A panel of 31 single-locus microsatellite markers for population genetic analysis in Brassica napus and cross-species amplification in Cruciferae

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Introduction

The Brassica genus contains many crop species with important agronomic traits. The relationships of the six cultivated Brassica species were described as “the triangle of U” (U 1935). Brassica genomes have been denoted as the A, B and C genomes for the three diploids, B. rapa (AA; 2n = 20), B. nigra (BB; 2n = 16) and B. oleracea (CC; 2n = 18), respectively. The three amphidiploid species, B. napus (AACC; 2n = 38), B. carinata (BBCC; 2n = 34), and B. juncea (AABB; 2n = 36), were formed through interspecific hybridization between any of the above diploid species (Prakash & Hinata 1980; U 1935).

Microsatellites or simple sequence repeats (SSRs), are arrays of short DNA motifs with 1-6 bp in length. They are interspersed within eukaryotic genomes and highly variable, abundant, highly polymorphic, and inherited in a co-dominant mode (Gupta & Varshney 2000). SSRs have been isolated and characterized from B. napus, B. nigra and B. rapa (Lowe et al. 2002, 2004; Plieske & Struss 2001; Suwabe et al. 2002; Szewc-McFadden et al. 1996; Westman & Kresovich 1999). The application of these SSR markers enables the identification of genes and QTLs underlying key traits for application in molecular breeding and germplasm enhancement (Lombard & Delourme 2001; Piquemal et al. 2005; Qiu et al. 2006).

Comparative mapping using molecular markers demonstrated that the Brassica species contain extensively triplicated counterparts of the corresponding homologous segments of the Arabidopsis thaliana genome, thereby suggesting that diploid Brassica species may have evolved through a hexaploid ancestor (Cavell et al. 1998; Parkin et al. 2002). Many genes occur 4-8 times in the genome of B. napus (Parkin et al. 2005; Schanz et al. 2006). Therefore, it is difficult to discriminate alleles from specific locus, and to precisely evaluate the population parameters such as the number of alleles, allele frequencies and heterozygosity at each locus in population genetics and association mapping studies using natural populations with multi-loci RFLP or SSR makers. Thus, single-locus molecular markers will be very useful for genetic diversity analysis and association mapping. We recently developed 627 GSS-SSRs from B. napus (Cheng et al. 2009). From which, a panel of 31 single-locus SSR markers were selected to examine their potential usefulness in assessing genetic diversity, phylogenic relationship in 96 accessions of B. napus germplasm, which included important oilseed rape cultivars from China, Europe, Australia, Canada and Japan.

Materials and methods

Plant materials and DNA extraction

Fresh leaf samples were collected from 96 accessions of B. napus germplasm, which included important oilseed rape cultivars from China, Europe, Australia, Canada and Japan, 6 Brassica species, B. rapa (AA; 2n = 20), B. nigra (BB, 2n = 16), B. oleracea (CC, 2n = 18), B. napus (AACC, 2n = 38), B. juncea (AABB, 2n = 36) and B. Carinata (BBCC, 2n = 34), and other Cruciferous species, including Raphanus satiius L (RR, 2n = 18), Isatis ingigotica (II, 2n = 14), Orychophragmus violcaeus (OO, 2n = 24), and Arabidopsis (2n = 10) growing in HZAU, Wuhan. Total DNA was extracted from young leaves using the cetyltrimethylammonium bromide (CTAB) method (Li and Quiros 2001).

SSR analysis

A panel of 31 single-locus SSR markers were selected from our previous study (Cheng et al., 2009). PCR amplification was performed in a 10 μL reaction volume containing 50 ng genomic DNA, 1 × Taq Buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μM each primer, 0.25 U Taq DNA polymerase (Fermentas). The reaction mixture was initially denatured at 94 °C for 5 min, followed by 40 cycles of amplification at 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min in a MyCycler thermal cycler (Bio-Rad, Wuhan, China). The PCR products were separated on 6% denaturation polyacrylamide gel, and visualized by silver-staining (Sanguinetti et al. 1994). The PCR analysis were repeated at least two times to ensure the reproducibility.

Data analysis

The polymorphic information contents (PIC) (Botstein et al. 1980) was calculated based on the formula: $PIC_i = 1 - \sum_{j=1}^{m} p_{ij}^2 - \sum_{j=1}^{m-1} \sum_{k=j+1}^{m} 2p_{ij}^2 p_{ik}^2$, where p_{ij} is the frequency of the jth allele, and p_{ik} is the frequency of the kth allele. Observed heterozygosity (H_o), expected heterozygosity (H_e) and Hardy-Weinberg equilibrium (HWE) test for each locus were performed in these 96 accessions of B. napus cultivars using software PowerMarker V3.25 (Liu & Muse 2005). To depict phylogenic relationship of these 96 B. napus accessions, a dendrogram based on Nei's dissimilarity matrices (Nei & Li 1979) was constructed using a cluster analysis (UPGMA) with software NTsys 2.0f (Rohlf 2006).

Results and discussion

A total of 87 alleles were identified at 31 loci. The number of alleles varied from two to six. PIC ranged from 0.0587 to 0.7018, with an average PIC value of 0.3289. H_o varied from 0.0000 to 0.5269, while H_e ranged from 0.0605 to 0.7444. All loci significantly deviated from HWE after a Bonferroni correction, which may be attributed to the existence of undetected population structure that was caused by their distinct evolutionary pathway and breeding history of these cultivars (Pritchard et al. 2000).

At the genetic dissimilarity threshold of 0.57 and 0.52, six and four cultivars were clustered into two separate subgroups: “China-1” and “China-2”, respectively. Among the remaining 86 cultivars, 62 Chinese cultivars were clustered in a subgroup labeled "China-3" at a genetic distance of 0.51, which contained two cultivars from Europe, four from Canada, four from Australia and one from Japan, respectively. The remaining 4 Chinese cultivars were clustered into the "Mixed" subgroup with cultivars from Europe, Canada, Australia, indicating that these foreign cultivars were genetically distant from the Chinese cultivars (Fig. 1).

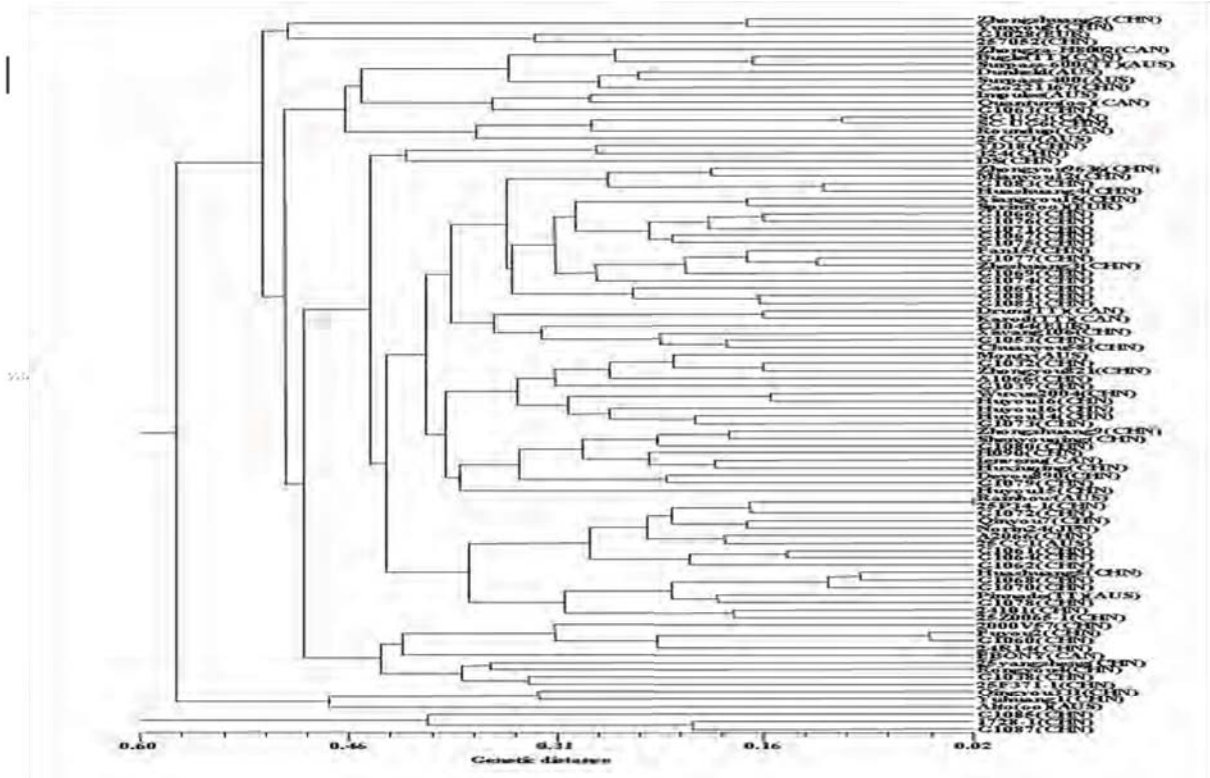

Fig. 1 Dendrogram based on the genetic distance matrix of 96 B. napus accessions using the UPGMA clustering method. CHN, means China; JPN, means Japan; CAN, means Canada; AUS, means Australia; EUR, means Europe. The arrow indicates the divergent point of subgroups.

Cross-species amplification was examined among six Brassica species, B. rapa (AA; 2n = 20), B. nigra (BB, 2n = 16), B. oleracea (CC, 2n = 18), B. napus (AACC, 2n = 38), B. juncea (AABB, 2n = 36) and B. Carinata (BBCC, 2n = 34), and other Cruciferous species, including Raphanus satiius L (RR, 2n = 18), Isatis ingigotica (II, 2n = 14), Orychophragmus violcaeus (OO, 2n = 24), and Arabidopsis (2n = 10) (Table 2). Of the 31 markers, 25 (80.7%), 31 (100%) and 28 (90.3%) amplified products from the

three diploid species, B. rapa, B. oleracea and B. nigra, respectively, and 31 (100%), 28 (90.3%) and 30 (96.8%) markers amplified products from the three amphidiploid species, B. napus, B. juncea and B. carinata (Table 1), respectively, indicating that the SSR markers developed from the genomic sequences of B. napus have high success rates of amplification in Brassica species. There have 29 (93.6%), 25 (80.7%), 25 (80.7%) and 24 (77.4%) markers successfully amplified at least one product from the genomes of R. sativas, Isatis ingigotica, Orychophragmus violcaeus, and Arabidopsis (Table 2), respectively, indicating that the SSR markers developed from B. napus have very good transferability across distantly related Cruciferous species. Twenty four (71.0%) markers amplified clear PCR products in all ten Cruciferous species, suggesting that the conservation of the genomes of the Brassica and Cruciferous species (Table 1). Three primer pairs (BnGMS88, BnGMS301 and BnGMS594) only amplified products from the genomes of B. oleracea, B. napus and B. carinata (Table 1), suggesting that these markers are C genome-specific. Of the 31 single-locus markers, BnGMS110, BnGMS148, BnGMS301, BnGMS390, BnGMS565 and BnGMS679 behave as multi-loci in some species(, while the remaining 25 markers (80.64%) are still single-locus in all other 9 Cruciferous species (B. rapa, B. nigra, B. oleracea, B. napus, B. juncea, B. Carinata, Raphanus satiius L, Isatis ingigotica, Orychophragmus violcaeus, and Arabidopsis) (Table 1), suggesting that which show the conservative of these markers across interspecies.

Disscussion

In present study, we have evaluated the genetic diversity and depicted the genomic ancestry of 96 B. napus cultivars using 31 single-locus SSR markers. The high rate of transferability and the nature of single-locus inheritance suggested these markers are very useful for population genetic analysis across the Cruciferae species.

Table 1 Cross-species amplification of the 31 B. napus GSS-SSR markers in Cruciferae species

Marker	Brassica						Other Cruciferea			
	Br	Bn	Bo	Bc	Bj	Bi	Rs	Ii	Ov	At
BnGMS6	S	S	S	S	S	S	S	S	S	S
BnGMS86	S	S	S	-	S	S	S	-	S	-
BnGMS88	-	S	S	S	-	-	-	-	-	-
BnGMS110	S	S	S	M	S	S	M	S	S	S
BnGMS148	S	S	S	M	S	M	S	S	S	S
BnGMS175	S	S	S	S	S	S	S	S	S	S
BnGMS198	S	S	S	S	S	S	S	S	S	S
BnGMS271	S	S	S	S	S	S	S	S	S	S
BnGMS301	-	S	M	S	-	-	-	-	-	-
BnGMS317	S	S	S	S	S	S	S	S	S	S
BnGMS319	S	S	S	S	S	S	S	S	S	S
BnGMS359	S	S	S	S	S	S	S	S	S	S
BnGMS380	S	S	S	S	S	S	S	S	S	S
BnGMS390	M	S	M	M	M	M	M	M	M	M
BnGMS415	-	S	S	S	S	S	S	-	-	-
BnGMS425	S	S	S	S	S	S	S	S	S	S
BnGMS446	S	S	S	S	S	S	S	S	S	S
BnGMS490	S	S	S	S	S	S	S	S	-	S
BnGMS510	-	S	S	S	S	S	S	S	S	S
BnGMS511	S	S	S	S	S	S	S	S	S	S
BnGMS564	-	S	S	S	S	S	S	-	-	-
BnGMS565	S	S	M	M	M	M	M	M	M	M
BnGMS581	S	S	S	S	S	S	S	S	S	S
BnGMS583	S	S	S	S	S	S	S	S	S	-
BnGMS594	-	S	S	S	-	-	-	-	-	-
BnGMS629	S	S	S	S	S	S	S	S	S	S
BnGMS636	S	S	S	S	S	S	S	S	S	S
BnGMS646	S	S	S	S	S	S	S	S	S	S
BnGMS676	S	S	S	S	S	S	S	S	S	S
BnGMS662	S	S	S	S	S	S	S	S	S	S
BnGMS679	S	S	S	M	S	M	S	S	S	S

S, single-locus; M, multi-loci; -, no amplification