Theor Appl Genet (2004) 109:160–166
DOI 10.1007/s00122-004-1610-3

ORIGINAL PAPER

V. Sabharwal · M. S. Negi · S. S. Banga · M. Lakshmikumaran

Mapping of AFLP markers linked to seed coat colour loci in *Brassica juncea* (L.) Czern

Received: 20 November 2003 / Accepted: 19 January 2004 / Published online: 16 March 2004

Abstract Association mapping of the seed-coat colour with amplified fragment length polymorphism (AFLP) markers was carried out in 39 *Brassica juncea* lines. The lines had genetically diverse parentages and varied for seed-coat colour and other morphological characters. Eleven AFLP primer combinations were used to screen the 39 *B. juncea* lines, and a total of 335 polymorphic bands were detected. The bands were analysed for association with seed-coat colour using multiple regression analysis. This analysis revealed 15 markers associated with seed-coat colour, obtained with eight AFLP primer combinations. The marker E-ACA/M-CTG$_{350}$ ex-plained 69% of the variation in seed-coat colour. This marker along with markers E-AAC/M-CTC$_{235}$ and E-AAC/M-CTA$_{250}$ explained 89% of the total variation. The 15 associated markers were validated for linkage with the seed-coat colour loci using a recombinant inbred line (RIL) mapping population. Bands were amplified with the eight AFLP primer combinations in 54 RIL progenies. Of the 15 associated markers, 11 mapped on two linkage groups. Eight markers were placed on linkage group 1 at a marker density of 6.0 cM, while the remaining three were mapped on linkage group 2 at a marker density of 3.6 cM. Marker E-ACA/M-CTG$_{350}$ co-segregated with *Gene1* controlling seed-coat colour; it was specific for yellow seed-coat colour and mapped to linkage group 1. Marker E-AAC/M-CTC$_{235}$ (AFLP8), which had been studied previously, was present on linkage group 2; it was specific for brown seed-coat colour. Since AFLP markers are not adapted for large-scale applications in plant breeding, it is important to convert these to sequence-characterised amplified region (SCAR) markers. Marker E-AAC/M-CTC$_{235}$ (AFLP8) had been previously converted into a SCAR. Work is in progress to convert the second of the linked markers, E-ACA/M-CTG$_{350}$, to a SCAR. The two linked AFLP markers converted to SCARs will be useful for developing yellow-seeded *B. juncea* lines by means of marker-assisted selection.

Communicated by H.F. Linskens

V. Sabharwal
TERI School of Advanced Studies (TERI SAS),
Lodhi Road, Darbari Seth Block, Habitat Place,
110 003 New Delhi, India

M. Lakshmikumaran (✉)
Plant Molecular Biology Laboratory,
Bioresources and Biotechnology Division,
The Energy and Resources Institute (TERI),
Lodhi Road, Darbari Seth Block, Habitat Place,
110 003 New Delhi, India
e-mail: malaks@teri.res.in
Tel.: +91-11-24682100
Fax: +91-11-24682144

S. S. Banga
Department of Plant Breeding,
Punjab Agricultural University,
141 004 Ludhiana, India

Introduction

Seed coat colour in *Brassica* varies from yellow to brown with intermediate shades. The yellow-seeded varieties have inherent advantages over their dark-seeded counterparts in both qualitative and quantitative terms. It is easy to determine the degree of ripeness of yellow seed because the occurrence of chlorophyll is not masked by the colour of the seed coat. A high chlorophyll content in the seeds of mustard causes discoloration of oil, which subsequently needs to be removed by technical processing (Jönsson 1977). As yellow-seeded varieties have a lower chlorophyll content, the problem of chlorophyll discoloration is avoided. Consequently, the yellow-seed trait is economically beneficial to the oil industry. Seeds of yellow-seeded cultivars of rape (*Brassica rapa*) and rapeseed (*B. napus*) have a 2.5% (Daun and De Clereq 1988) higher oil content than those of the dark-seeded varieties. Yellow seeds of *B. rapa* and *B. napus* have a 5% (Stringam et al 1974) and 3–4% (Shizadegan and Robbelen 1985) lower fibre content, respectively, compared with those of dark-seeded varieties. They may also have a 2.6–5% higher protein content than dark seeds (Shizadegan and Robbelen 1985; Liu et al 1991). Higher protein

and lower crude fibre content are desirable traits in rapeseed meal, which is the most important fodder product of mustard.

Most of the varieties grown for commercial cultivation are dark-seeded, and there are only very few yellow- or light-seeded varieties. The yellow-seeded *B. rapa* variety Candle has been released for commercial cultivation in Canada (Stringam 1980). The yellow-seeded rape varieties released for commercial cultivation in India are YsPb (yellow sarson) and YID. All of the *B. juncea* varieties grown for commercial cultivation are brown-seeded. Attempts have been made to develop artificially synthesised yellow-seeded varieties of *B. rapa*, *B. napus* and *B. juncea* (Jönsson 1975; Abraham and Bhatia 1986; Rahman 2001) by using the yellow-seeded forms that exist in the natural germplasm of *Brassica* species. However, breeding for yellow-seeded varieties is complicated by multiple gene inheritance, allotetraploidy, maternal effect and environmental factors (Shizadigan 1986; Van Deynze and Pauls 1994). The identification of markers linked to seed coat colour will facilitate the breeding of yellow-seeded varieties in *Brassica*. The inheritance pattern of seed coat colour has been studied to understand the genetics of control, which will help in localizing regions of the genome that control this trait. Seed coat colour in *B. rapa* has been shown to be controlled by one (Teutonico and Osborn 1994) to multiple genes (Schwetka 1982). In *B. napus* a three-gene model with the maternal genotype controlling seed coat colour has been proposed (Shirzadegan 1986, Van Deyzne et al. 1995). The inheritance of seed coat colour in *B. juncea* has been described by Vera and Woods (1982) and Anand et al. (1985). An exclusive maternal inheritance was observed and the brown seed coat was dominant over yellow. A segregation ratio of 15 brown: 1 yellow indicated a digenic control of the trait.

Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers linked to genes controlling seed coat colour in *B. napus* have been identified in two different doubled-haploid (DH) mapping populations (Van Deynze et al. 1995; Somers et al. 2001). In another study, a number of RAPD markers for seed coat colour were identified using *B. rapa-B. alboglabra* addition lines (Chen et al. 1997). In *B. rapa*, Teutonico and Osborn 1994) mapped a locus controlling seed coat colour to linkage group 5. The bulked segregant analysis (BSA) combined with amplified fragment length polymorphism (AFLP) technology has been used to identify markers linked to seed coat colour in *B. juncea* (Negi et al. 2000). A recombinant inbred line (RIL) mapping population was used for this purpose, and several linked markers were identified. One AFLP marker linked to the seed coat colour trait was converted to a sequence characterized amplified region marker (SCAR). The investigation reported here is an extension of the work carried out by Negi et al. (2000). We have used AFLP markers to screen the genotype of *B. juncea* lines varying for seed coat color and having multiple ancestry. The multiple regression approach was employed to determine the associations between AFLP markers and the seed-coat color trait. The associations were further validated for linkage on the RIL mapping population. This study led to the identification of eight additional markers linked to seed-coat colour loci in *B. juncea.* The position of a one of the genes controlling seed-coat colour was also mapped.

Materials and methods

Plant material

Thirty-six *Brassica juncea*. (L.) Czern breeding lines and three exotic accessions, all having multiple ancestry, were analysed for association with seed coat colour. The plant material varied with respect to seed-coat colour and various morphological traits such as erucic acid content and glucosinolate content. Detailed descriptions of the lines and accessions are give in Table 1. The lines were scored visually for seed-coat colour. The three exotic accessions and 17 of the *B. juncea* lines were yellow-seeded, while the remaining 19 lines were brown-seeded. The breeding lines were obtained from PAU (Punjab Agricultural University). The exotic accessions were maintained at TERI (The Energy and Resources Institute), New Delhi. Validation of the associated markers was carried out on a RIL mapping population that was generated and maintained at TERI, New Delhi. A single plant of *B. juncea* cv. Skorospieka was crossed as female parent to *B. juncea* cv. RH30. A segregating population was obtained by selfing a single F_1 plant. In total, 144 segregating F_2 individuals were obtained, and these were selfed to generate F_3 families. These were further advanced by single-seed descent. The two parents selected represent two genetically and morphologically diverse accessions that differ for a number of traits. Skorospieka is tall, yellow-seeded, late-flowering and resistant to white rust; RH30 is short, brown-seeded, early-flowering and susceptible to white rust. The F_6 individuals were scored visually for seed-coat colour. DNA was extracted from young and lyophilised leaves of 54 individual plants using a modified CTAB method (Weising et al. 1995).

AFLP analysis

The AFLP procedure was performed following the protocol developed by Vos et al. (1995) with minor modifications (Das et al 1999). All of the reagents required for the AFLP analysis were obtained from Life Technologies (Gaithersburg, Md.). The PCR reactions were performed in a GeneAmp PCR-9700 thermal cycler. Following the restriction digestion of genomic DNA (300 ng) with restriction enzymes *Eco*RI and *Mse*I, adaptors specific to *Eco*RI and *Mse*I overhangs were ligated. Pre-amplification of the adaptor-ligated DNA was performed using A- and C-selective nucleotides (*Eco*RI+A and *Mse*I+C) under the following cycling parameters: 20 cycles of 30 s at 94°C, 60 s at 56°C and 60 s at 72°C. Selective amplification was conducted using pre-amplified DNA, diluted 50 times, as a template. Primers with three selective bases at their 3′ ends were employed and *Eco*RI primer was end-labeled with γ-[P^{32}]-ATP. The cycling parameters were: one cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temperatures were reduced by 0.7°C per cycle during the first 12 cycles, and then 23 cycles were performed at 94°C for 30 s, 55°C and 72°C for 60 s. The samples were size fractionated on 6% polyacrylamide gel and autoradiographed.

Multiple regression analysis

Associations between the AFLP markers and the phenotypic trait, seed-coat colour, were established using the multiple regression approach (Virk et al. 1996). The trait was treated as a dependent

Table 1 List of *Brassica juncea* lines used in the association mapping study

Line no.	Name	Trait	Origin
1	QM-1	Brown, low erucic acid[a]	India
2	QM-3	Brown, low erucic acid[a]	India
3	CM-16-3	Brown, low erucic acid[a]	India
4	HLM-3	Brown, low erucic acid[a]	India
5	HLM-31	Brown, low erucic acid[a]	India
6	RLM-619-6-13-7	Brown, low erucic acid[a]	India
7	RNDR-190-80	Brown, low erucic acid[a]	India
8	RLC-1359-40-2	Brown, low erucic acid[a]	India
9	RRL-1359-18	Brown, low erucic acid[a]	India
10	RRL-1359-12	Brown, low erucic acid[a]	India
11	MCN-4	Brown, high erucic acid	India
12	RC-981	Brown, high erucic acid	India
13	BIO-467-95	Brown, high erucic acid	India
14	DHR-9601	Brown, high erucic acid	India
15	HUM-9801	Brown, high erucic acid	India
16	LM-100-22	Brown, high erucic acid	India
17	NQM-20	Brown, high erucic acid	India
18	NR-3326	Brown, high erucic acid	India
19	DNAWF-1	Brown, high erucic acid	India
20	YSRL-9-18-9	Yellow, low erucic acid[a]	India
21	YSRL-9-18-1(BC_4-3-2)	Yellow, low erucic acid[a]	India
22	YSRL-9-18-1(BC_4-7-13)	Yellow, low erucic acid[a]	India
23	KLM-159	Yellow, low erucic acid[a]	India
24	KLM-131	Yellow, low erucic acid[a]	India
25	KLM-152	Yellow, low erucic acid[a]	India
26	YSRL-9-18-2	Yellow, low erucic acid[a]	India
27	KLM-153	Yellow, low erucic acid[a]	India
28	KLM-132	Yellow, low erucic acid[a]	India
29	KLM-142	Yellow, low erucic acid[a]	India
30	KLM-152-A	Yellow, low erucic acid[a]	India
31	KLM-151	Yellow, low erucic acid[a]	India
32	KLM-153-A	Yellow, low erucic acid[a]	India
33	KLM-126	Yellow, low erucic acid[a]	India
34	KLM-163	Yellow, low erucic acid[a]	India
35	KLM-143	Yellow, low erucic acid[a]	India
36	CM-21-9	Yellow, low erucic acid[a]	India
37	Zem	Yellow, low erucic acid[a]	Australia
38	Skorospieka II	Yellow, low erucic acid[a]	Russia
39	Donskaja IV	Yellow, low erucic acid[a]	Russia

[a] Less than 2% erucic acid

variable and the various AFLP marker genotypes (scored as 0 and 1) as independent variables. The analysis was based on the model $Y=a+b_1m_1+b_2m_2+.....b_jm_j+.....+b_nm_n+d+e$, which relates the variation in the dependent variable (Y=accession means as a quantitative trait) to a linear function of a set of independent variables, m_j, representing the AFLP markers. The terms b_j are the partial regression coefficients that specify the empirical relationships between Y and m_j, d represents the residual left after regression and e is the random error of Y that includes environmental variation. The stepwise method of SPSS was employed to determine the most appropriate model. A one-variable (i.e. marker) model was first assessed, and the marker with the highest r^2 was identified. The second variable was then added, and the best two-variable model was selected using the criteria of the largest r^2. The model fitting was continued until all significant variation in Y was exhausted. Fisher's F-test was carried out for determining the best-fit model. The analysis was carried out at 5% level of significance.

Linkage analysis

Genetic segregation data for AFLP markers linked with seed-coat colour among 54 RILs (45 brown-seeded and 9 yellow-seeded) was analysed using MAPMAKER ver. 3.0 (Lander et al. 1987; Lincoln et al. 1992). A molecular marker linkage map was constructed using a maximum recombination fraction of 0.4 and a LOD threshold of 3.0. Two-point estimates were used to designate preliminary linkage groups, and multipoint analysis was used to designate the most-likely locus orders. The map distances were converted to centiMorgans (cM) using the Kosambi function (Kosambi 1944).

Results

Association mapping

Association mapping of seed-coat colour with AFLP markers was carried out using 39 *B. juncea* lines of genetically diverse parentages. They varied with respect to seed-coat colour and other morphological traits such as erucic acid content and glucosinolate content. Twenty *B. juncea* lines were yellow-seeded, while the remaining 19 were brown-seeded. Eleven AFLP primer combinations were employed, and a total of 335 polymorphic bands were detected. The bands were analysed for association with seed-coat colour using multiple regression analysis. Fifteen markers showed significant association and were generated with eight AFLP primer combinations. The eight primer combinations and the size of associated

Table 2 AFLP markers associated with seed coat colour and their squared regression values

Marker	Coefficient of determination (R^2)	Associated seed coat colour
E-ACA × M-CTG$_{350}$	0.69	Yellow
E-AAC × M-CTC$_{235}$	0.834	Brown
E-AAC × M-CTA$_{250}$	0.892	Brown
E-ACA × M-CTG$_{200}$	0.925	Yellow
E-ACC × M-CAT$_{200}$	0.942	Brown
E-ACC × M-CTA$_{300}$	0.955	Yellow
E-AAC × M-CAC$_{250}$	0.983	Yellow
E-ACC × M-CAT$_{300}$	0.985	Yellow
E-ACC × M-CTA$_{150}$	0.987	Brown
E-ACC × M-CAT$_{150}$	0.99	Brown
E-AAC × M-CAC$_{200}$	0.991	Brown
E-ACC × M-CAT$_{180}$	0.991	Brown
E-AGC × M-CTA$_{180}$	0.992	Yellow
E-ACC × M-CAT$_{250}$	0.993	Brown
E-AGG × M-CTA$_{300}$	0.995	Brown

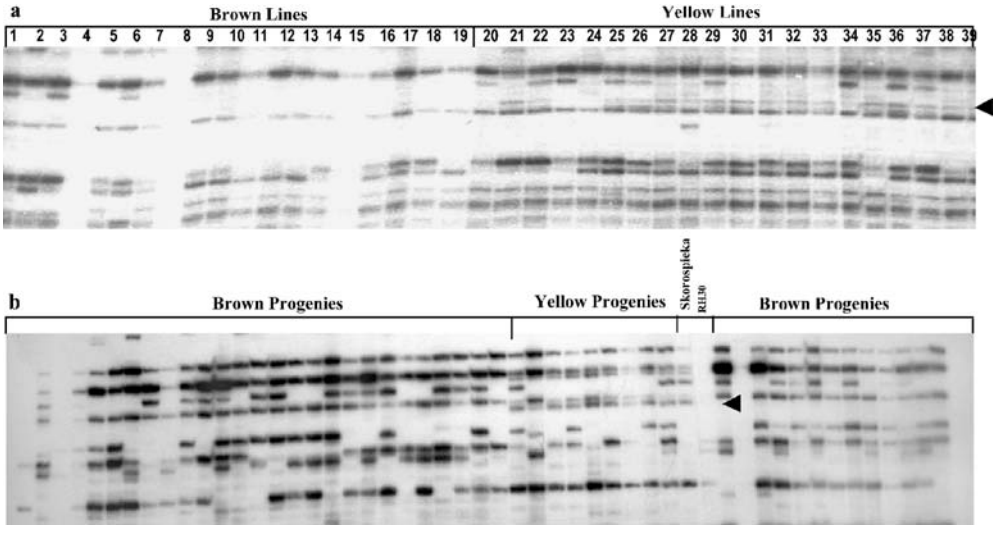

Fig. 1 **a** A portion of the AFLP profile generated with primer combination E-ACA × M-CTG in a *Brassica juncea* natural population consisting of 39 lines having genetically diverse parentages and varying in seed coat colour. The 350-bp marker associated with yellow seed coat colour is indicated by an *arrowhead*. **b** A portion of the AFLP profile generated with primer combination E-ACA × M-CTG in a *B. juncea* RIL mapping population consisting of nine yellow- and 45 brown-seeded progenies. The 350-bp marker linked with seed coat colour loci is indicated by an *arrowhead*

markers amplified are shown in Table 2.Of the 15 AFLP markers, six amplified in the yellow lines while the remaining nine showed amplification mainly in the brown lines (Table 2). A representative AFLP profile generated by employing the primer combination E-ACA/M-CTG in the 39 *B. juncea* lines is shown in Fig. 1a. This primer combination amplified marker E-ACA/M-CTG$_{350}$ in 17 of 20 yellow lines, but the marker was absent in all of the brown lines. E-ACA/M-CTG$_{350}$ (indicated by an arrow in Fig. 1a) explained 69% of the variation in seed-coat colour and, along with the markers E-AAC/M-CTC$_{235}$ and E-AAC/M-CTA$_{250}$, explained 89% of the total variation. These 15 markers together explained 99% of the trait with only a negligible portion (1%) remaining unexplained (Table 2).

Linkage mapping

The 15 molecular markers showing association with seed-coat color in the *B. juncea* natural population were validated for linkage in a RIL mapping population, which consisted of nine yellow- and 45 brown-seeded RIL progenies. The associated markers were amplified in the 54 individual RIL progenies and in the two parents with the eight AFLP primer combinations. One of the markers generated by employing the primer combination E-ACA/M-CTG is shown in Fig. 1b (arrow). This marker, designated E-ACA/M-CTG$_{350}$, was 350 bp in size. It was amplified in the yellow-seeded parent and all of the yellow-seeded progenies but was absent in the brown-seeded parent and brown-seeded progenies. No recombinants were obtained, and the marker co-segregated with the gene for seed-coat colour, designated *Gene1*. Similarly, the remaining 14 markers were validated by gen-

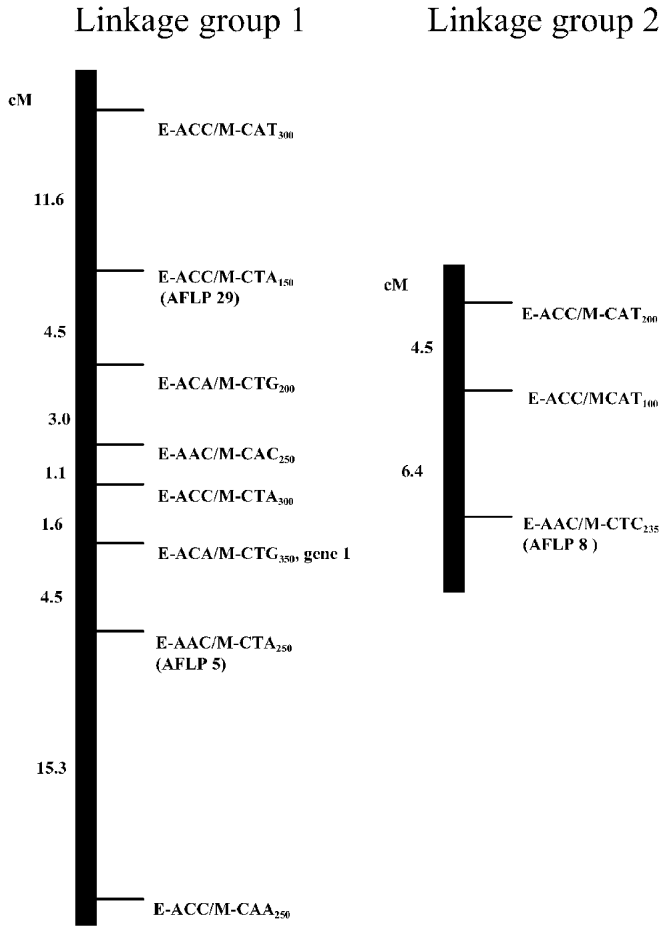

Fig. 2 A genetic linkage map of 11 AFLP markers and the *Gene1* locus associated with seed coat colour in *B. juncea*

erating their AFLP profiles with the seven other primer combinations. Analysis of the data for the presence/absence of bands detected by all eight primer combinations was carried out on MAPMAKER ver. 3.0 at a minimum LOD score of 3.0 and at a maximum distance of 40 cM. The linkage map for these markers is shown in Fig. 2. Eight AFLP markers were placed on linkage group 1. The entire interval mapped with AFLP markers around *Gene1* for seed-coat colour was 41.5 cM in length with a marker density of 6.0 cM. Marker E-ACA/ M-CTG$_{350}$, (Fig. 1b), which is specific for yellow-seed coat colour, co-segregated with *Gene1* and was mapped on linkage group 1. Markers E-ACC/M-CTA$_{300}$ and E-AAC/M-CTA$_{250}$ flanked *Gene1* at a distance of 1.6 cM and 4.5 cM, respectively. Marker E-ACC/M-CTA$_{300}$ was present in the yellow-seeded lines, while E-AAC/M-CTA$_{250}$ was present in the brown-seeded ones. Other markers present on this linkage group were E-ACC/M-CAT$_{300}$, E-ACC/M-CTA$_{150}$, E-ACA/M-CTG$_{200}$, E-AAC/M-CAC$_{250}$ and E-ACC/M-CAA$_{200}$, which were present at a distance of 21.8, 10.2, 5.7, 2.7 and 19.8 cM, respectively, from *Gene1*. Linkage group 2 comprised markers E-AAC/M-CTC$_{235}$, E-ACC/M-CAT$_{100}$ and E-ACC/M-CAT$_{200}$. These markers covered 10.9 cM at a marker density of 3.6 cM. The second gene controlling seed-coat colour in *B. juncea* could not be mapped as the light reflectance values for seed-coat colour, explaining the bimodal nature of trait inheritance, were not available.

Discussion

In the present study association mapping was successfully employed for the identification of markers associated with the seed-coat colour trait. Fifteen markers were found to be associated significantly and explained 99% of the trait. Of these three markers E-ACA/M-CTG$_{350,}$ E-AAC/M-CTC$_{235}$ and E-AAC/M-CTA$_{250}$ explained 89% of the total variation. Marker E-ACA/M-CTG$_{350}$ explained 69% of the variation in seed-coat colour. It was amplified in 17 of the 20 yellow-seeded lines and was absent in all of the brown-seeded lines. Absence of the marker in the four yellow lines could be due to the presence of different alleles for seed-coat colour at this locus in these *B. juncea* lines. However, due to our lack of knowledge of the exact parentage of these lines the exact cause of the absence of markers in some of the lines cannot be ascertained. It is possible that these lines were derived from parents distinct from those of other lines. Upon linkage analysis using RILs, this marker found to be amplified in all of the yellow-seeded progenies and was absent from all of the brown-seeded ones. This suggests that the alleles for yellow seed-coat colour in the RILs derived from *B. juncea* cvs. RH30 and Skorospieka are the same as those amplified in 17 of the 20 *B. juncea* lines taken for the association mapping study. Association mapping was carried out keeping in view its advantages over conventional mapping strategies. The strategy for association mapping makes use of natural populations or genetic resources. This makes the process faster and less labor intensive than conventional mapping which employs experimental populations such as DH, RIL, backcross and F_2 populations. Further, the linkage disequilibrium is maximised as only genetic markers in close linkage with the trait are identified. This is because the plants tested are products of several breeding cycles, and several opportunities for recombination between the gene and markers have occurred. Unlike conventional breeding strategies, the prediction of plant seed-coat colour at the seedling stage can be carried out on plants of any parental origin and is not affected by the genetic background. This makes the marker-assisted selection programme, which employs markers derived from association mapping more practical. Association mapping has been used extensively in medical genetics but has not been applied in plant genetics. The few reports on association mapping in plants include those on the association of variation with flowering time in maize (Thornsberry et al. 2001), association with morphological traits in rice (Virk et al. 1996), association with salt tolerance and ecogeography in barley (Pakniyat et al. 1997), association with biotic and abiotic stress tolerance in barley (Ivandic et al. 2003)

and association with adaptation to low winter temperatures (Skot et al. 2002).

Linkage mapping studies were carried out to find the centiMorgan distances of the 15 associated markers with respect to genes for seed-coat colour. A RIL mapping population was employed. The advantages of this population over others for mapping and tagging of genes has been discussed by Kole et al. (1997). As compared to segregating populations such as an F_2 or backcross, RILs are immortal. Although DHs are also immortal and can be generated in less time than RILs, it is difficult to produce a DH population in *B. juncea* as the protocol for microspore culture in this species is not well-established. Moreover, the advanced generations of RILs undergo a larger number of meioses and, consequently, more recombination events than the DHs. This increases the linkage disequilibrium between the marker and the loci for the trait of interest and thus enables finer mapping in RILs than in DHs.

In the previous study by Negi et al. (2000), three AFLP markers were identified as being tightly linked to the seed-coat colour trait and specific for brown-seeded individuals. These markers were E-AAC/ M-CTA_{250}, E-AAC/M-CTC_{235} and E-ACC/M-CTA_{150}, designated as AFLP-5, AFLP-8 and AFLP-29, respectively (Negi et al. 2000). Scoring of these markers in a segregating population easily distinguished yellow- and brown-seeded *B. juncea* and also differentiated between homozygous (BB) and heterozygous (Bb) brown-seeded individuals. In addition to the three markers identified by Negi et al. (2000), we identified eight more linked markers in this study. These markers were distributed on two linkage groups, which is in agreement with the fact that in *B. juncea* two independent recessive genes govern the yellow seed-coat colour trait (Vera and Woods 1982; Anand et al. 1985). The three tightly linked AFLP markers identified by Negi et al. (2000) were placed on two linkage groups. Linkage group 1 had the markers E-AAC/ M-CTA_{250} (AFLP-5) and E-ACC/M-CTA_{150} (AFLP-29) at a distance of 4.5 cM and 9.7 cM, respectively, from *Gene1*. Linkage group 2 had the marker E-AAC/M-CTC_{235} (AFLP-8), which was converted to a SCAR by Negi et al. (2000). Of the eight additional markers identified in the present study, two were mapped between *Gene1* and marker E-ACA/M-CTG_{200} (AFLP-5): marker E-ACA/M-CTG_{350} co-segregated with *Gene1*, while marker E-ACC/M-CTA_{300} was mapped at a distance of 1.6 cM from *Gene1*.

Studies have also been carried out in *B. napus* and *B. rapa* to identify markers linked to seed coat colour. RFLP markers were employed by Van Deynze et al. (1995) in a DH population for this purpose. This study identified a quantitative trait locus (QTL) that explained 24% of the phenotypic variation in the population. In another study by Somers et al. (2001), 11 RAPD markers linked with the *pigment1* gene in *B. napus* were identified. These markers were distributed on three linkage groups. One of these markers, UBC282, explained 72.3% of the phenotypic variation. These results are contradictory to the results of Van Deynze et al. (1995) who showed that a major gene in *B. napus* controls a large part of variation in seed-coat colour. In the study by Chen et al. (1997), one marker closely linked to the seed-coat colour gene in *B. rapa* and *B. alboglabra* lines was identified and the chromosomal position assigned. In another study, Teutonico and Osborn (1994) reported a 3:1 ratio for segregation of yellow:brown seeds in *B. rapa*, and the locus controlling the seed-coat colour gene was mapped to linkage group 5.

Markers linked to agronomic traits have the potential to be employed in map-based cloning and marker-assisted selection (MAS) programmes. AFLP markers are generally expensive to generate, dominant in nature and involve the use of radioactivity. This limits their large-scale application in marker-assisted plant breeding. For practical applications in MAS, these dominant markers need to be converted to codominant SCAR markers (Paran and Michelmore 1993). The PCR-based assay for SCAR is rapid and non-radioactive and can be used on crude DNA preparations. Maternal inheritance, environmental effects and the recessive character of the yellow-seed coat colour trait do not affect this marker (Negi et al. 2000). However, it is difficult to convert AFLP markers to SCAR markers mainly due to the small size of the former (50–400 bp). They do not reveal polymorphism upon conversion to SCAR (Negi et al. 2000). It is easier to convert RAPD markers to SCAR markers as the former generate fragments in the size range of 500–1,500 bp. In order to convert AFLP markers to useful SCAR markers it is necessary to clone the flanking regions. The PCR walking approach has proven to be extremely useful for the isolation of large-sized fragments adjacent to the AFLP markers (Negi et al. 2000). Work is in progress to convert the marker E-ACA/ M-CTG_{350}, which is tightly linked to *Gene1* and amplifies only in the yellow lines, to a SCAR marker that could then be employed along with E-AAC/M-CTC_{235} (AFLP-8), which has previously been converted to SCAR, in MAS and thus accelerate the breeding programme. Since the two markers are present on two linkage groups, they may be useful for map-based cloning of the two genes responsible for controlling seed-coat colour loci in *B. juncea*.

Acknowledgements The authors are grateful to Dr. R.K. Pachauri, Director General, TERI, and Dr. T.P. Singh, Director, TERI School of Advanced Studies, for their kind support and encouragement. The Senior Research Fellowship from the Council of Scientific and Industrial Research to Vaishali Sabharwal is duly acknowledged.

References

Abraham V, Bhatia CR (1986) Development of strains with yellow seedcoat in Indian mustard (*Brassica juncea* Czern. and Coss.). Plant Breed 97:86–88

Anand IJ, Reddy WR, Rawat DS (1985) Inheritance of seed colour in mustard. Indian J Genet 45:34–37

Chen BY, Jorgensen RB, Cheng BF, Heneen WK (1997) Identification and chromosomal assignment of RAPD marker linked

with a gene for seed coat colour in a *Brassica campestris-alboglabra* addition line. Hereditas 126:133–138
Das S, Rajagopal J, Bhatia S, Srivastava PS, Lakshmikumaran M (1999) Assessment of genetic variation within *Brassica campestris* cultivars using AFLP and RAPD markers. J Biosci 24:433–440
Daun JK, DeClercq DR (1988) Quality of yellow and dark seeds in *Brassica campestris* canola varieties Candle and Tobin. J Am Oil Chem Soc 65:122–126
Ivandic V, Thomas WTB, Nevo E, Zhang Z, Forster BP (2003) Associations of simple sequence repeats with quantitative trait variation including biotic and abiotic stress tolerance in *Hordeum spontaneum*. Plant Breed 122:291–378
Jönsson R (1975) Yellow-seeded rape and turnip rape. II. Breeding for improved quality of oil and meal in yellow-seeded materials. J Swed Seed Assoc 85:271–275
Jönsson R (1977) Breeding for improved oil and meal quality in rape (*Brassica napus* L.) and turnip rape (*Brassica campestris* L.). Hereditas 87:205–218
Kole C, Kole P, Vogelzang A, Osborn TC (1997) Genetic linkage map of a *Brassica rapa* recombinant inbred population. J Hered 88:553–557
Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175
Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L. (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Lincoln S, Daley M, Lander E (1992) Constructing genetic map with MAPMAKER/EXP. 3.0 Technical report, 3rd edn. Whitehead Technical Institute, Cambridge, Mass.
Liu H, Han J, Hu X (1991) Studies on the inheritance of seed coat colour and other related characters of yellow seeded *B. napus*. In: Wratten N, Salisbury PA (eds) Proc 8th Int Rapeseed Congr, vol. 5. The Regional Institute, Gosford, Australia, pp 1438–1444
Negi MS, Devic M, Delseny M, Lakshmikumaran M (2000) Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to SCAR marker for rapid selection. Theor Appl Genet 101:146–152
Pakniyat H, Powell W, Baird E, Handley LL, Robinson D, Scrimgeaur, Nevo E, Hackett CA, Caligari PDS, Forster BP (1997) AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. Genome 40:332–341
Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew genes in lettuce. Theor Appl Genet 85:985–993
Rahman MH, Joersbo M, Poulsen MH (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breed 120:473–475
Schwetka A (1982) Inheritance of seed color in turnip rape (*Brassica campestris* L.). Theor Appl Genet 62:161–169
Shirzadegan M (1986) Inheritance of seed colour in *Brassica napus* L. Z Pflanzenzuecht 96:140–146
Shirzadegan M, Robbelen G. (1985) Influence of seed colour and hull proportion on the quality properties of seeds in *Brassica napus* L. Fette Seifen Anstrichm 87:235–237
Skot L, Hamilton NRS, Mizen S, Chorlton KH, Thomas ID (2002) Molecular genoecology of temperature response in *Lolium perenne*: 2. Association of AFLP markers with ecogeography. Mol Ecol 11:1865–1876
Somers DJ, Rakow G, Prabhu VK, Friesen KRD (2001) Identification of a major gene and RAPD markers for yellow seed coat colour in *B. napus*. Genome 44:1077–1082
Stringam GR (1980) Inheritance of seed color in turnip rape. Can J Plant Sci 60:331–335
Stringam GR, McGregor DI, Pawlowski SH (1974) Chemical and morphological characteristics associated with seedcoat colour in rapeseed. In: Wratten N, Salisbury PA (eds) Proc 4th Intl Rapeseed Conf. The Regional Institute, Gosford, Australia, pp 99–108
Teutonico RA, Osborn TC (1994) Mapping of RFLP and quantitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus, B.oleracea* and *Arabidopsis thaliana*. Theor Appl Genet 89:885–894
Thornsberry JM, Goodmam MM, Doebley J, Kresovich S, Nielsen D, Buckler IV ES (2001) Dwarf 8 polymorphisms associate with variation in flowering time. Nat Genet 28:286–289
Van Deynze AE, Pauls KP (1994) The inheritance of seed colour and vernalization requirement in *Brassica napus* using doubled haploid populations. Euphytica 74:77–83
Van Deynze AE, Landry BS, Pauls KP (1995) The identification of restriction fragment length polymorphisms linked to seed colour genes in *Brassica napus*. Genome 38:534–537
Vera CL, Woods DL (1982) Isolation of independent gene pairs at two loci for seed coat color in *Brassica juncea*. Can J Plant Sci 62:47–50
Virk PS, Brian V, Ford-lloyd, Jackson MT, Pooni HS, Clemeno TP, Newbury HJ (1996) Predicting quantitative variation within rice germplasm using molecular markers Heredity 76:296–301
Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuipers M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414
Weising K, Nybom H, Wolff K, Meyer W (1995) DNA fingerprinting in plants and fungi. CRC Press, Boca Raton