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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).

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.

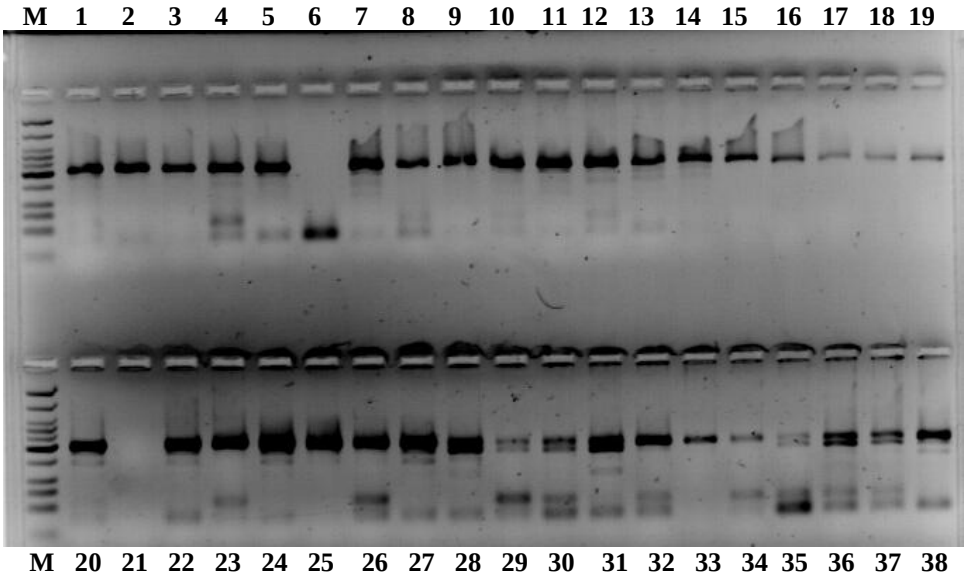

Fig. 1. SSR banding patterns of 38 *Brassica* genotypes generated by SSR primer Ol10-H02. Lanes represent M-Molecular marker, 1-12 *B. carinata*, 13-34 *B. juncea*, 35-38 *B. napus*. Molecular weights of marker bands are 766, 500, 350, 300, 250, 200, 150, 100, 75, 50 and 25, respectively.

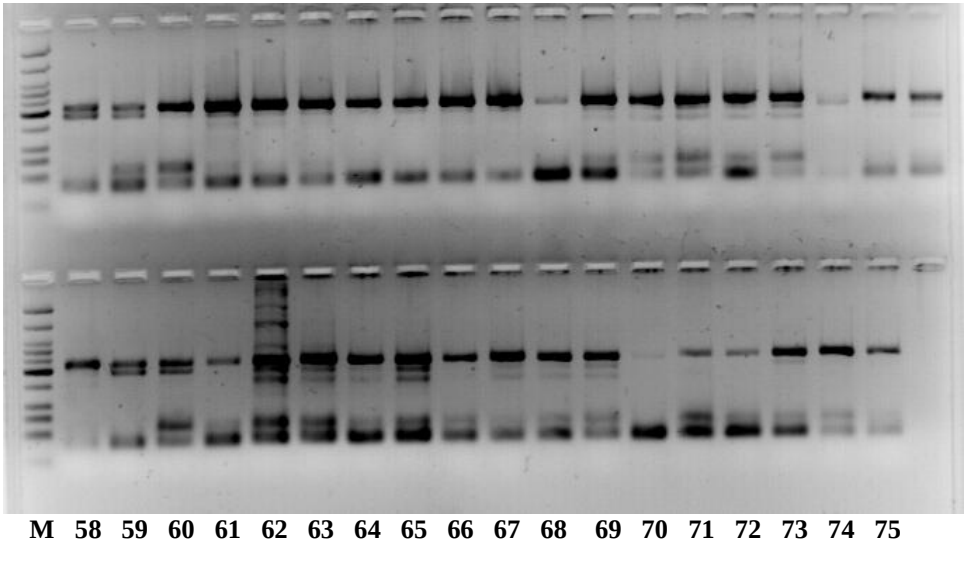

Fig. 2. SSR banding patterns of 37 *Brassica* genotypes generated by SSR primer Ol10-H02. Lanes represent M-Molecular marker, 39-57 *B. napus*, 58-70 *B. rapa*, 71-75 *B. nigra*. Molecular weights of marker bands are 766, 500, 350, 300, 250, 200, 150, 100, 75, 50 and 25, respectively.

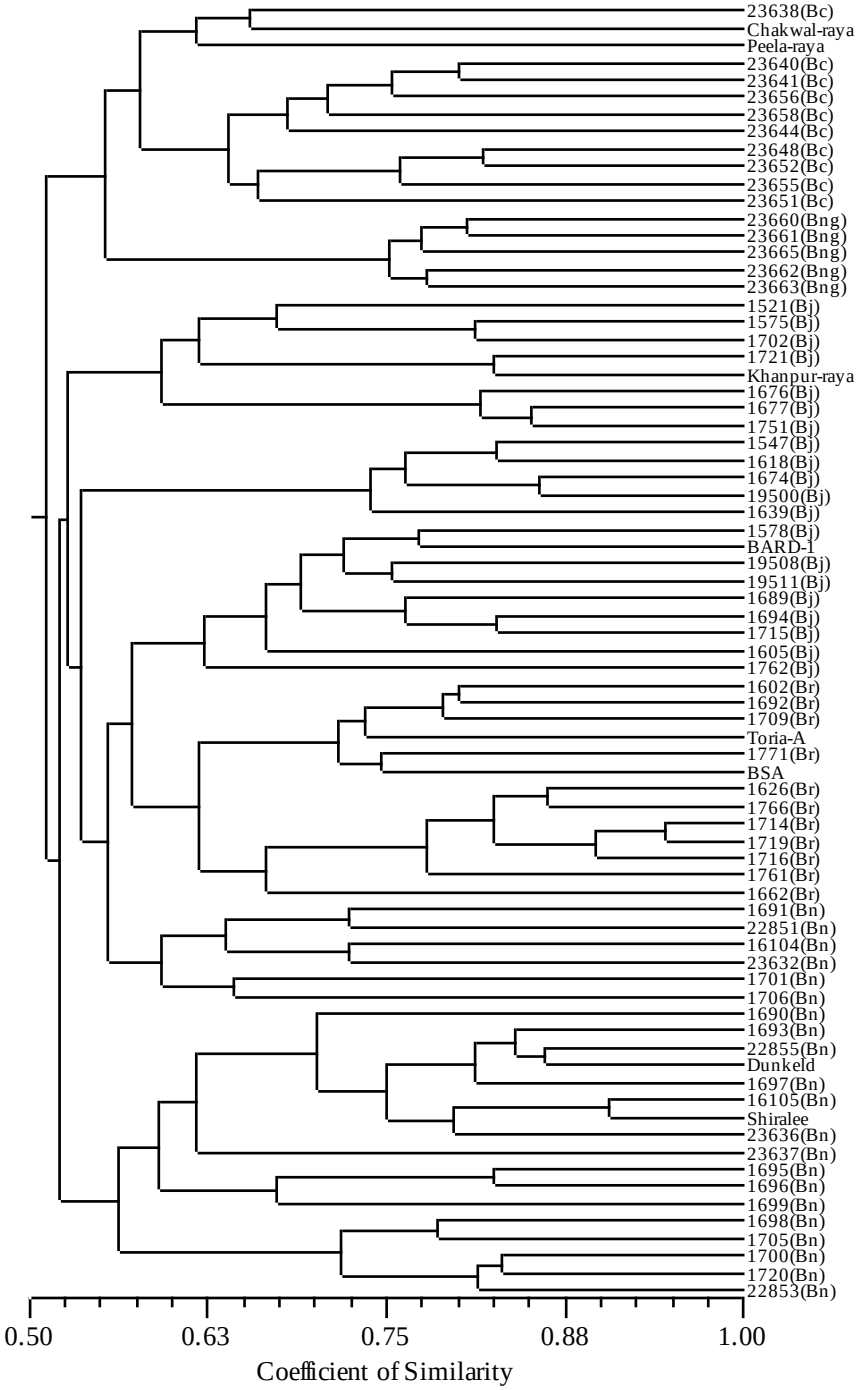

Fig. 3. Dendrogram of 75 *Brassica* genotypes based on database derived SSR markers. Bc denotes *Brassica carinata*, Bng for *B. nigra*, Bj for *B. juncea*, Br for *B. rapa* and Bn for *B. napus*.

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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