Mapping and tagging of agrnomically important genes in *Brassica juncea*

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Abstract

Brassica juncea (mustard) is a major oilseed crop of the Indian sub-continent. The two major breeding objectives in this crop are - increase in productivity and improvement in oil and meal quality. Two major gene pools have been identified in mustard – the Indian gene pool and the east European pool. The east European gene pool contains many desirable traits such as low erucic acid, low glucosinolates, yellow seed coat color, resistance to white rust and yield-enhancement component traits. However, the east European varieties are ill adapted to Indian agro-climatic conditions. Transfer of desirable genes from east European gene pool to Indian gene pool would require precise transfer of these genes/QTL through marker assisted breeding To tag and map the agronomically important genes, a high-density molecular map consisting of 1432 markers has been developed using AFLP, RFLP and SSR markers. On the basis of the comparative mapping of common RFLP and SSR markers, the 18 linkage groups of *B juncea* map have been assigned to 10 LGs of A genome (*B. rapa*) and 8 LGs of B genome (*B. nigra*). Using this map, two gene loci controlling erucic acid synthesis have been mapped by candidate gene approach through identification of SNPs in FAE gene. The two gene loci controlling seed coat color have been tagged by SSR markers. The markers developed for these two traits are now being used in ourbreeding programme. At present molecular mapping of genes involved in glucosinolate biosynthesis pathway is in progress. Six *GSL-ELON* (four belonging to 'A' genome and two belonging to 'B' genome) and four *GSL-ALK* (two each from 'A' and 'B' genome) have been mapped in *B. juncea* through either SNPs or intron spanning length polymorphism markers. Validation of these markers with trait variation is in progress for the identification of the markers to be used in the marker assisted breeding.

Key words: *Brassica juncea*, Glucosinolates, *GSL-ELON*, *GSL-ALK*

Introduction

Brassica juncea (Indian mustard) is the major oil-yielding *Brassica* species of India and is grown in around five million hectares in the north-western part of the country. The conventional plant breeding has contributed significantly in the development of many high yielding varieties in *B. juncea* by exploiting the genetic variability that existed among the adapted pool of elite germplasm. As a result, it has resulted in the narrowing down of the genetic base among the Indian cultivars. Apart from improvement in productivity, another important objective in *B. juncea* breeding is the development of '00' cutivars as all the cultivars grown in India are high in erucic acid (~50%) and glucosinolates (>100micromoles/g of defatted meal). Genetic improvements could be possible through incorporation of desirable genes either from the exotic germplasm or from the wild relatives of *B. juncea*. *B. juncea* germplasm could broadly be classified into two distinct gene pools on the basis of genetic diversity- the exotic east European types consisting of lines belonging to eastern Europe, Canada and Australia and the Indian types (Pradhan et al 1993; Srivastava et al 2001). The east European germplasm contains many desirable traits such as resistance to white rust (*Albugo candida*), low erucic and low glucosinolate, yellow seed coat color and many yield enhancing components. As the exotic germplasm is unadaptive to Indian agro-climatic conditions, attempts to use these directly in the pedigree breeding programmes have been unsuccessful. One of the ways to circumvent this problem would be to use DNA markers tightly linked to the genes of interest for precision breeding. To map and tag the genes involved with some of the important agronomic traits, we have constructed a high density molecular map of *B. juncea* consisting of 1432 markers consisting of AFLP, RFLP and SSR markers (Pradhan et al 2003) using a doubled haploid population derived from a cross between Varuna (belonging to Indian gene pool) and Heera (belonging to eastern European gene pool).

Molecular mapping and tagging of erucic acid trait in *B. juncea* was carried out by candidate gene approach. Two QTLs responsible for the variation in seed erucic acid content were assigned to two linkage groups of the *B. juncea* map. Amplification and sequencing of Fatty acid elongase 1 (FAE 1) gene, which is reported to be involved in the elongation of 18:1 to 22:1, identified two FAE1 genes in both high and low erucic acid mustard lines. On alignment, 4 SNPs in gene 1 (FAE1.1) and 3 SNPs in the gene 2 (FAE1.2) of the two FAE1 genes were identified. Using SNuPE method of SNP genotyping these two genes were mapped to two independent loci which co-segregated with two QTLs (LG17 of A genome and LG3 of B genome) governing the trait variation (Gupta et al 2004).

Yellow seed coat color in *B. juncea* is controlled by two genes. Microsatellite markers were used to map and tag these two independent loci. Using bulk segregant analysis, three microsatellite markers (Ra2-A11, Na10-A08 and Ni4-F11) were identified which showed strong association with seed coat color. Based on the marker genotyping data, the two seed coat color genes were placed with markers Ra2-A11 and Na10-A08 on LG1 of A genome and marker Ni4-F11 on LG2 of B genome in

the linkage map of *B. juncea* (Padmaja et al 2005).

The SNP markers for the erucic acid trait and the microsatellite markers for the seed coat color are being used for marker assisted selection in our back-cross breeding programme. In this paper we report mapping and tagging of the genes involved in the glucosinolate biosynthetic pathway.

Materials and Methods

Two candidate genes viz., GSL-*ELONG* (a member of MAM gene family) and GSL-*ALK* (2-oxoglutarate-dependent dioxigenase) which are involved in aliphatic GSL biosynthetic pathway were taken up for tagging genes involved in glucosinolate biosynthesis through candidate gene approach. On the basis of the reported sequences of these genes from Arabidopsis and other *Brassica* species, consensus degenerate primers were synthesized. These primers were used to amplify these genes from the low and high glucosinolate parents. The amplified fragments were either sequenced directly or were first cloned and then sequenced. Sequences were analysed using software DNAstar. SNPs or insertion/deletions were identified by aligning the sequences of Varuna and Heera.

Results and discussion

Amplification of GSL-*ELONG* gene using the degenerate primers in the two diploid progenitor parents of *B. juncea* resulted in four different gene sequences from *B. rapa* and two from *B. nigra*. Based on the sequence information obtained for each gene in the diploid progenitor parents, the gene specific primers were designed and used to amplify the corresponding GSL-*ELONG* genes from *B. juncea* parents Varuna (high GSL) and Heera (low GSL). The sequence information from the high and low GSL parents was aligned to detect SNPs or Indel so that it could be used for mapping these genes. Out of the six *B. juncea* GSL-*ELONG* genes, two *B. rapa* specific genes BjGSL-*ELONG*-R4 and BjGSL-*ELONG*-R5 mapped at the same position in LG6 of A genome while the remaining two *B. rapa* specific genes BjGSL-*ELONG*-R1 and BjGSL-*ELONG*-R3 mapped to LG10 and LG16 of A genome, respectively. The two *B. nigra* specific GSL-*ELON* genes, BjGSL-*ELON*-N1 and BjGSL-*ELON*-N2, were mapped to LG11 and LG12 of B genome (Table 1). Mapping of BjGSL-*ELONG*-R4 and R5 at the same position in LG 6 indicates that these genes might have resulted due to gene duplication and diverged from each other during the course of evolution.

Table 1. Type of polymorphism used to map six GSL-*ELON* genes and their positions in *B. juncea* map of Pradhan et al (2003)

Genes	Type of polymorphism	Position of the polymorphism	Mapped to LG	Position in LG (cM)
GSL-*ELON*-R1	In/del	Intron 1	LG10 (A)	84.2
GSL-*ELON*-R3	SNP	Exon 1	LG16 (A)	37.0
GSL-*ELON*-R4	In/del	Intron 4	LG6 (A)	28.4
GSL-*ELON*-R5*	In/del	Intron 5	LG6 (A)	28.4
GSL-*ELON*-N1	SNP	3′ flank	LG11 (B)	89.3
GSL-*ELON*-N2	SNP	3′ flank	LG12 (B)	117.5

* GSL-*ELON*-R5 is a truncated gene

Use of GSL-*ALK* gene specific primers on two diploid progenitor species resulted in amplification of two genes each from *B. rapa* and *B. nigra* parents. Amplification of these four genes in *B. juncea* revealed polymorphism in three genes between Varuna and Heera. These three genes were mapped to LG6 (GSL-*ALK*-R1) and LG1(GSL-*ALK*-R2) of A genome and LG5 (GSL-*ALK*-N1) of B genome of *B. juncea* map (Table 2).

Table 1. Type of polymorphism used to map three GSL-*ALK* genes and their positions in *B. juncea* map of Pradhan et al (2003)

Genes	Type of polymorphism	Position of the polymorphism	Mapped to LG	Position in LG (cM)
GSL-*ALK*-R1	SNP	5′ flank	LG6 (A)	64.2
GSL-*ALK*-R2	In/del	5′ flank	LG1 (A)	0.6
GSL-*ALK*-N1	SNP	5′ flank	LG5 (B)	80.8
GSL-*ALK*-N2	No polymorphism	-	-	-

Recently, the gene sequence of GSL-*PRO* gene involved in the biosynthesis of aliphatic glucosinolates has been reported. Efforts are on to map this gene to *B. juncea* map and also to validate the genes with trait variations through the use of different mapping populations. It will lead to development of efficient co-dominant markers for marker-assisted transfer of low glucosinolate trait to Indian *B. juncea* lines.

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