)	PARC/JICA	003154(01)	Pakistan	46 Km S to Bannu
61.	1692(Br)	PARC/KUJ	002265(11)	Pakistan	Grain Market
62.	1709(Br)	CRP/Fodder & Forages	23	Pakistan	NARC, Islamabad
63.	1714(Br)	CRP/Fodder & Forages	63	Pakistan	NARC, Islamabad
64.	1716(Br)	CRP/Fodder & Forages	109	Pakistan	NARC, Islamabad
65.	1719(Br)	CRP/Fodder & Forages	231	Pakistan	NARC, Islamabad
66.	1761(Br)	PARC/JICA	003090(02)	Pakistan	23 Km N Talagang
67.	1766(Br)	PARC/PGRI	003394(04)	Pakistan	Market
68.	1771(Br)	PARC/PGRI	003790(01)	Pakistan	Farm land
69.	23631(Br)	AARI, Faisalabad	BSA	Pakistan	Faisalabad
70.	23630(Br)	AARI, Faisalabad	Toria-A	Pakistan	Faisalabad
71.	23660(Bng)	PGRC	CN 31735	Canada	107 Science Place, Saskatoon
72.	23661(Bng)	PGRC	CN 32267	Canada	107 Science Place, Saskatoon
73.	23662(Bng)	PGRC	CN 34037	Canada	107 Science Place, Saskatoon
74.	23663(Bng)	PGRC	CN 40227	Canada	107 Science Place, Saskatoon
75.	23665(Bng)	PGRC	CN 105398	Canada	107 Science Place, Saskatoon

Table 2. List of microsatellite markers used for diversity analysis of *Brassica* germplasm.

Microsatellite	Forward Primer	Reverse Primer	Motif	Repeat No.
SSR Na10-A08	CATGGTTAAAACAATGGCCC	CAAGAAACACCATCATTTCTCA	CT	21
SSR Na10-A09	TCTTGAGCAAAGAAACTTGG	CAAACTGAGCCATACACAAAGG	GA	26
SSR Na10-B01	CAAGTGTCTGCTAGGTGGGG	TCGATCGAAGAAACCAGACC	GA	46
SSR Na10-B04	GCGTCGAGAGAGATCGAGAG	CTCACCGTCACTGCTTCATC	GA	40
SSR Na10-B07	GCCTTAGATTAGATGGTCGCC	ACTTCAGCTCCGATTTGCC	CT	29
SSR Na10-B08	AGAGAAAAACACTTCCCGCC	GTGAGCTTTGCGAAACACG	CT	38
SSR Na10-B10	GTCGGGTTTGAGTGAGTTGG	CATCGCAGATCCTTCTCTCC	GGC\A\T	18
SSR Na10-B11	TTTAACAACAACCGTCACGC	CTCCTCCTCCATCAATCTGC	CT	28
SSR Na10-C01	TTTTGTCCCACTGGGTTTTC	GGAAACTAGGGTTTTCCCTTC	GA	19
SSR Na12-A02	AGCCTTGTTGCTTTTCAACG	AGTGAATCGATGATCTCGCC	CT	16
SSR Na12-A07	TCAAAGCCATAAAGCAGGTG	CATCTTCAACACGCATACCG	GT	11
SSR Na12-A08	AACACTTGCAACTTCATTTTCC	CATTGGTTGGTGAATTGACAG	GA	28
SSR Na12-E02	TTGAAGTAGTTGGAGTAATTGGAGG	CAGCAGCCACAACCTTACG	TTG	13
SSR Na12-D04	ACGGAGTGATGATGGGTCTC	CCTCAATGAAACTGAAATATGTGTG	CA	11
SSR Na12-F03	GGCGACATAGATTTGAACCG	TCCACTTTCTCTCTCTTCCCC	GA	35
SSR Ni4-D09	AAAGGACAAAGAGGAAGGGC	TTGAAATCAAATGAGAGTGACG	CT	25
SSR Ol10-B01	CCTCTTCAGTCGAGGTCTGG	AATTTGGAAACAGAGTCGCC	GA	20
SSR Ol10-F11	TTTGGAACGTCCGTAGAAGG	CAGCTGACTTCGAAAGGTCC	GGC	7
SSR Ol10-F12	TCCATGTTTCATGTTGGAGG	CTCTCCGGCTTCACTTTCC	CT	64
SSR Ol10-H02	AACAGGAAGAAACGACGAGG	AGAGAGCCATGAGAAGCACC	GGC	8
SSR Ra2-A11	GACCTATTTTAATATGCTGTTTTACG	ACCTCACCGGAGAGAAATCC	CT	51
SSR Ra2-E11	GGAGCCAGGAGAGAAGAAGG	CCCAAAACTTCCAAGAAAAGC	CT	24
SSR Ra2-F11	TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC	CT	34
SSR Ra2-E03	AGGTAGGCCCATCTCTCTCC	CCAAAACTTGCTCAAAACCC	CT	18
SSR Ra3-H12	ATAATGGCTGCTGCAGGTTC	CCTACTACAACTATACGTTATGCCTG	GT	38

Results

Microsatellites distribution within the species of Brassicaceae family: Twenty five microsatellite-specific primer pairs were analyzed in order to estimate the distribution of the microsatellites among different *Brassica* species. The amplified fragments were classified as microsatellite-specific amplification products if the size of the fragments was similar to the expected size. All the primer pairs that could be used successfully in *B. rapa* were examined in other *Brassica* species. More than 90% of them were able to amplify corresponding microsatellite regions in all *Brassica* species analyzed (Figs. 1 & 2).

Genetic diversity: Twenty five microsatellite-specific primer pairs were tested for their efficiency in detecting polymorphisms among 75 *Brassica* genotypes. A total of 228 polymorphisms were observed among the varieties and lines. The number of alleles per microsatellite locus varied from 2 to 7. Most of the primer pairs detected two alleles (35%), 23% three, 21% four and 15% five alleles. Six alleles were detected by 4% and seven by 2% of them. The gene diversity of microsatellite markers varied form 0.05 to 0.90 with an average of 0.50. Using 228 polymorphisms the estimated genetic distances revealed by the markers were, on average, 0.48 among 75 *Brassica* genotypes. All 75 accessions were discriminated in a dendrogram (Fig. 3) constructed on the basis of shared fragments. The 75 genotypes were classified into seven groups, representing *B. rapa, B. napus, B. carinata, B. nigra, and B. juncea*. The major groups were further divided into eight sub-groups. Pair wise similarity matrices were calculated for every genotype. The similarity values ranged from 0.47 to 0.82 for *B. carinata*, 0.31 to 0.85 for *B. juncea*, 0.27 to 0.86 for *B. napus*, 0.39 to 0.88 for *B. rapa* and 0.28 to 0.81 for *B. nigra*. The number of alleles per microsatellite locus varied from 2 to 4. The size of the alleles ranged from 150 to 318 bp.

Discussion

All the microsatellite primer pairs that could amplify corresponding loci in *B. rapa* were compatible with those in other species, although this was not the case for other microsatellite primer pairs. This finding suggests that the corresponding microsatellite regions present in *B. rapa* and other *Brassica* species are widely conserved in the family Cruciferae. In the present study, we describe the potential utilization of some of the microsatellites as molecular markers in cruciferous species. Microsatellites of *B. rapa* are one of the best sources for developing DNA markers suitable for *Brassica* genetic studies, and should provide a reliable and effective means for comparative genomics in *Brassica*. The distribution of the microsatellite loci among different species of the *Brassicaceae* family showed a high variation potential, especially among the species of the triangle of U as previously discussed by Plieseke & Struss (2001). Assignment of alleles to the mapped loci described by Lowe *et al*., (2002, 2004) was not always possible because of the high allelic diversity and variation in allele sizes in comparison to the published data. This may be due to differences in PCR conditions leading to more stringent amplification. Also, for SSR analysis using a sequencer with better resolution, much more information can be extracted from the PCR products compared to what is possible from gel electrophoresis. Since most of the amplifying primer pairs in our study gave strong bands and the amplification products were polymorphic between the species, they are suitable as STS markers in genetic analyses.

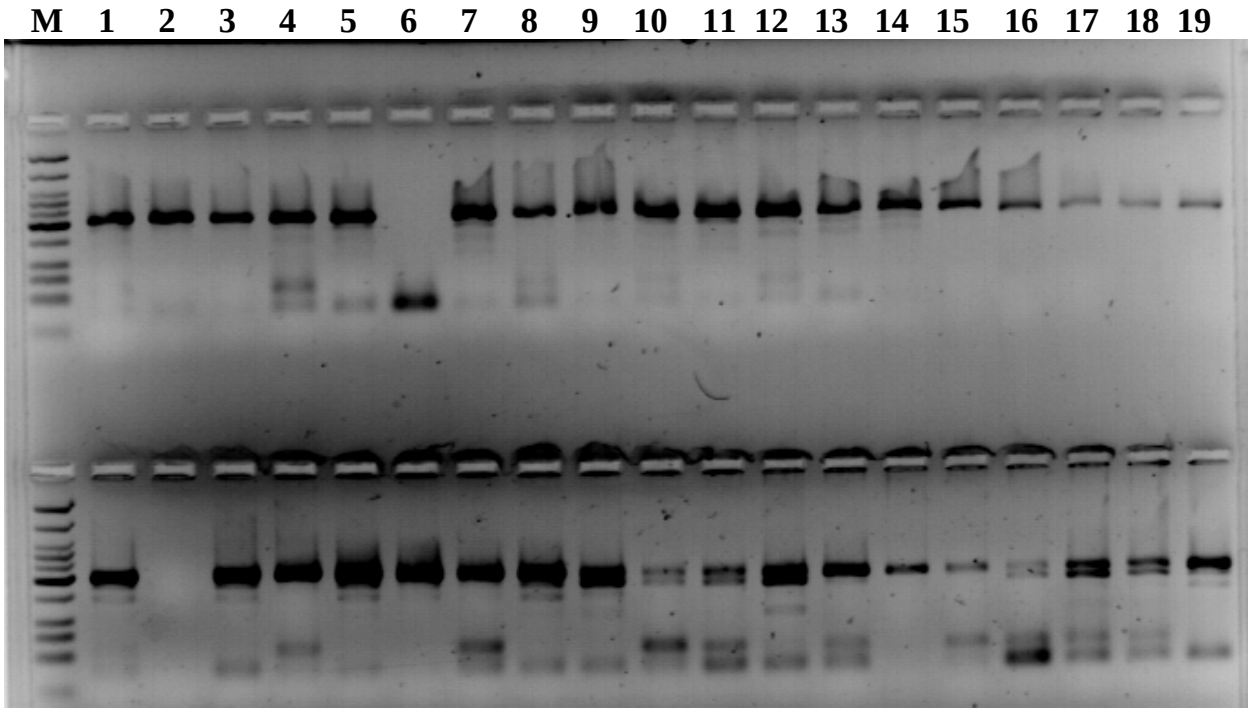

Fig. 1. SSR banding patterns of 38 *Brassica* genotypes generated by SSR primer Ol10-H02. Lanes represent M-Molecular marker, 1-12 *B. carinata*, 13-34 *B. juncea*, 35-38 *B. napus*. Molecular weights of marker bands are 766, 500, 350, 300, 250, 200, 150, 100, 75, 50 and 25, respectively.

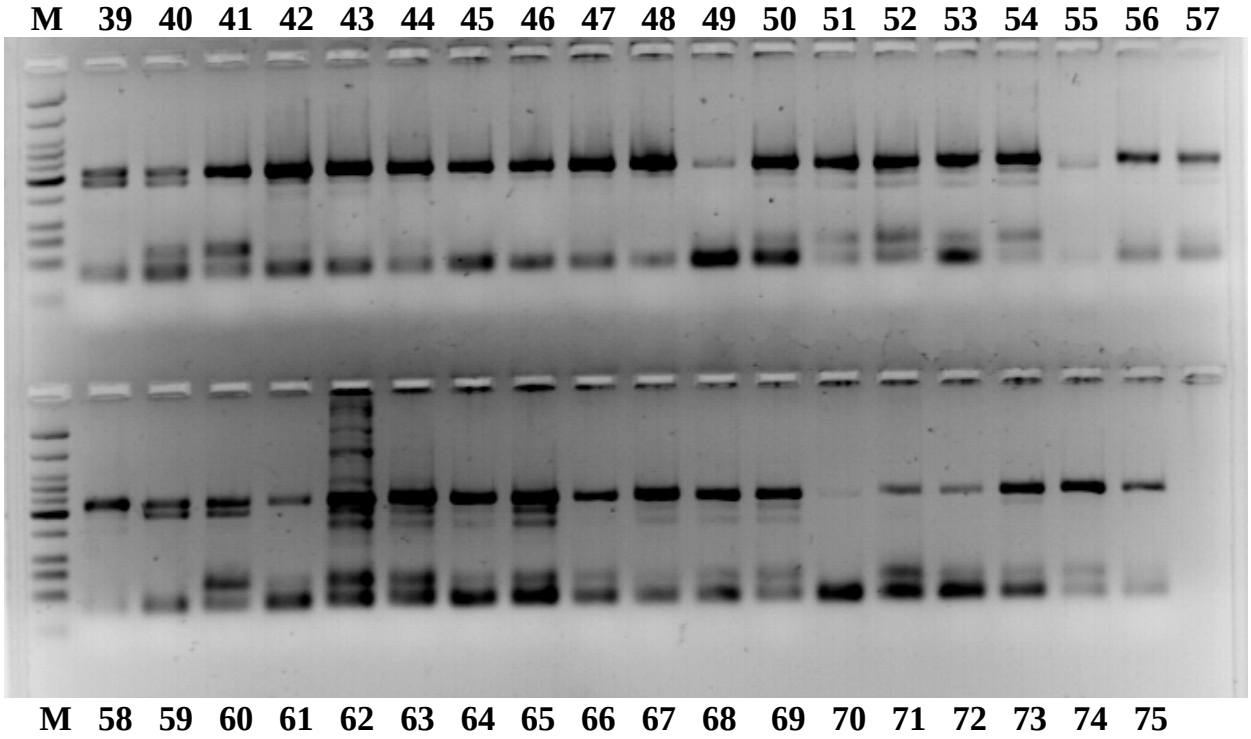

Fig. 2. SSR banding patterns of 37 *Brassica* genotypes generated by SSR primer Ol10-H02. Lanes represent M-Molecular marker, 39-57 *B. napus*, 58-70 *B. rapa*, 71-75 *B. nigra*. Molecular weights of marker bands are 766, 500, 350, 300, 250, 200, 150, 100, 75, 50 and 25, respectively.

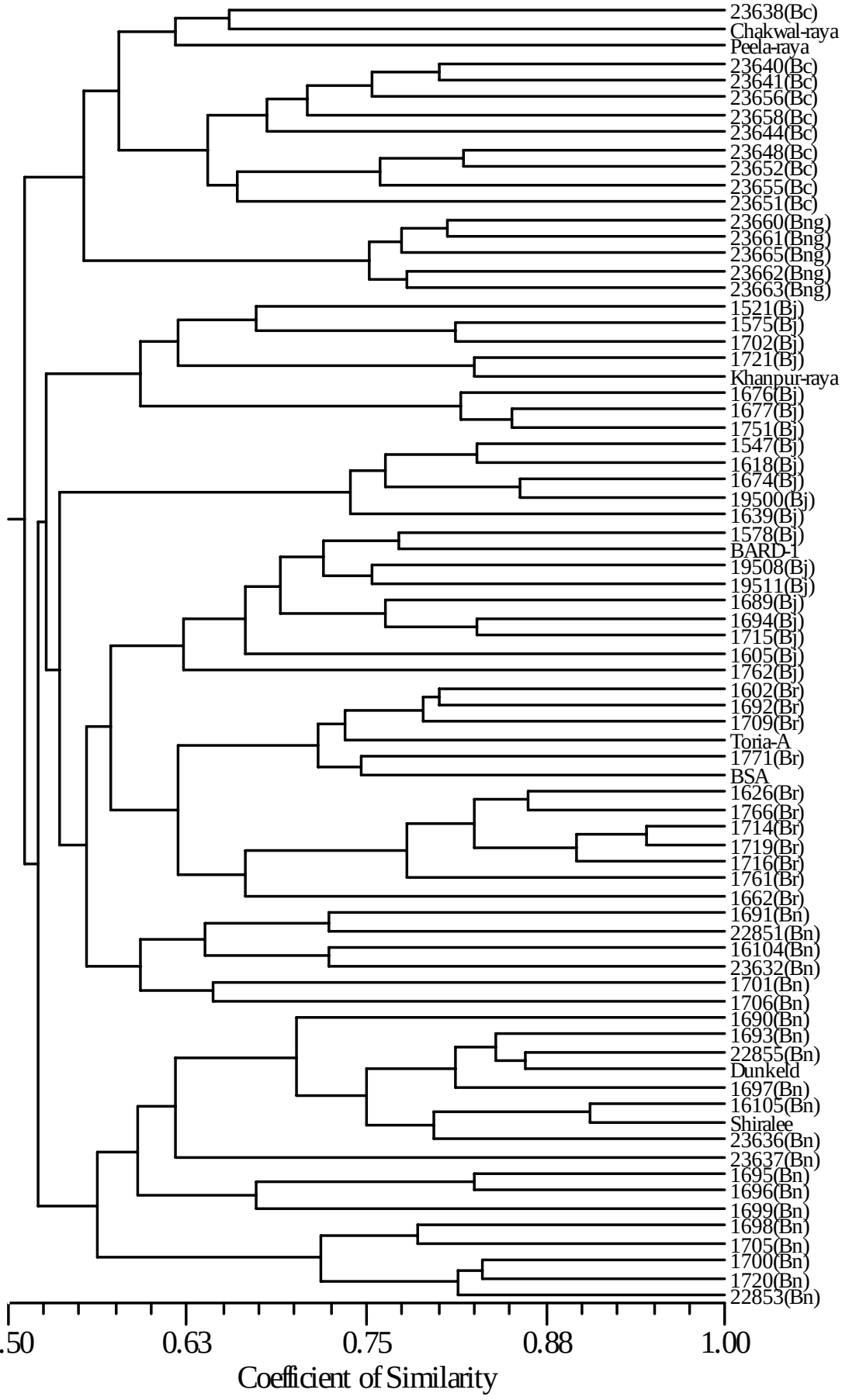

Fig. 3. Dendrogram of 75 *Brassica* genotypes based on database derived SSR markers. Bc denotes *Brassica carinata*, Bng for *B. nigra*, Bj for *B. juncea*, Br for *B. rapa* and Bn for *B. napus*.

This study shows the usefulness of SSR markers in the genetic identification of oilseed *Brassica* germplasm. The results in this report will permit to establish a set of microsatellite primers that can be used for several important aspects of various breeding strategies e.g., selecting appropriate parents for hybrids of different *Brassica* species and for monitoring hybridity level.

One of the most important concerns of the hybrid breeders has been the selection of appropriate donors and the prediction of hybrid performance. Enhancement of genetically diverse gene pools is an essential requirement in hybrid breeding. The importance of the genetic diversity of the parents for the expected heterosis of their hybrids is well known. Molecular markers have been previously used for estimation of genetic diversity, and the prediction of hybrid performance and heterosis (Melchinger, 1993; Becker & Engqvist, 1995; Xiao *et al*., 1998). Becker & Engqvist (1995) found a correlation between the genetic distance based on RAPD markers and heterosis for leafy dry matter in rapeseed, whereas Xiao *et al*., (1998) could not find a significant correlation between microsatellite heterogeneity and the prediction of heterosis in rice. Nevertheless, they found that the genetic diversity among the parental lines is related to heterosis. In our study, microsatellite markers were able to detect a high level of polymorphism among the genotypes used. Thus the results demonstrate high efficiency of microsatellite markers for monitoring genetic diversity and provide a reliable and an effective means for predicting heterosis in *Brassica*.

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References

Alamerew, S., S. Chebotar, X. Huang, M. Roder and A. Borner. 2004. Genetic diversity in Ethiopean hexaploid and tetraploid wheat germplasm assessed by microsatellite markers. *Genet. Resour. Crop. Evol*., 51: 559-567.

Babula, D., M. Kaczmarek, A. Barakat, M. Delseny, C.F. Quiros and J. Sadowski. 2003. Chromosomal mapping of *Brassica oleracea* based on ESTs from *Arabidopsis thaliana*: Complexity of the comparative map. *Mol. Gen. Genomics*, 268: 656-665.

Becker, H. and G.M. Engqvist. 1995. The potential of re-synthesized rapeseed for hybrid breeding. Proc 9th Int Rapeseed Cong., GCIRC, Cambridge, England, pp. 113-115.

Broun, P. and S.D. Tanksley. 1996. Characterization and genetic mapping of simple repeat sequences in the tomato genome. *Mol. Gen. Genet*., 250: 39-49.

Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot and A. Vignal. 1996. A comprehensive genetic map of human genome based on 5264 microsatellites. *Nature*, 380: 152-154.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. *Ecology*, 26: 297-302.

Dieterich, W.F., J. Miller, R. Steen, M.A. Merchant, D. Damron-Boles and Z. Husain. 1996. A comprehensive genetic map of the mouse genome. *Nature*, 380: 149-152.

Kole, C., P.H. Williams, S.R. Rimmer and T.C. Osborn. 2002. Linkage mapping of genes controlling resistance to white rust (*Albugo Candida*) in *B. rapa* (syn. *campestris*) and comparative mapping to *Brassica napus* and *Arabidopsis thaliana*. *Genome*, 45: 22-27.

Kresovich, S., A.K. Szwew-McFadden, S.M. Bliek and J.R. McFerson. 1995. Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). *Theor. Appl. Genet*., 91: 206-211.

Lagercrantz, U. 1998. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusion and frequent rearrangements. *Genetics*, 150: 1217-1228.

Lagercrantz, U. and D. Lydiate. 1996. Comparative genome mapping in *Brassica*. *Genetics*, 144: 1903-1910.

Landry, B.S., N. Hubert, R. Crete, M.S. Chiang, S.E. Lincoln and T. Etoh. 1992. A genetic map for *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping of resistance genes to race 2 of *Plasmodiophora brassicae* (Wornin). *Genome*, 35: 409-420.

Lowe, A.J., A.E. Jones, A.F. Raybould, M. Trick, C.L. Moule and K.J. Edwards. 2002. Transferability and genome specificity of a new set of microsatellite primers among *Brassica* species of the U triangle. *Mol. Ecol. Notes*, 2: 7-11.

Lowe, A.J., C.L. Moule, M. Trick and K.J. Edwards. 2004. Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. *Theor. Appl. Genet*., 108: 1103-1112.

Melchinger, A.E. 1993. Use of RELP markers for analysis of genetic relationships among breeding materials and prediction of hybrid performances. In: *International Crop Science* I. Crop Science Society of America, Madison, Wisconsin, pp. 621-628.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Res*., 10: 4321-4325.Nei, M. and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Nat. Acad. Sci. USA*, 76: 5269-5273.

Parkin, I.A.P., D.J. Lydiate and M. Trick. 2002. Assessing the level of collinearity between *Arabidopsis thaliana* and *Brassica napus* for *A. thaliana* chromosome 5. *Genome*, 45: 356-366.

Plieske, J. and D. Struss. 2001. Microsatellite markers for genome analysis in *Brassica.* I.: Development in *Brassica napus* and abundance in *Brassicaceae* species. *Theor. Appl. Genet*., 102: 689-694.

Quiros, C.F., F. Grellet, J. Sadowski, T. Suzuki, G. Li and T. Wroblewski. 2001. *Arabidopsis* and *Brassica* comparative genomics: sequence, structure and gene content in the ABI-Rps2-CK1 chromosomal segment and related regions. *Genetics*, 157: 1321-1330.

Ryder, C.D., L.B. Smith, G.R. Teakle and G.J. King. 2001. Contrasting genome organization: Two regions of *Brassica oleracea* genome compared with collinear regions of *Arabidopsis thaliana* genome. *Genome*, 44: 808-817.

Sambrook, J., E.F. Frithsch and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Springs Harbor Lab. Press, New York.

Scheffler, J.A., A.G. Sharpe, H. Schmidt, P. Sperling, I.A.P. Parkin, D.J. Lydiate and F. Heinz. 1997. Desaturase multigene families of *Brassica napus* arose through genome duplication. *Theor. Appl. Genet*., 94: 583-591.

Somers, D.J., G. Rakow, V.K. Prabhu and R.D. Friesen. 2001. Identification of a major gene and RAPD marker for yellow seed coat colour in *Brassica napus*. *Genome*, 44: 1077-1082.

Song, K.M., J.Y. Suzuki, M.K. Slocum, P.H. Williams and T.C. Osborn. 1991. A linkage map of *Brassica rapa* synonym *campestris* based on restriction fragment length polymorphism loci. *Theor. Appl. Genet*., 82: 296-304.

Tautz, D. and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Res*., 25: 4127-4138.

Xiao, C., L. Wu and J.L. Wu. 1998. SSR heterogenic patterns of parents for marketing and predicting heterosis in rice breeding. *Mol. Breed*., 4: 263-268.

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