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Mapping and tagging of seed coat colour and the identification of microsatellite markers for marker-assisted manipulation of the trait in *Brassica juncea*

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Abstract Microsatellite marker technology in combination with three doubled haploid mapping populations of *Brassica juncea* were used to map and tag two independent loci controlling seed coat colour in *B. juncea*. One of the populations, derived from a cross between a brown-seeded Indian cultivar, Varuna, and a Canadian yellow-seeded line, Heera, segregated for two genes coding for seed coat colour; the other two populations segregated for one gene each. Microsatellite markers were obtained from related *Brassica* species. Three microsatellite markers (Ra2-A11, Na10-A08 and Ni4-F11) showing strong association with seed coat colour were identified through bulk segregant analysis. Subsequent mapping placed Ra2-A11 and Na10-A08 on linkage group (LG) 1 at an interval of 0.6 cM from each other and marker Ni4-F11 on LG 2 of the linkage map of *B. juncea* published previously (Pradhan et al., Theor Appl Genet 106:607–614, 2003). The two seed coat colour genes were placed with markers Ra2-A11 and Na10-A08 on LG 1 and Ni4-F11 on LG 2 based on marker genotyping data derived from the two mapping populations segregating for one gene each. One of the genes (*BjSC1*) co-segregated with marker Na10-A08 in LG 1 and the other gene (*BjSC2*) with Ni4-F11 in LG 2, without any recombination in the respective mapping populations of 130 and 103 segregating plants. The identified microsatellite markers were studied for their length polymorphism in a number of yellow-seeded eastern European and brown-seeded Indian germplasm of *B. juncea* and were found to be useful for the diversification of yellow seed coat colour from a variety of sources into Indian germplasm.

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Introduction

Improvement in the quality of oil and meal of oilseed mustard, *Brassica juncea*, is one of the important breeding objectives for breeders of this crop. Oil and meal quality could be improved by developing canola-quality mustard lines containing less than 2% erucic acid in the seed oil and less than 30 μmol/g glucosinolate in the oil-free meal (Potts et al. 1999). The quality of canola mustard lines can be further improved through the development of yellow-seeded mustard cultivars, which are known to contain less fibre and more protein, both of which enhance its dietary feed value in broilers (Simbaya et al. 1995; Slominski 1997; Slominski et al. 1999). In addition, the seeds of yellow-seeded lines have been shown to have a higher oil content (Shirzadegan and Robbelen 1985) than those of brown-seeded lines, with the oil of the former having a better market value due to its bright yellow colour.

Most of the eastern European types of *B. juncea* lines are yellow-seeded, while varieties extensively grown in India are brown-seeded. The brown seed coat colour in *B. juncea* is controlled by two independently segregating dominant genes with duplicate effect (Vera et al. 1979; Vera and Woods 1982). Yellow seeds are produced when both the loci are in a homozygous recessive condition, and the maternal genotype influences the expression of the trait. Due to its recessive nature coupled with maternal influence, the transfer of yellow seed coat colour is difficult, since the trait has to be mobilized into Indian cultivars from un-adapted exotic sources of eastern European origin through backcross breeding. The use of co-dominant DNA markers tagged to this trait would help breeders to apply marker-assisted

selection (MAS) in backcross breeding, thereby obviating the need of selfing after every backcrossing.

The seed coat colour trait has been mapped in *B. napus* with restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and amplified (A)FLP markers (Van Deynze et al. 1995; Somers et al. 2001; Zhi-wen et al. 2005) and in *B. juncea* with AFLP markers (Negi et al. 2000; Lionneton et al. 2004; Sabarwal et al. 2004). For practical breeding purposes, these dominant RAPD and AFLP markers need to be converted to PCR-based co-dominant markers before they can be recommended for MAS. The PCR-based co-dominant microsatellite markers (or simple sequence repeats, SSR) have been shown to have great deal of potential in genome mapping and gene tagging (Goldstein and Schlötterer 1999). Among the cultivated *Brassica* species, a large number of SSR markers have been developed from amphidiploid *B. napus* (AACC) and the diploids *B. rapa* (AA), *B. nigra* (BB) and *B. oleracea* (CC), and many of these markers have been shown to be applicable within and between different *Brassica* species (Lowe et al. 2002, 2004; Plieske and Struss 2001; Saal et al. 2001; Suwabe et al. 2002). *B. juncea* (AABB) is an amphidiploid, and to date no SSR marker has been developed in this species. Hence, the SSR markers developed in its diploid progenitor species, *B. rapa* (Suwabe et al. 2002) and *B. nigra* (Lowe et al. 2004), could be utilized for mapping and tagging genes in *B. juncea*.

The objectives of this study were to determine the inheritance pattern of seed coat colour in *B. juncea* through the use of different segregating populations and to develop allele-specific molecular tags for this trait using SSR markers. Three SSR markers tightly linked to seed coat colour trait were identified and mapped to two linkage groups of the *B. juncea* map published earlier (Pradhan et al. 2003). The wider applicability of these tagged markers was tested by genotyping a large number of genetically diverse *B. juncea* genotypes that show contrasts with respect to the seed coat colour trait.

Materials and methods

Plant material and mapping population

Three mapping populations comprising microspore-derived doubled-haploid (DH) plants originating from F_1 of crosses between brown- and yellow-seeded parents were used for mapping and tagging the seed coat colour trait in *Brassica juncea*. Population 1 consisted of 123 DH lines derived from cross between a brown-seeded Indian cultivar, Varuna, and a yellow-seeded canola quality line, Heera. This population was earlier used for the construction of a linkage map in *B. juncea* (Pradhan et al. 2003). The DH lines of populations 2 (130 lines) and 3 (103 lines) were derived from two crosses, involving two brown-seeded DH lines—designated as 4-4-2 and 16-4-8, respectively—crossed to a common yellow-seeded DH line, DH 26. The pedigree and genesis of these mapping populations are given in Fig. 1. A set of 27 *B. juncea* genotypes consisting of 12 brown- and 15 yellow-seeded lines/cultivars belonging to the Indian and the eastern European gene pools (Srivastava et al. 2001) were used (Table 1) to test the applicability of linked markers for marker-assisted transfer of the seed coat colour trait.

DNA was isolated from well-expanded leaves of field-grown plants following the methodology of Rogers and Bendich (1994). For carrying out bulk segregant analysis (BSA; Michelmore et al. 1991), equal amounts of DNA from eight brown- and eight yellow-seeded DH lines were pooled to constitute the brown and yellow bulks, respectively. Seed coat colour was assessed by visual observation from open-pollinated matured seeds harvested from field-grown DH lines.

Microsatellite markers and linkage analysis

The primer sequences of 332 microsatellite markers—106 from *B. rapa*, 98 from *B. nigra* and 128 from *B. napus*—available in the Brassica DB database (http://www.brassica.info/) were custom synthesized from Microsynth AG (Balgach, Switzerland). DNA amplifica-

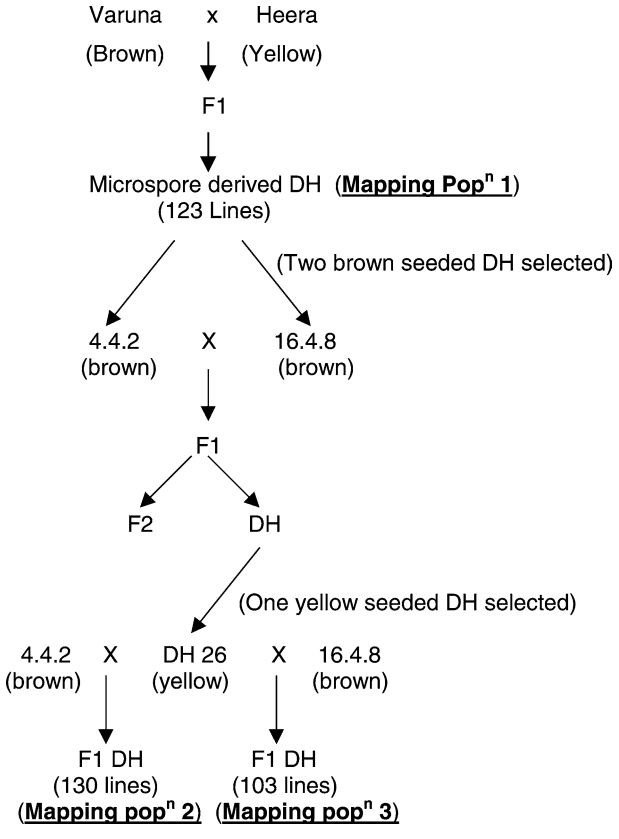

Fig. 1 Schematic representation of the pedigree of the genetic stocks and mapping populations used for mapping and tagging of seed coat colour trait in *Brassica juncea*

Table 1 *Brassica juncea* germplasm used to test the wider applicability of two microsatellite markers tagged to two genes for the seed coat colour trait and their allelic variability

Sl no.	Name of the lines /cultivars	Origin	Fragment size amplified by Na10-A08 (bp)	Fragment size amplified by Ni4-F11 (bp)
	Brown lines			
1	Varuna	India	202	153
2	Kranti	India	202	153
3	RH-30	India	202	151
4	RC 781	India	198	153
5	T-6342	India	198	155
6	Pusa bold	India	202	153
7	RLM 619	India	202	153
8	SEJ 2(1)	India	202	155
9	Rajat	India	202	151
10	Yi Man Feng Wai	China	202	151
11	Shi Yian Ku youCai	China	202	153
12	PNMB	India	198	153
	Yellow lines			
13	DYJ 1	India	146	161
14	YRT-3	India	146	163
15	BJ 24-5	India	146	161
16	Heera	Canada	146	161
17	Cutlass	Canada	146	161
18	VNIIMK II	Russia	146	161
19	Skorospieka	Russia	146	165
20	Donskaja IV	Russia	146	161
21	Kranodraaskaja	Russia	146	165
22	Niesopaycoszi-zagaja	Poland	146	163
23	Malopoloska	Poland	146	161
24	IR-7-26917	Germany	146	163
25	Zem 1	Australia	146	161
26	Zem-84-500	Australia	146	161
27	Chang Yang Huanzai 37	China	146	161

tion was carried out in 20-µl of reaction mixture containing 30 ng template DNA, 5 pmol primers, 100 µ*M* each of the dNTPs, 1.5–2.5 m *M* $MgCl_2$, 1× PCR buffer and 1 U *Taq* DNA polymerase (Invitrogen, UK). The following PCR profile was used in a Perkin Elmer Thermal Cycler 9700 (Foster City, Calif.): an initial denaturation at 94°C for 3 min, followed by 45 cycles of 94°C for 15 s, 52–62°C for 15–30 s (according to the T_m of the primer), 72°C for 30 s, with a final extension at 72°C for 5 min. Amplification products were resolved in either 2% agarose gel or 4–5% MetaPhor agarose gels (FMC BioProducts, Rockland, Me.). In those cases where scorable polymorphism was not clearly discerned either by agarose or metaphor agarose gels, it was detected through capillary electrophoresis in an automated genotyper (Avant 3100 model; Applied Biosystems, Foster City, Calif.) using fluorescence-labelled forward primers with FAM (6-carboxyfluorescein) at the 5′ end.

Genetic segregation data of the identified markers showing association with seed coat colour was tested for goodness of fit (χ^2 test) according to the expected Mendelian inheritance. The markers were mapped to the existing *B. juncea* map (Pradhan et al. 2003) using mapping population 1 following the mapping criteria described by Pradhan et al. (2003).

Results

Inheritance of seed coat colour

The observation of a 3 brown:1 yellow segregation in the F_1 DH from the cross between Varuna and Heera (Table 2) confirmed earlier observations that the brown seed coat colour in *B. juncea* is controlled by two independent dominant genes with duplicate effect. The observation of a 15 brown:1 yellow segregation in the F_2 and a 3 brown:1 yellow segregation in the F_1 DH between the two brown-seeded DH lines 4-4-2 and 16-4-8 and their subsequent segregation of 1 brown:1 yellow in each of the F_1 DH lines from the crosses between 4-4-2 (brown) and DH 26 (yellow) and between 16-4-8 (brown) and DH26 (yellow) indicated that the two brown genes in the 4-4-2 and 16-4-8 DH lines are segregating independently (Table 2).

Screening of microsatellite markers and BSA for seed coat colour

Of the 332 primer pairs tested on the parental lines Varuna and Heera, 203 primer pairs showed amplification products, of which 137 primer pairs exhibited single band products while the remaining primer pairs amplified more than one band. Polymorphism between Varuna and Heera was recorded for 64 microsatellites, of which 49 microsatellites showed single band polymorphism. Microsatellites showing single band polymorphism between the two parental lines Varuna and Heera

Table 2 Segregation analysis and χ^2 test for fit to ratio for the seed coat colour trait from segregating F_1 DHs and the F_2 derived from different crosses in *B. juncea*

Cross	Generation	Observed frequency, brown:yellow	Total	Expected ratio	χ^2
Varuna (brown) × Heera (yellow)	F_1 DH	88:35	123	3:1	0.786
4-4-2 (brown) × 16-4-8 (brown)	F_1 DH, F_2[a]	51:21 231:17	72, 248	3:1, 15:1	0.667, 0.149
4-4-2 (brown) × DH 26 (yellow)	F_1 DH	68:62	130	1:1	0.492
16-4-8 (brown) × DH 26 (yellow)	F_1 DH	44:59	103	1:1	1.64

[a]Due to maternal influence, F_3 seeds were visually scored for seed coat colour

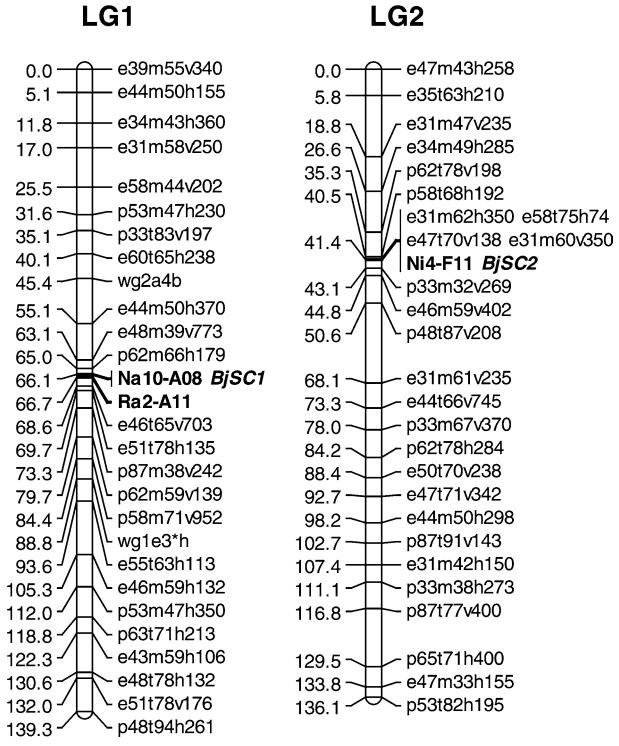

Fig. 2 A genetic linkage map of three microsatellite markers (Ra2-A11, Na10-A08 and Ni4-F11) tightly linked to two seed coat colour genes (*BjSc1* and *BjSc2*) mapped to LG 1 and LG 2 of the *B. juncea* map, respectively. Other representative markers shown in these linkage groups were earlier mapped by Pradhan et al. (2003)

were used for identifying markers associated with the seed coat colour trait through BSA.

Application of BSA to the pooled DNA from the three mapping populations revealed polymorphism between the brown and yellow bulk by three microsatellites markers, Ra2-A11, Na10-A08 and Ni4-F11. In the parental lines, markers Ra2-A11, Na10-A08 and Ni4-F11 amplified a fragment of 342 bp, 202 bp and 153 bp, respectively, from brown-seeded Varuna and 242 bp, 146 bp and 161 bp, respectively, from yellow-seeded Heera. In the BSA from the mapping population 1 (Fig. 1), the brown bulk amplified both Varuna- and Heera-specific fragments, whereas the yellow bulk amplified only the Heera-specific fragment for all the three microsatellite markers. This discrepancy was not observed when amplification was carried out with the pooled DNA from mapping populations 2 and 3. In mapping population 2 (Fig. 1), the brown and yellow bulks amplified the Varuna- and Heera-specific fragment for the Ra2-A11 and Na10-A08 markers, respectively, and there was no polymorphism between the brown and yellow bulks for the Ni4-F11 marker amplifying only the Heera-specific allele of 161 bp. On the other hand, the brown and yellow bulks from mapping population 3 (Fig. 1) revealed a type of polymorphism similar to that observed between Varuna and Heera for the Ni4-F11 microsatellite marker. No polymorphism was detected between the two bulks when amplification was carried out with Ra2-A11 and Na10-A08; in these cases, only an amplified product of the Heera-specific allele of 242 bp and 146 bp, respectively, was revealed. The markers were also amplified from one accession each of *B. rapa* (cv. Pusa kalyani) and *B. nigra* (cv. IC 257) to determine the genome specificity of these three markers. Ra2-A11 and Na10-A08 showed amplification from *B. rapa*, whereas Ni4-F11 showed amplification only with *B. nigra* DNA. On the basis of the above observations, we assumed that the bands detected by Ra2-A11and Na10-A08 are A-genome specific and that the band detected by Ni4-F11 is specific to the B genome. Hence, the Varuna-type alleles amplified from Ra2-A11 and Na10-A08 were designated as 'A' and those from Ni4-F11 as 'B', and Heera-type alleles were designated as 'a' and 'b', respectively.

Genotyping and mapping of three microsatellite markers in *B. juncea*

A total of 120, 130 and 103 DH lines from mapping populations 1, 2 and 3, respectively, were genotyped with all three microsatellite markers. In population 1, all three markers segregated to give a genotypic ratio of 1:1 (data not shown). Microsatellite markers Ra2-A11 and Na10-

Table 3 Segregation data from mapping populations 2 and 3 following genotyping with three microsatellite markers and their association with seed coat colour trait

Marker	Marker genotype	Observed genotypic and phenotypic frequency			
		Population 2		Population 3	
		Genotypic frequency	Phenotypic frequency B:Y[a]	Genotypic frequency	Phenotypic frequency B:Y[a]
Ra2-A11	AA	69	68:1	0	–
	aa	61	0:61	103	44:59
	Total	130	68:62	103	44:59
Na10-A08	AA	68	68:0	0	–
	aa	62	0:62	103	44:59
	Total	130	68:62	103	44:59
Ni4-F11	BB	0	–	44	44:0
	bb	130	68:62	59	0:59
	Total	130	68:62	103	44:59

[a]B, brown; Y, yellow

Table 4 Co-segregation of the marker genotype with the seed coat colour trait in mapping population 1

Marker genotype (Ra2-A11/Ni4-F11)	Number of individuals	Seed coat colour	Marker genotype (Na10-A08/Ni4-F11)	Number of individuals	Seed coat colour
AABB	19	Brown	AABB	18	Brown
AAbb	33	Brown	AAbb	33	Brown
aaBB	33	Brown	aaBB	34	Brown
aabb	35	Yellow	aabb	35	Yellow

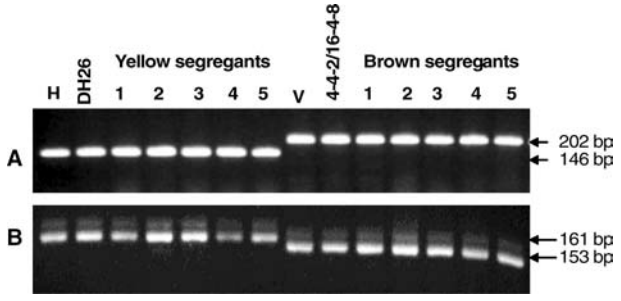

Fig. 3 A representative gel of the amplified products with microsatellite markers Na10-A08 (**A**) and Ni4-F11 (**B**) from yellow-seeded Heera (*H*) and DH 26 and from brown-seeded Varuna (*V*), 4-4-2 (**A**) and 16-4-8 (**B**) and from five each of yellow-seeded and brown-seeded DH segregants from the cross 4-4-2 and DH 26 (**A**) and 16-4-8 and DH 26 (**B**)

A08 mapped to linkage group (LG) 1 at an interval of 0.6 cM based on the observation that these two markers differ from each other for one recombination event, and Ni4-F11 mapped to LG 2 of the existing *B. juncea* map (Pradhan et al. 2003) (Fig. 2). On the other hand, population 2 showed a genotypic ratio of 1:1 when amplified from Ra2-A11 and Na10-A08 and population 3 showed a 1:1 segregation for Ni4-F11. No segregation was observed for Ni4-F11 in population 2 and for Ra2-A11 and Na10-A08 in population 3 (Table 3).

Co-segregation of markers with the seed coat colour trait

The association of markers with the seed coat colour was initially studied in the mapping population 1. The DH lines were classified into four marker genotypic classes—AABB, AAbb, aaBB and aabb. Co-segregation of different genotypes with seed coat colour indicated that all the 35 yellow-seeded segregants were of the aabb genotype and that all of the brown-seeded segregants were of the AABB, Aabb and aaBB genotypes (Table 4). This observation established the fact that one seed coat colour gene is tightly linked to Ra2-A11 and Na10-A08 on LG 1 and that the other seed coat colour gene is linked to Ni4-F11 on LG 2. However, the relative positions of the two seed coat colour genes with reference to Ra2-A11 and Na10-A08 on LG 1 and with reference to Ni4-F11 on LG 2 could not be established due to our inability to identify recombinants among the brown segregants in mapping population 1. Hence, we used the genotyping data of microsatellite markers Ra2-A11 and Na10-A08 from mapping population 2 and of Ni4-F11 from mapping population 3 to identify recombinants as each of these populations segregated for two different genes for seed coat colour. In the 130 DHs of mapping population 2, we identified one recombinant with Ra2-A11 (of the 69 DH individuals having the AA marker genotype, 68 were brown-seeded and one was yellow-seeded; Table 3) and no recombinant with Na10-A08. On the other hand, genotyping of the 103 DH lines of mapping population 3 did not yield a single recombinant with Ni4-F11 (Table 3). Hence, the two seed coat colour genes, *BjSC1* and *BjSC2*, were mapped at the same positions with Na10-A08 in LG1 and with Ni4-F11 in LG2, respectively (Fig. 2). The co-segregation patterns between the marker and the trait in a set of five yellow-seeded and five brown-seeded individuals from mapping populations 2 and 3 are shown in Fig. 3. Amplification of 20 randomly selected F_2 individuals from a cross between 4-4-2 and DH 26 by microsatellite marker Na10-A08 and from a cross between 16-4-8 and DH 26 by microsatellite marker Ni4-F11 resulted in the successful identification of the heterozygotes, with a marker genotypic segregation of 1:2:1 (data not shown) with both markers.

Wider applicability of microsatellite markers tagged to seed coat colour

The primer sequence of the three microsatellite markers tagged to two seed coat colour genes in *B. juncea* are

Table 5 Primer sequences of the three microsatellite markers tagged to seed coat colour trait in *B. juncea* and their amplification products

Primer name[a]	Sequence[b]	Product size (bp)
Ra2-A11	F: 5′-GACCTATTTTAATATGCTGTTTTACG-3′; R: 5′-ACCTCACCGGAGAGAAATCC-3′	Varuna, 342; Heera, 242
Na10-A08	F: 5′-CATGGTTAAAACAATGGCCC-3′; R: 5′-CAAGAAACACCATCATTTCTCA-3′	Varuna, 202; Heera, 146
Ni4-F11	F: 5′-CGTAAGTTTCAATTGTCAACGG-3′; R: 5′-TCGTACGAAACAATCAACGG-3′	Varuna, 153; Heera, 161

[a]http://www.brassica.info
[b]F, Forward primer; *R* reverse primer

shown in Table 5. Two of the markers, Ra2-A11 and Na10-A08, present on LG 1 showing linkage with one of the two genes for seed coat colour are dinucleotide CT microsatellites with repeat numbers of $(CT)_{51}$ and $(CT)_{21}$ and amplify fragments of 332 bp and 167 bp in their originator species, *B. rapa* and *B. napus*, respectively. The third microsatellite marker, Ni4-F11, shows tight linkage with the second gene of seed coat colour trait on LG 2; it is a dinucleotide GA microsatellite with repeat numbers of $(GA)_{45}$ and amplifies a 195-bp fragment in its originator species, *B. nigra*.

The validity of the Na10-A08 and Ni4-F11 microsatellite markers for their wider applicability was tested on a set of genetically diverse germplasm consisting of 12 brown-seeded and 15 yellow-seeded *B. juncea* lines (Table 1). DNAs from these 27 lines were amplified by Na10-A08 and Ni4-F11 and genotyped in the automated genotyper using fluorescent primers. All of the 12 brown-seeded lines were of the AABB genotype and all the 15 yellow-seeded lines were of the aabb genotype. However, we observed allelic variation among brown germplasm for Na10-A08 and in both the brown and yellow germplasm for Ni4-F11 microsatellite markers. With respect to the brown germplasm, we observed two alleles (202 bp and 198 bp) for Na10-A08, whereas a total of six alleles—three each in the brown- (151, 153 and 155 bp) and yellow- (161, 163 and 165 bp) seeded germplasm were recorded for Ni4-F11 (Table 1).

Discussion

Negi et al. (2000) developed an AFLP-converted, co-dominant SCAR marker (SCM08) linked to the seed coat colour trait in *B. juncea* and reported its wider applicability in MAS. The applicability of the SCM08 co-dominant marker was tested in our DH mapping population 1. Although we observed similar parental polymorphism as that observed by Negi et al. (2000) in their parental lines, we found no association between the SCM08 marker and seed coat colour. The seed coat colour trait revealed a segregation of 3 brown:1 yellow in both the Varuna-type marker genotype and the Heera-type marker genotype, which shows independent assortment between the marker and the seed coat colour. The SCM08 marker, therefore, could not be used for marker-assisted transfer of yellow seed coat colour trait from Heera to Varuna.

The two-gene inheritance of seed coat colour and the subsequent segregation of two genes to two different genetic stocks (4-4-2 and 16-4-8) indicated that the two genes for seed coat colour are either present on two different chromosomes or quite far from each other on the same chromosome. Among the brown-seeded lines, the intensity of brownness was so similar that it was difficult to distinguish genotypes with two dominant genes from those with one dominant gene by the naked eye. This explains the amplification of both brown- and yellow-specific marker alleles in the brown-seeded bulk of population 1 used for BSA, which represented a mixture of 3 AABB, 3 AAbb and 2 aaBB genotypes. However, this discrepancy was not present when we applied BSA to the two other populations, as population 2 contained only AAbb brown-seeded individuals and population 3 contained only aaBB brown-seeded individuals.

We also observed that the populations 2 and 3 were useful in the proper placement of seed coat colour genes with reference to their respective linked markers since the identification of recombinants among the brown-seeded DH lines of population 1 was difficult due to segregation of two dominant genes with duplicate effect. We identified two microsatellite markers, Na10-A08 and Ni4-F11, showing perfect co-segregation with one each of the two genes of seed coat colour trait. We based our identification on the fact that no recombinant was identified from the 130 DH lines with Na10-A08 in mapping population 2 and from the 103 DH lines with Ni4-F11 in mapping population 3. The two markers mapped to LG 1 and LG 2 of our *B. juncea* map (Pradhan et al. 2003), respectively, and are present in the regions that are highly saturated with AFLP markers. Lionneton et al. (2004) recently mapped two loci of seed coat colour in *B. juncea* using AFLP markers. In their map, the two loci were mapped to LG 3 and LG 6, to the regions with a high density of AFLP markers. We could not compare the two maps because the AFLP nomenclature adopted by Lionneton et al. (2004) differs from the that which we use (Pradhan et al. 2003). However, the mapping of SCM08 (Negi et al. 2000) to LG 13 of our map indicated that this SCAR marker is not linked to seed coat colour trait in *B. juncea* (map not shown).

The application of these two markers to diverse germplasm of *B. juncea* with respect to seed coat colour indicated that brown-seeded cultivars contained brown-specific marker alleles and yellow-seeded lines contained yellow-specific marker alleles at both the loci. We also observed allelic variation for both microsatellite markers in brown and yellow germplasm. Allelic variation in microsatellites has been shown to be of common occurrence in crop plants (Udupa et al. 1999; Xu et al. 2004). Since the length variations we observed were multiples of two nucleotides, it is presumed that these length variations could be due to the variation in the number of dinucleotide repeats in this microsatellite. However, there was clear-cut polymorphism between brown and yellow lines, indicating that all of these germplasm lines could be effectively used for marker-assisted manipulation of the seed coat trait using these tagged microsatellite markers.

This is the first report of the application of co-dominant microsatellite markers for molecular mapping and tagging of an agronomically important trait in *B. juncea*. The mapping and validation studies provided in this study indicate that the two co-dominant microsatellite markers Na10-A08 and Ni4-F11 can be effectively used for marker-assisted transfer of the yellow seed coat colour trait in *B. juncea* from a wide source of germ-

plasm into a large number of agronomically important lines of the Indian gene pool. High-throughput screening of segregating populations could be achieved by a non-gel-based fluorescent method. Moreover, we have a highly saturated map consisting of 52 AFLP markers flanking the Ra2-A11 and Na10-A08 microsatellite markers and 30 AFLP markers flanking the Ni4-F11 satellite marker within a distance of 5 cM in each of the linkage groups of the map constructed from mapping population 1 (Pradhan et al. 2003). This information could be effectively utilized for high-resolution mapping and map-based cloning of the candidate gene.

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References

Goldstein DB, Schlötterer C (1999) Microsatellites: evolution and applications. Oxford University Press, Oxford

Lionneton E, Aubert G, Ochatt S, Merah O (2004) Genetic analysis of agronomic and quality traits in mustard (*Brassica juncea*). Theor Appl Genet 109:792–799

Lowe AJ, Jones AE, Raybould AF, Trick M, Moule CJ, Edwards KJ (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 AJ, Moule C, Trick M, Edwards KJ (2004) Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor Appl Genet 108:1103–1112

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by BSA: a rapid method to detect markers in specific genome regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Negi MS, Devic M, Delseny M, Lakshmikumaran M (2000) Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection. Theor Appl Genet 101:146–152

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

Potts D, Rakow G, Males D (1999) Canola quality *Brassica juncea*, a new oilseed crop for the Canadian prairies. In: Proc 10th Int Rapeseed Congr. Canberra (available on CD-ROM)

Pradhan AK, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D (2003) A high-density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor Appl Genet 106:607–614

Rogers SO, Bendich AJ (1994) Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin SB, Schilperoot RA (eds) Plant molecular biology manual. Kluwer, Dordrecht, pp 1–8

Saal B, Plieske J, Hu J, Quiros CF, Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. II. Assignment of rapeseed microsatellites to A and C genomes and genetic mapping in *Brassica oleracea* L. Theor Appl Genet 102:695–699

Sabarwal V, Negi MS, Banga SS, Lakshmikumaran M (2004) Mapping of AFLP markers linked to seed coat colour loci in *Brassica juncea* (L.) Czern. Theor Appl Genet 109:160–166

Shirzadegan M, Robbelen G (1985) Influence of seed colour and hull proportions on quality properties of seeds in *Brassica napus* L. Fette Seifen Anstrichm 87:235–237

Simbaya J, Slominski BA, Rakow G, Campbell LD, Downey RK, Bell JM (1995) Quality characteristics of yellow seeded *Brassica* seed meal: protein, carbohydrates and dietary fibre components. J Agric Food Chem 43:2062–2066

Slominski BA (1997) Development in the breeding of low fibre rapeseed/canola. J Anim Feed Sci 6:303–317

Slominski BA, Simbaya J, Campbell LD, Rakow G, Guenter W (1999) Nutritive value for broilers of meal derived from newly developed varieties of yellow seeded canola. Anim Feed Sci Technol 78:249–262

Somers DJ, Rakow G, Prabhu KV, Friesen KRD (2001) Identification of a major gene and RAPD markers for yellow seed coat colour in *Brassica napus*. Genome 44:1077–1082

Srivastava A, Gupta V, Pental D, Pradhan AK (2001) AFLP-based genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of *Brassica juncea*. Theor Appl Genet 102:193–199

Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098

Udupa SM, Robertson LD, Weigand F, Baum M, Kahl G (1999) Allelic variation at $(TAA)_n$ microsatellite loci in a world collection of chickpea (*Cicer arietinum*) germplasm. Mol Gen Genet 261:354–363

Van Deynze AE, Landry BS, Pauls KP (1995) The identification of restriction fragment length polymorphisms linked to seed colour genes in *Brassica napus*. Genome 38:534–542

Vera CL, Woods DL (1982) Isolation of independent gene pairs at two loci for seed coat colour in *Brassica juncea*. Can J Plant Sci 62:47–50

Vera CL, Woods DL, Downey RK (1979) Inheritance of seed coat colour in *Brassica juncea*. Can J Plant Sci 59:635–637

Xu K, Deb R, Mackill DJ (2004) A microsatellite marker and a co-dominant PCR based marker for marker assisted selection for submergence tolerance in rice. Crop Sci 44:248–253

Zhi-wen L, Ting-dong F, Jin-xing T, Bao-yuan C (2005) Inheritance of seed colour and identification of RAPD and AFLP markers linked to seed colour gene in rapeseed (*Brassica napus* L.). Theor Appl Genet 110:303–310