Theor Appl Genet (2004) 108:743–749
DOI 10.1007/s00122-003-1481-z

ORIGINAL PAPER

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Molecular tagging of erucic acid trait in oilseed mustard (*Brassica juncea*) by QTL mapping and single nucleotide polymorphisms in *FAE1* gene

Received: 30 June 2003 / Accepted: 17 September 2003 / Published online: 16 October 2003

Abstract Molecular mapping and tagging of the erucic acid trait (C22:1) in *Brassica juncea* was done by a candidate gene approach. Two QTLs underlying the variation of seed erucic acid content were assigned to two linkage groups of a *B. juncea* map using a doubled haploid (DH) mapping population derived from high × low erucic acid F_1 hybrid. Two consensus primers corresponding to the full-length Fatty Acid Elongase 1 (*FAE1*) gene, reported to be involved in the elongation of C18:1 to C22:1, were designed. PCR amplification and subsequent cloning and sequencing identified two *FAE1* genes (*FAE1.1* and *FAE1.2*) in both high and low erucic acid mustard lines. Sequence alignment of corresponding *FAE1* genes between high and low erucic acid mustard lines identified four substitution type single nucleotide polymorphisms (SNPs) in *FAE1.1* and three in *FAE1.2*. Using the SNuPE method of SNP genotyping, these two genes were mapped to two independent loci that co-segregated with the two QTLs governing the erucic acid trait. Association of wild (*E1E2*) and mutant (*e1e2*) haplotypes of two *FAE1* genes with erucic acid variation in two segregating populations revealed that the *e1e1e2e2* genotype identified low erucic acid individuals (<2%) and *E1E1E2E2* identified individuals with highest erucic acid content (>40%). The *E1e1E2e2* heterozygote was found to be intermediate in phenotype. The applicability of these SNPs in marker-assisted manipulation of the erucic acid trait was verified by genotyping a set of contrasting germplasm of *B. juncea* belonging to two distinct gene pools (Indian and east European) and other oil-yielding *Brassica* species.

Communicated by C. Möllers

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Introduction

The presence of high concentrations of very long-chain fatty acids, especially erucic acid (C22:1), in the seed of oil-yielding *Brassica* species has been reported to be nutritionally undesirable (Beare et al. 1963). Hence, emphasis was placed upon the development of low erucic acid (<2%) rapeseed-mustard. Whereas success has already been achieved in *B. napus*, work on breeding of zero erucic acid mustard (*B. juncea*) was initiated following the identification of low erucic lines Zem 1 and Zem 2 by Kirk and Oram (1981). As in *B. napus* (Stefansson and Hougen 1964), the inheritance of the erucic acid trait in *B. juncea* was shown to be controlled by two genes with additive effects, zero erucic being recessive in expression. It was also demonstrated that the Indian geographical group of *B. juncea* (with ~50% erucic acid) contains alleles for a high erucic acid level at two loci, whereas the eastern European group (with <25% erucic acid) possesses alleles for a high erucic acid level at one locus only (Kirk and Hurlstone 1983).

B. juncea constitutes the second most important oilseed crop of India, and all the varieties currently being grown are high erucic types. Low erucic acid mustard can be developed by the introduction of recessive alleles from donor varieties like Zem1 or its derivative line, Heera (Sodhi et al. 2002), through backcross breeding with an Indian variety as the recurrent parent. However, this is a lengthy process and necessitates selfing of every backcross generation and identifying zero erucic plants in the segregating population through analysis of the fatty acid profile of single cotyledons of individual seeds by gas chromatography (GC). On the other hand, the development of molecular markers either tightly linked to the erucic acid trait or within the candidate genes will allow selection of lines with low erucic alleles in the heterozygous state during backcross, thereby obviating the need of selfing every backcross generation and extensive GC analyses. Furthermore, the population size for screening will be reduced by a quarter for two-gene materials (the

Indian types) and by a half for one-gene materials (the eastern European types).

Fatty Acid Elongase 1 (*FAE1*) has been shown to be the candidate gene for elongation of C18:1 (oleic acid) to C22:1 (erucic acid) through the characterization of mutants deficient in the long-chain fatty acids (James and Dooner 1990; Kunst et al. 1992; Lemieux et al. 1990). The *FAE1* gene was first cloned from *Arabidopsis* and it was proposed that it encodes a β-ketoacyl CoA synthase (KCS) that is involved in the first step of the fatty acid elongation pathway (James et al. 1995). The functional role of the *FAE1* gene was ascertained by genetic transformation of a zero erucic acid rapeseed (Lassner et al. 1996). In *B. napus*, Barret et al. (1998) isolated two rapeseed cDNAs of gene-encoding KCS by using sequence information from the *Arabidopsis FAE1* gene and mapped one of them to one of the two erucic acid loci in the *B. napus* genome. Subsequently, Fourmann et al. (1998) mapped two *FAE1* genes in *B. napus* (*FAE1.1* and *FAE1.2*) by detecting polymorphism through polyacrylamide gel electrophoresis from the amplification products of the partial *FAE1* genes. These two genes were found to co-segregate with two QTLs (Jourdren et al. 1996) controlling erucic acid content. However, the tagging information generated by Fourmann et al. (1998) may have limited application in the erucic acid breeding programme of *B. napus* because of: (1) low throughput genotyping as the polymorphism detection was gel-based, and (2) absence of a molecular tag for low and high alleles in the *FAE1.2* locus as the polymorphism was detected using two low erucic acid lines. In addition, the tagging information of erucic acid from *B. napus* may not be applicable to *B. juncea* as only one of the diploid progenitor species, *B. rapa*, is the common parent in the two allotetraploid *Brassica* species (U 1935).

We report here the isolation of two full-length *FAE1* genes in *B. juncea* from one high and one low erucic acid mustard line and the detection of single nucleotide polymorphisms (SNPs) in the two genes by sequence comparison. These two genes have been mapped to *B. juncea* genome and their association with the erucic acid trait has been studied. We have also studied the genic and allelic divergence of *FAE1* genes by using diverse germplasm of mustard and other *Brassica* species to discern the wider applicability of the identified SNPs for marker-assisted manipulation of the erucic acid trait in *Brassica* species.

Materials and methods

Plant material and mapping populations

The two *B. juncea* genotypes used for the isolation of the *FAE1* genes were Varuna (high erucic acid type containing ~47% erucic acid) and Heera (free from erucic acid). Mapping and tagging of the erucic acid trait was done by using two different segregating populations. One was a mapping population of 123 doubled haploid (DH) lines derived from F_1 of a cross between Varuna and Heera, which was earlier used in the laboratory for construction of a *B. juncea* map (Pradhan et al. 2003). The second population, comprised of 40 BC_5F_2 plants segregating for erucic acid, was derived from a cross of Varuna×Heera, where Varuna was the recurrent parent. This BC_5F_2 population was derived from a single BC_4F_2 zero erucic acid plant. The different oil-yielding *Brassica* species used in the present study were maintained by selfing for several generations.

Cloning and sequencing of *FAE1* gene

DNA was isolated from the mature leaves following Rogers and Bendich (1994). The two consensus primers designed for amplification of the full-length *FAE1* gene from both Varuna and Heera were based on the *FAE1* sequences of *Arabidopsis* (James et al. 1995; accession number U29142) and rapeseed (Barret et al. 1998; accession number U50771). The forward and reverse primers used for the amplifications were 5′ ATGACGTCCATTAACGTAAAGCTCC 3′ and 5′ ATTAGGACCGACCGTTTTGGACA 3′, respectively. PCR reactions were performed in a 50-µl final volume containing 200 ng of genomic DNA, 200 µM dNTPs, 20 pmole of each primer, 1 unit of Titanium *Taq* polymerase (Clontech) and 1× Titanium *Taq* polymerase buffer with the following reaction conditions: 95°C for 3 min, followed by 30 cycles with 95°C for 30 s and 68°C for 2.5 min and final extension 68°C for 3 min. The amplified fragments were resolved in 1% agarose gel, eluted and purified using GFX PCR DNA gel band purification kit (Amersham Pharmacia) and were cloned in PCR script SK^+ (Stratagene). Presence of the insert was ascertained by restriction analysis with *Hin*dIII enzyme. To avoid PCR-based mutations, cloning was done from three independent amplifications from both Varuna and Heera DNA. Four positive clones from each amplification product were sequenced on an ABI 310 sequencer (Applied Biosystems). Sequences were analysed using software DNAstar. SNPs were detected by aligning the sequences of Varuna and Heera.

QTL mapping of erucic acid trait and linkage analysis by SNP genotyping

Erucic acid content was determined by GC (Perkin Elmer) either using the seeds or single cotyledons of the seedlings following the procedure developed by Thies (1971). For erucic acid determination of BC_5F_2 seeds, the seeds were germinated on filter paper in dark for 48 h and one of the cotyledons was removed for erucic acid analysis by GC. The remaining cotyledon with embryo was planted in the field, and the DNA was isolated from the leaves of randomly selected 40 plants. QTL detection of the erucic acid trait was done by interval mapping using the programme MapQTL version 3.0 (Van Ooijen and Malipaard 1996). SNP genotyping was done by single nucleotide primer extension method (SNuPE) in the ABI 310 sequencer using SNaPshot multiplex kit (Applied Biosystems). The genotypic data of the mapping popu-

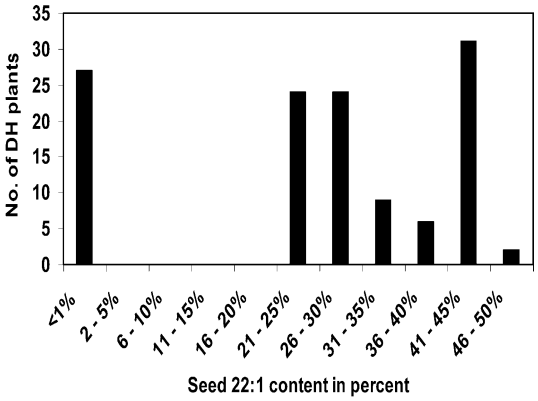

Fig. 1 Frequency distribution of seed erucic acid content (C22:1) of 123 double haploid (DH) mapping population derived from a Varuna × Heera cross

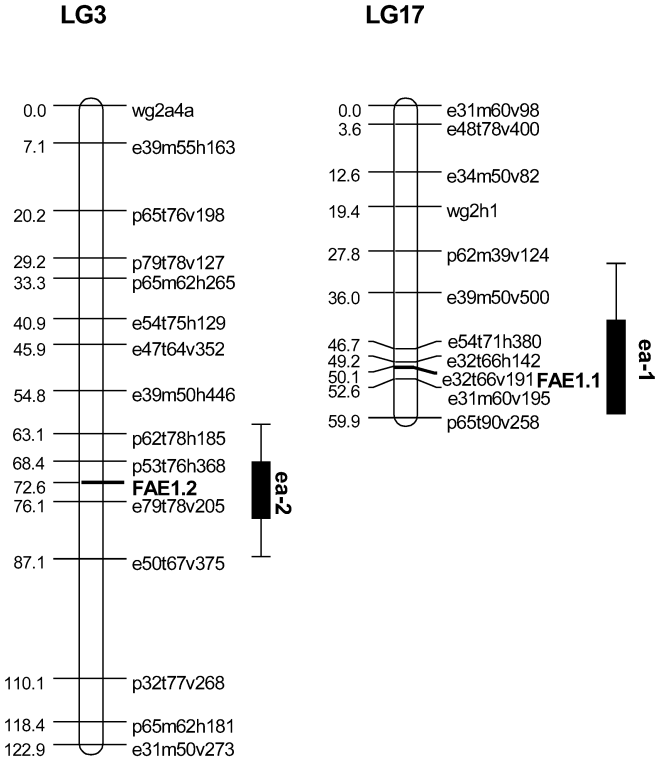

Fig. 2 The position of two QTLs, *ea-1* and *ea-2* (*black rectangular boxes*), controlling the seed erucic acid content on LG 17 and LG 3, respectively, of *Brassica juncea* map. The *FAE1.1* and *FAE1.2* also mapped to LG17 and LG 3, coinciding with *ea-1* and *ea-2* QTLs, respectively

lation for the *FAE1* genes were subjected to linkage analysis using the program JoinMap version 2.0 (Stam 1993; Stam and Van Ooijen 1996) as described by Pradhan et al. (2003).

Results

QTL mapping of loci controlling erucic acid content

The 123 DH lines earlier used for the construction of a linkage map in *B. juncea* (Pradhan et al. 2003) were used for detecting QTL(s) for erucic acid variations. The phenotypic distribution of erucic acid content in 123 DH lines indicated that 27 lines had erucic acid content less than 1%, while the remaining lines had more than 21% erucic acid. Although there were four distinct classes of phenotypes (<1%, 21–25%, 26–30% and 41–45%), the distribution showed overlap among the high erucic acid phenotypes (Fig. 1). However, the low erucic acid class (<1%) was well defined and the segregation revealed a perfect fit to the expected 3:1 ratio (high:low). The framework map of *B. juncea* constructed with 199 informative markers (Pradhan et al. 2003) was used for QTL mapping of the erucic acid trait. Two linkage groups (LGs), LG 17 and LG 3, detected one QTL each for the erucic acid trait. In LG 17, one erucic acid QTL (*ea-1*) was detected at a peak LOD score of 20.5, explaining approximately 60% of the total phenotypic variance. The second QTL (*ea-2*) in the LG 3 was detected at a peak LOD score of 10.2 that explained approximately 38% of the total phenotypic variance (Fig. 2). Analysis showed that the two loci have unequal contribution to erucic acid level in *B. juncea*.

Sequence divergence in the two *FAE1* loci

The PCR amplification of *FAE1* gene revealed the presence of a single band of around 1.5 kb in the high erucic acid mustard line Varuna. The amplified products were cloned and checked for the presence of *FAE1* gene by digesting the plasmids with *Hin*dIII. Upon digestion, four types of fallouts (as opposed to the two types that could have been expected, depending upon the orientation of the inserts) were obtained (data not shown). On sequencing all these four types of clones, we identified two *FAE1* genes (designated as *FAE1.1* and *FAE1.2*). The two *FAE1* genes differed from each other in nucleotide sequences at 67 positions. There was loss of a HindIII site in the *FAE1.1* gene at position 1144 and at position 1415 in *FAE1.2*. Both *FAE1.1* (accession number AJ558197) and *FAE1.2* (accession number AJ558198) were 1,521-nucleotides long and showed 99.5% and 93.8% sequence homology, respectively, with the *B. napus FAE1* gene (Barret et al. 1998). Isolation and characterization of *FAE1* gene from low erucic acid mustard line Heera also revealed the presence of two genes (*FAE1.1* and *FAE1.2*) of the same length as was observed in case of Varuna.

SNP detection in the two *FAE1* genes between high and low erucic acid mustard

Sequence alignment of *FAE1.1* gene between Varuna and Heera detected four SNPs due to transition type base substitutions at positions 591 (G→A), 735 (C→T), 968

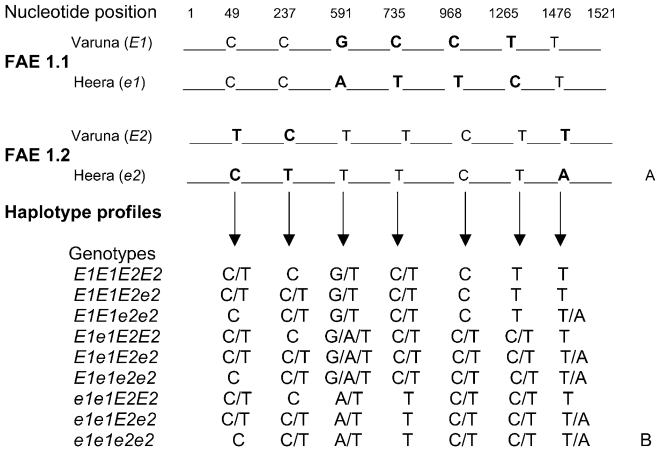

Fig. 3 Position of seven SNPs in two *FAE1* genes (**A**) and the haplotype profiles of nine genotypes expected from segregation of two independent loci (**B**)

(C→T) and 1265 (T→C) (Fig. 3A), of which the SNPs at positions 968 and 1265 resulted from replacement polymorphism. Three substitution SNPs were detected in the *FAE1.2* at position 49 (T→C) and 237 (C→T) that were transitional types and a transversional type at position 1476 (T→A) (Fig. 3A). Of these three SNPs, the SNP at position 49 resulted from replacement polymorphism. Assuming *E1* as wild (Varuna) and *e1* as mutant (Heera) haplotype based on 4 SNPs of *FAE1.1* and *E2* as wild (Varuna) and *e2* as mutant (Heera) haplotype based on 3 SNPs of *FAE1.2*, the expected SNP profiles of nine genotypes possible between the two unlinked genes have been shown in Fig. 3B.

SNP genotyping and mapping of *FAE1* genes

The 123 DH lines earlier used for QTL analysis of erucic acid trait were used for mapping the two *FAE1* genes. Of the 123 DH lines genotyped for four SNPs (591 $_{G\rightarrow A}$, 735 $_{C\rightarrow T}$, 968 $_{C\rightarrow T}$ and 1265 $_{T\rightarrow C}$) of *FAE1.1*, 68 were of *E1* type with a haplotype structure of G/T - C/T - C - T and 55 of *e1* type with a haplotype structure of A/T -T - T/C - C/T (Fig. 3B). On the other hand, genotyping with three SNPs (49 $_{T\rightarrow C}$, 237 $_{C\rightarrow T}$ and 1476 $_{T\rightarrow A}$) of *FAE1.2* detected 67 DH lines as *E2* type with a haplotype structure of T/C - C - T, and 56 as *e2* type with a haplotype structure of C - T/C - A/T, respectively (Fig. 3B). Both the genes showed 1:1 segregation for the two parental alleles ($P<0.05$). On mapping, *FAE1.1* mapped to LG 17 at a position coinciding with the highest LOD value (20.5) of *ea-1* QTL, and *FAE1.2* mapped to LG 3 at a position coinciding with highest LOD value (10.2) of *ea-2* QTL in *B. juncea* map (Pradhan et al. 2003). The map positions of these two *FAE1* genes along with two erucic acid QTLs have been shown in Fig. 2.

Table 1 Mean seed erucic acid content of different genotypic classes from F_1DH and BC_5F_2 segregating populations. Genotyping of different individuals in these two populations was done on the basis of seven single nucleotide polymorphisms (SNPs) of the two *FAE1* genes. The genotype of different individuals was determined on the basis of the observed haplotype profiles as shown in Fig. 3B

Genotypes	Number of individuals	Erucic acid (%) Mean±SD
F_1 DH		
E1E1E2E2	39	42.03±2.32
E1E1e2e2	29	29.64±1.74
e1e1E2E2	28	23.86±1.55
e1e1e2e2	27	0.24±0.39
BC_5F_2		
E1E1E2E2	4	46.16±0.94
E1E1E2e2	4	40.17±0.79
E1e1E2E2	4	38.84±1.06
E1E1e2e2	5	28.85±1.03
E1e1E2e2	6	30.10±0.94
e1e1E2E2	3	25.04±0.83
E1e1e2e2	5	18.17±1.04
e1e1E2e2	5	14.37±0.88
e1e1e2e2	4	0.14±0.17

Association of *FAE1* SNPs with C22:1 content

The association of two *FAE1* genes with C22:1 content was initially studied in the DH mapping population. The DH lines were classified into four genotypic classes as *E1E1E2E2, E1E1e2e2*, *e1e1E2E2* and *e1e1e2e2*, on the basis of the observed SNP profiles of the two *FAE1* genes (Fig. 3B). Of the 123 plants, 27 were of *e1e1e2e2* genotype, 28 of *e1e1E2E2* genotype, 29 of *E1E1e2e2* genotype and 39 were of *E1E1E2E2* genotype. Association of different genotypes with erucic acid content indicated that all the *e1e1e2e2* plants had an erucic acid content less than 1% with an average of 0.24%, and the plants genotyped as *E1E1E2E2* had the highest erucic acid content with an average of 42.03%. The other two genotypic classes, *e1e1E2E2* and *E1E1e2e2*, had an intermediate erucic acid content with an average of 23.86% and 29.64%, respectively (Table 1).

The association of the two *FAE1* genes with erucic acid content was further studied by genotyping a segregating BC_5F_2 progeny of 40 individuals derived from a cross Varuna × Heera, with Varuna as recurrent parent. Genotyping of these 40 individuals by seven SNPs revealed identification of all nine genotypes on the basis of the observed haplotype profiles as shown in Fig. 3B. Association of genotypes with the erucic acid phenotype clearly showed that *e1e1e2e2* genotypes identified zero erucic types, while *E1E1E2E2* genotypes identified only highest erucic-type individuals. Depending on the number of wild-type alleles present, additive gene effect was observed on the erucic acid phenotype and the heterozygotes, *E1e1E2e2*, were found to be intermediate in phenotype (Table 1). The contribution of the *E1* allele was observed to be more than that of the *E2* allele as was detected by QTL analysis.

Table 2 The haplotype profiles of different lines of three oil-yielding *Brassica* species with variable seed erucic acid content on genotyping with seven SNPs of two *FAE1* genes of *B. juncea*

Lines	C22:1 content (%)	SNPs						
		FAE1.1				*FAE1.2*		
		591 G→A	735 C→T	968 C→T	1265 T→C	49 T→C	237 C→T	1476 T→A
B. juncea								
Pusa bold	46.73	G/T	C/T	C	T	C/T	C	T
Varuna	47.30	G/T	C/T	C	T	C/T	C	T
Donskaja IV	20.54	A/T	T	C/T	C/T	C/T	C	T
Cutlass	19.68	A/T	T	C/T	C/T	C/T	C	T
Zem 1	0.0	A/T	T	C/T	C/T	C	C/T	A/T
Heera	0.0	A/T	T	C/T	C/T	C	C/T	A/T
J 301	0.0	A/T	T	C/T	C/T	C	C/T	A/T
B. rapa								
Pusa gold	42.82	G	C	C	T	C	C	T
Candle	0.64	A	T	T	T	C	C	T
B. napus								
GSL 1	45.89	G/T	C/T	C	T	C	C	T
NU 98	0.0	A/T	T	C/T	T	C	C	T

Haplotype tagging of *FAE1* SNPs in different *Brassica* species

The association of the discerned SNPs with the erucic acid trait was verified by genotyping contrasting germplasm from three important oil-yielding *Brassica* species: *B. juncea*, *B. napus* and *B. rapa* (Table 2). In the case of *B. juncea*, it was observed that all the seven SNPs could be used to distinguish low erucic types from high erucic types. The low erucic types (Zem 1, Heera and J 301) detected SNP profiles of the low alleles *e1e2* for both the *FAE1* genes, while the Indian types (Varuna and Pusa bold) containing ~50% erucic acid detected SNP profiles of the high alleles *E1E2* for both the *FAE1* genes. On the other hand, the east European types (Donskaja IV and Cutlass) containing <25% erucic acid detected SNP profiles of the low allele *e1* for *FAE1.1* and the high *E2* for *FAE1.2* (Table 2).

Though all the seven SNPs of two *FAE1* genes were used for genotyping the *B. napus* and *B rapa* lines, the SNPs specific to the *FAE1.1* gene were found to show association between haplotype and erucic acid content. The low erucic acid *B. napus* and *B. rapa* lines detected *e1* allele, while the high erucic acid lines detected the *E1* allele, except for the fourth SNP (1265 $_{T\rightarrow C}$) that did not show polymorphism between high and low types (Table 2).

Discussion

The main aim of the present study was to develop markers for tagging the erucic acid trait so that the markers could be effectively used in the breeding programme for marker-assisted transfer of low- and high erucic acid traits in *B. juncea*. We followed the candidate gene approach using *FAE1* sequences, as this gene encodes a KCS that is involved in the elongation of oleic acid (18:1) to erucic acid (22:1). Isolation and characterization of full-length *FAE1* sequences from *B. juncea* revealed identification of two genes (*FAE1.1* and *FAE1.2*). Alleles encoding the two *FAE1* genes of the high erucic acid mustard line Varuna could be distinguished from the alleles of the low erucic acid mustard line Heera on the basis of the haplotype structure of four SNPs in *FAE1.1* and three SNPs in *FAE1.2* in the coding regions. The high sequence homology of *FAE1.1* with *B. napus FAE1* and subsequent association of *FAE1.1* SNPs with erucic acid content of *B. rapa* established the fact that the *FAE1.1* is specific to *B. rapa*, whereas *FAE1.2* is specific to *B. nigra* in the present study.

DNA markers, particularly anonymous markers such as RFLP, RAPD, AFLP and SSR, have been widely used for genome mapping and tagging of many agronomically important traits in plants (for a review, see Brar 2002). SNPs, on the other hand, have been found to be more abundant and can be used for germplasm fingerprinting, marker-assisted breeding and can potentially be used to create high-density genetic maps. Moreover, SNPs in the coding region (cSNPs) can be used as the most reliable markers in marker-aided selection and some of these may also have functional significance if the resulting amino acid change causes the altered phenotype.

Among the different methods of SNP genotyping (Syvänen 2001), allele-specific PCR (See et al. 2000; Tanhuanpää et al. 1998; Tanhuanpää and Schulman 2002) and the TaqMan allelic discrimination method (Meksem et al. 2001) have been used in gene mapping and marker-assisted breeding in plants. Allele-specific PCRs, in general, are gel-based and may not be applicable for high-throughput genotyping. Although, Meksem et al. (2001) have used the TaqMan allele discrimination method for high-throughput genotyping of a marker tightly linked to a gene imparting resistance to soybean cyst nematode, the different software used for allele discrimination on the basis of fluorescence intensity are

specifically designed for situations in which a single locus encodes for the trait. Hence, the software may be inadequate for accurate genotyping of a gene present in more than one copy.

We used the SNuPE method of SNP genotyping (Chen et al. 1999), as the advantages of this method are the speed and the accuracy of SNP detection, the low cost and ability to genotype many targets rapidly. It was also observed that this method is free from any ambiguities for SNP genotyping of a duplicate gene. For example, the SNP 591 $_{G\rightarrow A}$ of *FAE1.1* showed the expected G/T and A/T fluorescent peaks for allele G and A, respectively, for *FAE1.1* that contains T at the same position in *FAE1.2*. Recently, this method has also been used for high-throughput SNP genotyping in *Zea mays* (Batley et al. 2003). We did not observe any mis-incorporation of a base in any of the seven SNPs detected by SNuPE method as observed by Batley et al. (2003), since all the SNP primers were designed with an annealing temperature of around 50°C. We have also observed that by varying the length of the SNP primers it was possible to genotype more than one SNP in a single PCR reaction (multiplexing). In the present experiment, we did multiplexing of two SNP primers each with SNPs 735 $_{C\rightarrow T}$ and 968 $_{C\rightarrow T}$ of *FAE1.1* and with SNPs 49 $_{T\rightarrow C}$ and 237 $_{C\rightarrow T}$ of *FAE1.2*, respectively.

The unambiguous identification of low- and high erucic acid types in two different types of segregating populations and representative samples of *B. juncea* germplasm from the two diverse gene pools (Srivastava et al. 2001) revealed that the SNP markers identified in this study will have a wide application in marker-assisted breeding of the erucic acid trait in *B. juncea*. One of the reasons for this wider applicability could be that most of the present-day low erucic acid *B. juncea* lines are primarily derived from either Zem 1 or its sister line, Zem 2 (Bhat et al. 2002; Potts et al. 1999; Sodhi et al. 2002). In comparison, the observation of variations in the *FAE1.1* locus between *B. napus* detecting a new SNP at position 1020 $_{A\rightarrow G}$ (Fourmann et al. 1998) and *B. juncea* detecting a new SNP at position 1265 $_{T\rightarrow C}$ (the present study) and variations in *FAE1.2* locus that identified two different indels in two low erucic acid types in *B. napus* (Fourmann et al. 1998), indicates that many different low erucic acid donor sources might have been used by the breeders for development of low erucic acid cultivars in *B. napus*. However, the observation of a common SNP at position 968 $_{C\rightarrow T}$ in *FAE1.1* of *B. napus* (Fourmann et al. 1998) and in *B. juncea*, *B. napus* and *B. rapa* (in the present study) that results in a replacement polymorphism shows that this SNP could be one of the important polymorphisms for distinguishing the low from the high erucic types in *Brassica* species.

The accurate allele-specific tagging of the erucic acid trait with the SNP markers developed in the present study shows that these markers could be effectively used for marker-assisted transfer of the low erucic acid trait in *B. juncea*. The SNuPE method can be used in a high-throughput manner for genotyping the population segregating for a duplicate gene. However, one would need more than one SNP from each of *FAE1.1* and *FAE1.2* to identify all nine genotypes that are expected for a two-gene trait. Hence, the tagging information generated here will be utilized for precise transfer and diversification of low erucic acid traits to Indian *B. juncea* cultivars by backcross breeding.

Acknowledgements This work was supported by the National Dairy Development Board (NDDB), India and its subsidiary, Dhara Vegetable Oil and Food Company Ltd (DOFCO). SNP analysis was done on sequencer provided by NMITLI Programme of Council of Scientific and Industrial Research, Government of India.

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