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Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection

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Abstract A *Brassica juncea* mapping population was generated and scored for seed coat colour. A combination of bulked segregant analysis and AFLP methodology was employed to identify markers linked to seed coat colour in *B. juncea*. AFLP analysis using 16 primer combinations revealed seven AFLP markers polymorphic between the parents and the bulks. Individual plants from the segregating population were analysed, and three AFLP markers were identified as being tightly linked to the seed coat colour trait and specific for brown-seeded individuals. Since AFLP markers are not adapted for large-scale application in plant breeding, our objective was to develop a fast, cheap and reliable PCR-based assay. Towards this goal, we employed PCR-walking technology to isolate sequences adjacent to the linked AFLP marker. Based on the sequence information of the cloned flanking sequence of marker AFLP8, primers were designed. Amplification using the locus-specific primers generated bands at 0.5 kb and 1.2 kb with the yellow-seeded parent and a 1.1-kb band with the brown-seeded parent. Thus, the dominant AFLP marker (AFLP8) was converted into a simple codominant SCAR (Sequence Characterized Amplified Region) marker and designated as SCM08. Scoring of this marker in a segregating population easily distinguished yellow- and brown-seeded *B. juncea* and also differentiated between homozygous (BB) and heterozygous (Bb) brown-seeded individuals. Thus, this marker will be useful for the development of yellow seed *B. juncea* cultivars and facilitate the map-based cloning of genes responsible for seed coat colour trait.

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Introduction

Brassica juncea (AABB, 2n = 36) is an important oilseed crop of India and is grown widely throughout northern India. This crop is gaining importance in Canada and the USA as an alternative to rapeseed, *B. napus*. *B. juncea* has a number of valuable agronomic characteristics, which include tolerance to heat and drought and resistance to black leg disease. However, it has high amounts of erucic acid in the oil and high levels of glucosinolates in the seed meal, both of which are nutritionally undesirable. So, the development of a *B. juncea* that has low amounts of both these components has gained importance in recent years. Further, the quality and quantity of oil can be improved by the development of yellow-seeded cultivars in this species. Brown seeds produce a dull-coloured oil, which is rated inferior to the bright-yellow oil obtained from yellow-seeded *B. campestris*. It has been reported that the yellow-seeded varieties have an inherent advantage over the dark-seeded varieties as in the former there is a lower crude fiber content in the seed hull, a high protein content in the seed meal and a higher oil content in the seed (Stringman et al. 1974, Shirazdegan and Röbellen 1985). In addition, it is easier to determine the degree of ripeness in yellow seeds, as the occurrence of chlorophyll is not masked by the dark seed coat colour, thus making yellow seeds an economically important trait. Therefore, it is important to incorporate the yellow-seeded trait in the double-low lines of *B. juncea*. All the varieties grown commercially in this species are brown-seeded. No yellow-seeded varieties have been released so far although exotic cultivars such as Skorospieka, Donskaja and Zem exist in the germplasm collection as potential donors of the yellow-seeded trait in breeding programmes.

The seed coat colour trait in Brassica species has been reported to be recessive (Shirazdegan 1986). In *B.*

campestris a single gene was found to be responsible for seed coat colour (Chen and Heneen 1992). In *B. napus* it was shown that homozygous recessive genes at three loci (Shirazdegan 1986) determined the seed coat colour. In *B. juncea* two independent dominant genes with duplication effect (Anand et al. 1985, Chauhan and Kumar 1987) govern the seed coat trait. It is possible to assume that the genomes of *B. campestris* (AA) and *B. nigra* (BB) each harbour one of the two genes for seed coat colour of *B. juncea*. Chen and Heneen (1992) found that seed coat colour is mainly controlled by the maternal genotype but influenced by the interaction between the maternal endosperm and/or embryonic genotypes. The influence of environment on the seed colour has also been reported (Van Deyzne et al. 1993).

In recent years, a number of agronomic traits in Brassica species have been mapped using molecular markers. These include clubroot resistance (Voorips et al. 1997), white rust resistance (Kole et al. 1996; Prabhu et al. 1998), fatty acids (Fourmann et al. 1998, Hu et al. 1999); fertility restorer (Delourme et al. 1994) and self-incompatibility (Chyi et al. 1994). It is important to tag the genes responsible for seed coat colour because the latter is known to be highly influenced by environmental conditions and to have a complex mode of inheritance. In *B. napus*, (Van Deyzne et al. 1995) identified *a chalcone synthase* gene (RFLP, restriction fragment length polymorphism marker) linked to seed coat colour. In another study, a number of random amplified polymorphic DNA (RAPD) markers for seed coat colour were identified using *B. campestris – alboglabra* addition lines. One of the RAPD markers was closely linked to the seed coat colour gene and its chromosomal position assigned (Chen et al. 1997). In the study reported here, we have used bulked segregant analysis (BSA) combined with amplified fragment length polymorphism (AFLP) methodology to identify markers linked to seed coat colour in *B. juncea*. One of the AFLP markers linked to the seed coat colour trait was also converted into a sequence-characterized amplified region (SCAR) marker. For achieving this, we employed the polymerase chain reaction (PCR)-walking methodology developed by Siebert et al. (1995) to isolate genomic regions adjacent to the AFLP marker. Based on the sequence information, primers were designed for PCR amplification, which in turn generated amplification products polymorphic between yellow- and brown-seeded individuals. The conversion of a dominant AFLP marker into a simple, codominant SCAR marker has direct application for marker-assisted selection in plant breeding.

Materials and Methods

Plant Material

A single plant of the *B. juncea* (L.) Czern. (cv. Skorospieka) was crossed as a female parent to *B. juncea* (cv. RH30). A segregating population was obtained by selfing a single F_1 plant. One hundred and forty-four (144) segregating F_2 individuals were obtained, and these were selfed to generate F_3 families, which were further advanced by single seed descent. The two parents selected represent two genetically and morphologically diverse cultivars that differ in a number of traits. Skorospieka is tall, yellow seeded and late-flowering and is resistant to white rust, whereas RH30 is short, brown-seeded and early-flowering and is susceptible to white rust. The F_3 and F_4 individuals were scored visually for seed coat colour. DNA was extracted from individual plants using a modified CTAB method (Doyle and Doyle 1990). For bulked segregant analysis (BSA, (Michelmore et al. 1991), equivalent amounts of DNA from ten brown-seeded lines (F_4) and ten yellow-seeded lines (F_4) were pooled to create brown-seeded and yellow-seeded bulks, respectively. Both pools were analysed using AFLP methodology to identify putative markers linked to the seed coat colour trait.

AFLP analysis

AFLP fingerprints were generated based on the protocol described by Vos et al. (1995). Genomic DNA (250 ng) was restricted with *Eco*RI and *MseI* (2.5 U each) in a restriction buffer (50 m*M* TRIS HCL, pH 7.5, 50 m*M* Mg-acetate, 250 m*M* K-acetate) in a final volume of 25 µl. *Eco*RI and *Mse*I adapters were subsequently ligated to the digested DNA fragments. The adapter-ligated DNA was pre-amplified with AFLP primers each having one selective nucleotide using the following cycling parameter: 20 cycles of 30 s at 94°C, 60 s at 56°C and 60 s at 72°C. The pre-amplified DNA was diluted (1:50), and an aliquot was used for selective amplification with the *Eco*RI and *Mse*I primers having three selective nucleotides at the 3' ends. The following cycling parameter were used for selective amplification: 1 cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temperature was then lowered by 0.7° C per cycle during the first 12 cycles, and then 23 cycles were performed at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s. The reaction products were resolved on 5% polyacrylamide gels and autoradiographed.

Cloning of AFLP markers

The seven AFLP fragments (Table 1) that amplified specifically for either brown or yellow bulks were cloned for analysis. These AFLP bands were excised from dried polyacrylamide gels, re-hydrated in TE for 1 h at room temperature and transferred to 500 µl of elution buffer (0.5 *M* NH_4Ac, 10 m*M* magnesium acetate, 1 m*M* EDTA, pH 8.0, 0.1% SDS) at 37°C. From this 1.0 µl of supernatant was used as template for PCR amplification using primers and reaction conditions similar to those used for the AFLP reaction. The amplified products were electrophoresed on a 1.2% agarose gel. The bands were excised from the gel, and the DNA was cloned into the plasmid vector pGEM-T (Promega). Recombinant plasmid DNA was isolated and sequenced using the dideoxy method.

PCR-walking for the isolation of the region adjacent to the linked AFLP markers

PCR-walking (Siebert et al. 1995) was applied with modifications as suggested by Devic et al. (1997) for isolating genomic sequences flanking the AFLP markers (AFLP5, AFLP8 and AFLP29). Genomic libraries of RH30 were prepared by individually digesting 1.0 µg of DNA to completion with *Eco*RV, *Dra*I, *Pvu*II, *Sca*I and *Ssp*I. Oligonucleotide 1: 5'-CTAATACGACTCACTATAGGGCT CGAGCGGCCGCCCGGGGAGGT-3' and adapter oligonucleotide 2: 5'-P-ACCTCCCC-NH_2–3' were mixed to a final equimolar concentration of 50 µM and annealed by incubation in a boiling waterbath for 5 min followed by gradual cooling to allow formation of the adaptor duplex. The adapter duplex (5 µM final concentration) was ligated to 10 µl of the digested DNA at 16°C in the presence of 10 U T4 DNA ligase (Promega). The ligase was heat-inactivated at 70°C for 10 min. The walking step involved

Table 1 AFLP fragments specific for the bulks

Primer designation	Primer combination	AFLP marker designation	Approximate size of marker(bp)	Specific for
1E	E-AAC+M-CTA	AFLP-2	350	Yellow
1E	E-AAC+M-CTA	AFLP-5	250	Brown
1F	E-AAC+M-CTC	AFLP-8	235	Brown
4A	E-ACC+M-CAA	AFLP-10	150	Yellow
4A	E-ACC+M-CAA	AFLP-14	300	Brown
4D	E-ACC+M-CAT	AFLP-22	250	Brown
4E	E-ACC+M-CTA	AFLP-29	150	Brown

two rounds of PCR amplification of the adapter-ligated DNA. In the first PCR amplification step, primer AP1 was used in combination with AFLP clone-specific primer AFLP8F to amplify genomic regions flanking the AFLP8 marker.

AP1: 5'-GGATCCTAATACGACTCACTATAGGGC-3' (27 mer)

AFLP8F: 5'-CTTTCCAGACTCCCATTGGTGC-3' (22 mer)

The second PCR amplification was done using nested primer AP2 in conjunction with AFLP clone-specific primer WALK8F.

AP2 : 5'-CTATAGGGCTCGAGCGGC-3' (18 mer)

WALK 8F : 5'-GCGCGTCCCCTTCCAGAAGTGAAC-3' (24 Mer)

The PCR protocols followed were as described by Devic et al. (1997).

Conversion of AFLP marker into SCAR marker

The amplified products after PCR-walking were purified through wizard PCR-prep columns (Promega), ligated to the pGEM-T vector (Promega) and transformed into XL-1 blue cells. The recombinant plasmids were screened using the colony PCR method (Innes et al. 1990). The recombinant plasmids containing inserts were sequenced with an ABI prism automatic sequencer (Perkin Elmer) using a fluorescent dye terminator. Based on the sequence analysis of the cloned fragments obtained after PCR-walking, the SCAR primers were designed and synthesized.

SCM08: 5'-GAGCATCTAAACCGTCGTGCTTCC-3' (24 mer)

WALK8F : 5'-GCGCGTCCCCTTCCAGAAGTGAAC-3' (24 mer)

Using primer pair WALK8F and SCM08, were PCR-amplified genomic DNA from brown and yellow parents and individual lines. Genomic DNA (50-ng aliquots)was used in a standard PCR reaction containing 2.5 m*M* $MgCl_2$, 2.0 m*M* dNTPs and 0.5 U *Taq* polymerase. PCR conditions for amplification were 94°C for 3 min, followed by 40 cycles of 94°C for 45 s, 69°C for 45 s, 71°C for 1.5 min. The PCR products were visualized after agarose gel electrophoresis.

Results

Identification of brown and yellow lines for bulked segregant analysis (BSA)

Ten yellow-seeded (yellow) lines and ten brown-seeded (brown) ones were selected for BSA on the basis of segregation of seed coat colour in the F_2 and F_3 mapping populations. Of the 144 F_2 plants, 138 were brown-seeded and six were yellow-seeded. The Chi square test showed that the seed coat colour segregated in the ratio 15 (brown): 1 (yellow), confirming the digenic inheritance pattern of the trait. These six yellow-seeded lines maintained their seed coat colour in the F_3 generation, but some of the brown-seeded lines showed segregation. Thus, four additional lines were identified for yellow seed coat colour in F_3 generation to make a bulk of ten lines. Similarly, ten brown lines were selected on the basis of their seed coat colour being maintained in the F_2, F_3 and F_4 mapping populations.

Identification of AFLP markers linked to seed coat colour

To identify markers linked to seed coat colour we bulked DNA from yellow- and brown-seeded lines to create pools. A total of 32 primer combinations were tested on DNA from the parents Skorospieka (yellow-seeded) and RH30 (brown-seeded). Of these, 16 primer pairs (data not shown) which revealed polymorphisms between parents were selected for bulked segregant analysis (BSA). The primers amplified approximately 40–90 bands per assay. A total of 969 bands were obtained with the 16 different primer combinations and revealed 17–44% polymorphism between the parents. AFLP bands present in one pool and absent in the other were regarded as candidate markers linked to seed coat colour. Of the 16 primer combinations tested, 5 primer combinations produced DNA fragments present only in one pool and absent in the other. Seven AFLP bands specific either to the yellow or brown pool were identified (Table 1).

To confirm the linkage of candidate AFLP markers to seed coat colour loci, we screened 60 individual lines in the segregating F_4 population for polymorphism. Figure 1 shows a representative amplification profile with two different primer combinations. With the primer combination E-AAC/M-CTA a band at 250 bp (marked as an arrow, Fig.1) was present in brown-seeded parent RH30 and individuals which are brown-seeded; this was designated as AFLP5 (Table 1). This band was absent in the yellow parent and segregating lines having yellow seeds of *B. juncea*. With the primer combination E-AAC/M-CTC a fragment of 235 bp (shown as an arrow in Fig.1) was present in the brown-seeded parent and individual brown lines; this was designated as AFLP8. This latter band was absent in yellow parent and yellow-seeded segregating individuals (Fig. 1). Out of these 60 individual plants analysed, 3 plants showed recombination for both AFLP5 and AFLP8 markers, which accounts for 5% recombination. Similarly, the 150-bp band (AFLP29) specific for brown parent was absent in yellow parent and in all yellow-seeded individuals (Figure not shown). Of the 45 brown-seeded individuals it was present in 38 indi-

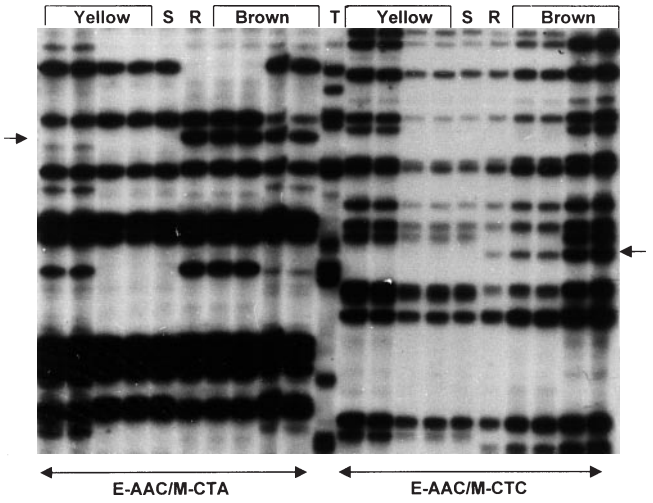

Fig. 1 A portion of the AFLP autoradiogram generated with primer combinations E-AAC/M-CTA and E-AAC/M-CTC for *B. juncea* DNA. The lanes correspond to yellow seed parent (*S*), brown-seed parent (*R*), segregating yellow (*yellow*)and brown (*brown*) individuals and tomato (*T*) DNA. The AFLP markers linked to brown seed coat colour are indicated by *arrows*

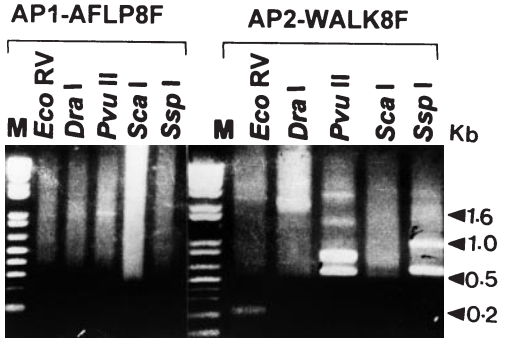

Fig. 2 Gel electrophoresis of the two-step PCR amplification products obtained after PCR walking on *B. juncea* (cv. RH30) genomic DNA. The two rounds of PCR amplification were carried out using primers AP1/AFLP8F and AP2/Walk8F with genomic DNA digested with *Eco*RV, *Dra*I, *Pvu*II, *Sca*I and *Ssp*I. *M* 1 kb marker (BRL).

vidual lines and absent in 7 lines (Figure not shown). This marker was absent in the yellow parent and in all 15 yellow individuals. In the case of markers AFLP2 and AFLP10 (Table 1) the bands were present in the yellow parent, yellow individuals and were also present in some of the brown seeded individuals. Thus, these were eliminated from further analysis. The markers AFLP14 and AFLP22, which were specific for the brown parent and brown pool, showed recombination. They were absent in a number of brown-seeded individuals and were thus not used for further analysis.

The markers tightly linked to seed coat colour, namely AFLP5, AFLP8 and AFLP29, were cloned with the objective of converting these into simple PCR-based markers. The resulting recombinant clones were amplified with the primer combination used to generate each AFLP fragment. The PCR products were checked on the sequencing gel to ensure that the inserts were of the same size as that of the AFLP fragments cloned. The cloned DNAs were sequenced to facilitate the analysis of these markers. Based on the sequence data of the three AFLP markers (AFLP5, AFLP8, and AFLP29) primers were designed for direct amplification of the corresponding loci from genomic DNA. Since the amplified fragments for these loci were small in size (<250 bp), it was not possible to detect polymorphism between yellow- and brown-seeded individuals (data not shown). Thus, it was decided to carry out PCR-walking to isolate fragments adjacent to the AFLP fragment.

PCR-walking

The PCR-walking approach was carried out to obtain fragments adjacent to the AFLP8 marker since this marker showed the least recombination and was tightly linked to seed coat colour. The genomic DNA isolated from the brown parent (RH30) was digested with five blunt end-generating restriction enzymes such as *Eco*RV, *Dra*I, *Pvu*II, *Sca*I and *Ssp*I. The adapter-ligated DNAs were used as templates in a two-step PCR. After primary amplification using AP1 and an AFLP clone-specific primer (AFLP8F), a smear was obtained (Fig. 2) in all the lanes. After the second PCR amplification using primers AP2 and Walk8F, a single band was obtained with *Eco*RV and *Dra*I, whereas 2 intense bands were obtained with *Pvu*II and *Ssp*I. The bands obtained with *Dra*I, *Pvu*II and *Ssp*I were cloned into the pGEM-T vector and sequenced. The ends of the sequence corresponded to either AP2 or the Walk8F primer, thus clearly indicating the authenticity of the walk upstream of the AFLP8 marker. Sequence analysis of the PCR-walking clones was compared to the database, and no homology was obtained.

Amplification of SCAR from genomic DNA

Based on the sequence flanking the AFLP8 marker, primers were designed for PCR amplification of genomic DNA. From the 235-bp AFLP fragment corresponding to AFLP8, nearly 1.0 kb of additional DNA sequence was obtained using the PCR-walking technique. Based on the sequence flanking the AFLP8 marker, primers were designed for direct amplification of genomic DNA as described in the Materials and methods. These primers were used to amplify DNA from the two parents (RH30 and Skorospieka) and 60 F_4 individuals segregating for the seed coat colour trait. As seen in Fig. 3, the yellow parent produced a strong band at 500 bp and a weak band at 1.2 kb (marked by arrows), whereas the brown parent produced a single intense band at 1.1 kb. All of the yellow-seeded individuals in the segregating populations showed an amplification pattern similar to that of the yellow parent (Fig. 3), whereas differences were observed in the

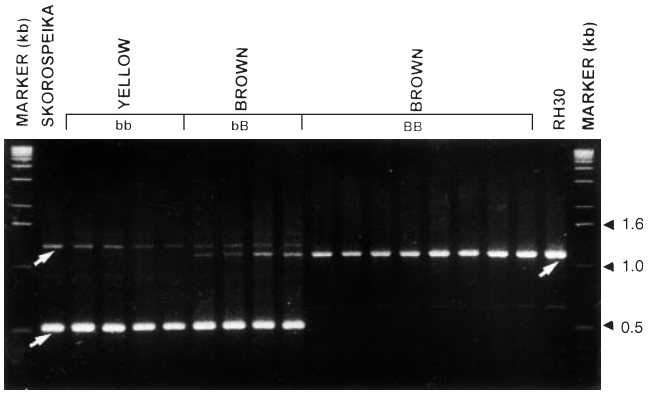

Fig. 3 Analysis of the PCR products obtained using the SCAR primers (walk8F and SCM08) on individual plants. Skorospieka is the yellow-seeded parent and RH30 is the brown-seeded parent. The F_4 individual lines are represented as yellow (*bb*), heterozygous brown (*bB*) and homozygous brown (*BB*). *Marker lane* corresponds to 1-kb marker (BRL)

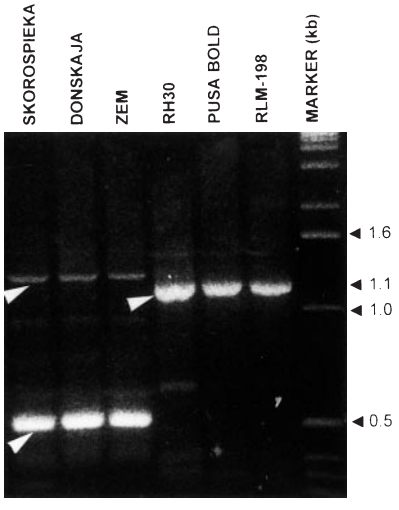

Fig. 4 Gel electrophoresis of the PCR products obtained after amplification using SCAR markers of *B. juncea* yellow-seeded varieties (Skorospieka, Donskaja and Zem) and brown seeded varieties (RH30, Pusa Bold and RLM-198). *Marker lane* corresponds to 1-kb ladder (BRL). The *arrowheads* indicate bands specific to either bb or BB alleles

banding pattern among the brown-seeded individuals (Fig. 3). This is due to the fact that the brown-seeded lines may be either homozygous (BB) or heterozygous (Bb). The homozygous (BB) brown individuals showed a pattern similar to that of the brown parent, whereas the heterozygous brown individuals produced 3 bands corresponding to the banding pattern of both the yellow and brown parent. A total of 60 segregating (F_4) individuals were screened with SCM08 and WALK8F, and three recombinant plants were identified, which were all brown seeded. These were the same individual plants which also showed recombination with AFLP8 marker. Thus, a dominant AFLP marker was converted into a co-dominant SCAR marker and was subsequently designated as SCM08.

This SCAR marker (SCM08) was extended to screen other accessions of *B. juncea* for detection of polymorphism (Fig. 4). The yellow-seeded Donskaja and Zem1 produced bands at 0.5 kb and 1.2 kb, which was similar to that of Skorospieka. The brown-seeded accessions, namely Pusa Bold and RLM198, produced a band at 1.1 kb, which was similar to that of RH30. Other yellow-seeded accessions (Draznaya, Vniimk, Malopolska) and brown-seeded accessions (Kranti, RLM514, Rohini, Pusa Barani) were also screened and produced banding patterns similar to those of the yellow and brown parents, respectively (data not shown).

Discussion

In this study, we have used a combination of bulked segregant analysis and AFLP technique to identify markers linked to the seed coat trait in *B. juncea*. Bulked segregant analysis (BSA) has been shown to be an efficient way to generate a number of markers linked to important agronomic traits (Michelmore et al. 1991). This technique has been used in conjunction with RAPD in a number of cases (Delourme et al. 1994; Jourden et al. 1996). However, in the present study we employed the AFLP technique along with the BSA as it offers the advantage of analysing a large number of markers in a single experiment and is highly reproducible (Powell et al. 1996). A high percentage of polymorphism (17–44%) was observed between the parents. This was expected as the brown-seeded parent (RH30) is an Indian accession, whereas the yellow-seeded parent is an exotic collection from Poland. These two accessions also vary for a number of morphological traits, such as plant height and flowering time. Between the bulks, seven markers were polymorphic, however three co-segregated with brown-seeded individuals in the segregating population. This established a tight linkage of these three AFLP markers to the seed coat colour trait in *B. juncea*, and all were associated with the dominant allele. This was expected, as the brown-seeded individuals were either Bb or BB, whereas the yellow-seeded ones were only bb. In earlier studies, a number of AFLP markers were shown to be linked to different traits; prominent among these are leaf rust resistance (Cervera et al. 1996), clubroot resistance (Voorips et al. 1997), potato virus Y resistance (Brigneti et al. 1997) and the sex locus in *Asparagus officinalis* (Reamon-Buttner et al. 1998).

Although we were able to identify a number of AFLP markers in a short period of time, AFLP markers are generally expensive to generate, are dominant in nature and involve the use of radioactivity, thus limiting their large-scale application in marker-assisted plant breeding. For ease of use, AFLP markers need to be converted into simple SCAR markers. This methodology involves characterization of the linked marker and the design of locus-specific primers (Paran and Michelmore 1993). The conversion of a linked marker to SCAR has been applied successfully in a number of cases involving RAPD markers (Naqvi and Chattoo 1996; Lahogue et al 1998;

Barret et al. 1998) and AFLP markers (Adam-Blondon et al. 1998; Bradeen and Simon 1998).

In our study, the polymorphic markers linked to seed coat colour that were obtained after AFLP analysis were in the size range of 150 to 300 bp. Based on their end sequences, PCR amplification of genomic DNA did not reveal any polymorphism, thereby demonstrating its inability to be directly used as SCAR markers. Similar results have been observed in potato by De Jong et al. (1997). RAPD markers are easy to convert to either SCARs or cleaved amplified polymorphic sequences (CAPSs) for rapid detection as RAPD fragments are generally in the size range of 500 to1500 bp (Barrett et al. 1998). However, in the case of AFLP markers, which are of 150 to 300 bp in size, it is essential to isolate the flanking regions for conversion to SCAR markers. We have used the PCR walking approach to isolate fragments adjacent to the AFLP marker. Other groups have employed inverse-PCR (I-PCR, Hartl and Ochmann 1996) to isolate the flanking regions for conversion to SCAR markers (Bradeen et al. 1998; De Jong et al. 1997), but PCR-walking has been shown to be a better approach than I-PCR to characterize adjacent regions (Devic et al. 1997), as it is possible to isolate large-sized fragments. Another advantage of PCR walking is that it is not hampered by flanking sequences and restriction endonuclease sites and it does not require optimization for each experiment. Thus, PCR-walking has proven to be extremely helpful for the isolation of large-sized fragments adjacent to the AFLP marker. The primers amplified products of different sizes in the yellow- and brown-seeded *B. juncea* parents and thus turned out to be a useful SCAR marker, namely SCM08. The primers for SCM08 produced two fragments of 0.5 kb and 1.2 kb in size in the yellow parent, whereas an intense band at 1.1 kb was generated with the brown parent. This clearly indicated the presence of two loci for the SCM08 marker, which may be due to the fact that *B. juncea* is an amphidiploid with *B. campestris* and *B. nigra* being the parental genomes. A similar observation was made on PCR-walking of the AFLP8 marker where two bands (Fig. 2) were obtained with *Pvu*II and *Ssp*I. This again confirmed duplication of the loci of interest. Recently, it has been shown by RFLP mapping that a number of loci are duplicated in the *B. juncea* (Cheung et al. 1997) genome. Similar duplications of many loci have also been reported in other *Brassica* species (Sadowski et al 1996). In *B. napus*, the SCAR primers amplified two loci for the dwarf *Bzh*-gene and were associated with the *B. campestris* and *B. oleracea* genomes, respectively (Barrett et al. 1998).

This SCM08 marker was extended to analyse the 60 individuals of a segregating *B. juncea* population. The simple PCR assay using the SCAR primers was useful as all the individuals could be classified as bb, Bb or BB. Thus, the SCAR marker has an advantage of being codominant and useful for screening heterozygotes. In the case of *B. napus*, a SCAR marker linked to the dwarf locus was codominant in nature and was able to easily distinguish the heterozygous plants (Barrett et al. 1998) for this locus. Similarly, heterozygotes were easily detected using AFLP markers for the sex locus in asparagus (Reamon-Buttner et al. 1998) and the Y2 locus for carotene accumulation in carrot (Bradeen and Simon 1998). Further, the SCM08 marker was successfully applied to screen other *B. juncea* accessions. The banding patterns obtained were the same, and they distinguished between the yellow and brown parental lines (Skorospieka and RH30) and the segregating populations. These results confirm the tight linkage of the SCAR marker (SCM08) to seed coat colour and indicate that this marker will be useful for marker-assisted breeding. Thus, the PCR-based assay for the SCM08 marker is a rapid, non-radioactive method and can be used on crude DNA preparations (unpublished results). Maternal inheritance, environmental effects and the recessive character of the yellow seed coat trait do not affect this marker. This marker, which is easy to use, will greatly expedite and facilitate *B. juncea* breeding programmes and will be useful for map-based cloning in *B. juncea*.

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