Addition of Restriction Fragment Length Polymorphism Markers to the Genetic Linkage Map of *Brassica rapa* L. (syn. *campestris*)

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Genetic linkage analysis of 151 restriction fragment length polymorphism (RFLP) loci, that included eight new loci, detected by the six probes in the present study, and four trait loci including seed colour, leaf pubescence, resistance to white rust caused by *Albugo candida* race-2 (*AC-2*) and race-7 (*AC-7*) employing the MAPMAKER/EXP 3.0 programme led to the development of 10 linkage groups (LGs) spanning over 44.4 centiMorgan (cM) to 130.4 cM containing 9 to 22 loci and two short LGs with two or three marker loci in *Brassica rapa*. The enriched map covers 993.1 cM of *B. rapa* genome with an average marker interval of 6.41. Eight new RFLP loci occupied new map positions on five linkage groups, LG 2, 3, 6, 8 and 9. Addition of these RFLP loci led to appreciable changes in the corresponding linkage groups and resulted in an increase of the total map length by 102.8 cM and of the marker interval by 0.35 cM. Interval mapping by using the computer programme MAPMAKER/QTL 1.1 for scanning the genetic map led to the detection of one major quantitative trait locus (QTL) in LG 4 and one minor QTL in LG 8 governing resistance to *AC-7*. Both QTLs contributed 7.89 to the interaction phenotype (IP) score with 96.3% genetic variation. The multi-locus model suggested additive gene action with 96.8% genetic variation.

Key words: Brassica rapa, RFLP, QTL, Linkage Map

Introduction

Brassica rapa L. syn. *campestris* is an important oilseed *Brassica* species having somatic chromosome complement of $2n = 2x = 20$, and it comprises the 'AA' genome. Molecular markers are used as reliable genetic land marks for genome analysis, including the construction of linkage maps, mapping of qualitative and quantitative traits, delineation of genome organization, and depiction of genome homology in several crop species. A high degree of natural polymorphisms detected by molecular genetic markers (Figdore *et al.*, 1988; Song *et al.*, 1990), appreciable gene homology, and conservation of the gene repertoire in *Brassica rapa* and its related genus *Arabidopsis thaliana* have stimulated active research on construction of genetic linkage maps, gene mapping and in-depth studies on genome organization and molecular phylogeny.

A handful of simply inherited trait loci (SITL) have been mapped in *B. rapa*, and several quantitative trait loci (QTL) have also been detected. These include SITL for erucic acid, seed colour, leaf pubescence, and resistance to white rust caused by the fungus *Albugo candida* (Teutonico and Osborn, 1994; Kole *et al*., 1996, 1997a, 2002a). Several QTL controlling vernalization requirement, flowering time, winter survival, freezing tolerance, degree of white rust resistance, and contents of palmitic, oleic and linolenic acid have been detected (Tanhuanpaa *et al*., 1995a, b, 1996; Teutonico and Osborn, 1995; Teutonico *et al.*, 1995; Osborn *et al*., 1997; Kole *et al*., 2001, 2002a, b). A major QTL controlling vernalization responsive flowering time has already been Mendelized (Kole *et al.,* 1997b, 2001).

Construction of genetic linkage maps relies on the choice of parental lines, segregating mapping population, and markers to reveal parental polymorphism. Immortal mapping population like

recombinant inbred lines (RILs) have several advantages over segregating mapping populations derived from single meiotic events, such as F_2 and back cross populations (Burr *et al.*, 1988). Recombinant inbred lines are immortal and facilitate the enrichment of existing map, saturation mapping, positional gene cloning, quantitative trait mapping, and marker-assisted selection. Several genetic linkage maps were developed in *B. rapa* using immortal and mortal mapping population (Song *et al.*, 1991; Teutonico and Osborn, 1994; Kole *et al.*, 1997a; Nozaki *et al.*, 1997; Zhang *et al.*, 2000; Yu *et al.*, 2003; Wang *et al.*, 2004; Kim *et al.*, 2006; Suwabe *et al.*, 2008). However, Kole *et al.* (1997a) constructed a map of *B. rapa* using an immortal population of 87 RILs. This map consisted of 143 restriction fragment length polymorphism (RFLP) loci and three SITL controlling seed colour, leaf pubescence and resistance to *A. candida* race-2 (*AC-2*) distributed on 10 linkage groups (LGs) and two short groups spanning over 890 cM (centiMorgan) with an average marker interval of 6 cM. Later Kole *et al.* (2002a) added another trait locus conferring resistance to *A. candida* race 7 (*AC-7*) in this map. The same map has been utilized to map several QTLs controlling white rust resistance, flowering time, vernalization requirement, and winter survival (Osborn *et al.*, 1997; Kole *et al.*, 2001, 2002a, b). Hence, addition of markers to this map based on permanent mapping population not only provide saturation of the existing map but also facilitate the detection of new QTL and markers linked to SITL.

In the present study, we used a RIL-based population, marker data (Kole *et al.*, 1997a) for addition of new marker loci to the existing genetic linkage map of *B. rapa*. Further the map was scanned employing interval mapping using trait data for white rust resistance.

Material and Methods

Mapping population

A recombinant inbred (RI) population consisting of 87 F_7 families developed by single-plant-descent of individual F_2 plants derived from a cross between an winter turnip rape variety, *PER*, and a spring sarson variety, *R500*, was used as the mapping population (Kole *et al.*, 1997a). These two parents differ contrastingly with regard to their response to several characters including white rust resistance, vernalization requirement, flowering time, winter survival, freezing tolerance, and self-incompatibility.

RFLP analysis

DNA was extracted from leaf tissues following the cetyl trimethyl ammonium bromide (CTAB) procedure as described by Kidwell and Osborn (1992). 5 μg of DNA from each RIL digested with either *Hind*III or *Eco*RI were electrophoresed in 0.8% agarose gels. The gels were washed with 0.25 M HCl for 10 min at room temperature and rinsed thrice with doubled distilled water (DDH_2O). The gels were denatured with a solution containing 0.5 M NaOH and 1.5 M NaCl for 30 min at room temperature and washed thrice with DDH_2O. The gels were neutralized in a solution containing 0.5 M Tris (pH 7.5) and 1.5 M NaCl for 30 min at room temperature. Southern blots of these gels were prepared (Southern, 1975) by neutral transfer onto Magna NT nylon membranes (MSI, Westboro, MA, USA). DNA was fixed to the membranes by UV-cross-linking (UV stratalinker 2400, Stratagene, USA) on autosetting followed by baking for 2 h at 90 °C in a vacuum oven.

Six DNA probes were used in this study. These included four cDNA and two gDNA probes. The two genomic DNA probes were from two libraries: (1) a *pst*I genomic DNA library (*wg8e3* clone) from the *B. napus* cultivar Westar (Thormann *et al.*, 1994) and (2) an *Eco*RI genomic DNA library from the *B. campestris* cultivar Tobin (*tg3c1* clone) that was constructed and screened essentially as described for the Westar genomic DNA library. Probes from these libraries were screened by hybridization to blots containing DNA from parental cultivars digested with *Hind*III or *Eco*RI. The cDNA probes of four cloned genes included *SLG6*, *COR47*, *BNC24B*, and *GS-KB6*. The cloned genes *BNC24B*, *GS-KB6* and *COR47* were screened for detection of polymorphisms between the parental cultivars using *Hind*III or *Eco*RI (Kole *et al.*, 1997a). The cDNA probe of the cloned gene *SLG6* from *B. oleracea* was digested with *Hind*III to screen the parents.

The DNA probes were radio-labelled by random priming in the presence of ^{32}P-dCTP (Feinberg and Vogelstein, 1983) and hybridized to Southern blots. RFLP analysis was done following essentially Teutonico and Osborn (1994). However, the blots in the present study were hybridized

in rotating bottles at 60 ºC. The stringency of the final wash varied from 0.1× to 0.4× SSC, all at 60 ºC, depending on the homology of the probe with *B. campestris*, 0.2× to 0.3× for *A. thaliana* probes (*COR47*, *GS-KB6*), and 0.2× to 0.4× for *B. oleracea* (*SLG6*) and *B. napus* (*BNC24B*) probes. After the probed blots were exposed to X-ray films, the blots were treated with 0.2 M NaOH to remove the probe and then neutralized in 0.5 M Tris-HCl, 0.1× SSC, 0.1% SDS, pH 7.5.

Linkage analysis and map construction

Linkage analysis of 151 RFLP loci including the genotype data for *wg8e3*, *tg3c1*, *SLG6*, *COR47*, *BNC24B*, and *GS-KB6* was done using the MAPMAKER/EXP 3.0 programme (Lincoln *et al.*, 1992). A minimum likelihood of odd (LOD) threshold of 3.0 and a maximum recombination fraction (RF) of 0.4 were used for grouping the marker loci into potential linkage groups. The order and relative distance of the marker loci for each linkage group were determined using three-point and multi-point analyses. The final linkage orders were verified with the 'ripple' command. Map distances in centiMorgans (cM) were expressed in 'Haldane mapping function' (Haldane, 1919).

QTL analysis

Interval mapping analysis (Lander and Botstein, 1989) was employed to locate QTLs controlling white rust resistance. For this purpose, mean interaction phenotype (IP) scores over four replications for 77 RILs were used as the phenotypic trait data. The computer programme MAPMAKER/QTL 1.1 was used for scanning the genome. A LOD threshold of 3.0 was used at the first step to explore the regions containing any putative major QTL associated with resistance. Only additive effects were estimated because there were no heterozygous marker loci included in the analysis. Search for additional marker loci associated with resistance was performed by fixing the interval with highest LOD score and rescanning the genome (Lincoln *et al.*, 1992) using an LOD threshold of 2.0 above the base line. The multi-locus model was constructed following Zeng (1994). The confidential intervals of individual QTL was deduced by fixing the left marker of the identified interval and using the map function of the programme. However, the final confidential intervals from the joint model were identified by fixing the intervals with the left markers and using the map function of the programme. The QTL regions were drawn on the corresponding linkage maps in such a way that their length indicated the confidential interval and their breadth suggested their contribution towards expression of the traits.

Results

Linkage mapping and map enrichment

RFLP analysis using six probes detected 12 marker loci. Of these 4 RFLP loci were mapped previously (Kole *et al.*, 1997a). The genotypic data of the 87 RI lines for five loci (*BNC24Ba*, *GS-KB6a*, *GS-KB6b*, *COR47a*, *tg3c1*) were similar to those scored by Kole *et al.* (1997a). The RFLP locus for *tg3c1*, however, remained unlinked in the previous mapping endeavour. This led to the detection of seven new RFLP loci (*SLG6a*, *SLG6b*, *COR47b*, *COR47c*, *BNC24Bc*, *GS-KB6c*, *wg8e3*) and one candidate locus (*tg3c1*) for mapping endeavour for the present pursuit. Linkage analysis of 155 marker loci including 151 RFLP loci and four trait loci using the MAPMAKER/EXP 3.0 programme with a minimum LOD threshold of 3.0 and a maximum recombination fraction of 0.4 led to the development of 10 major linkage groups (LG 1–LG 10) and two short groups (LG-A and LG-B). The new eight RFLP loci occupied a position in five linkage groups, LG 2, LG 3, LG 6, LG 8 and LG 9 (Fig. 1). The enriched map covers 993.1 cM of the *B. rapa* genome with an average marker interval of 6.41 (Table I). Addition of these new RFLP loci led to appreciable changes in the five linkage groups; the change in map length varied from 1.1 to 66.4 cM and that in marker interval from –0.454 to +3.637 cM (Table II). The centiMorgan distances of the majority of marker intervals were true to the corresponding recombination fractions in most of the cases. However, for long intervals the distances expressed in Haldane mapping function were apparently too long. In LG 3, the interval between *wg6c6* and *ec3 g7* was 41.6 cM. But the corresponding RF value was only 0.282. Similarly, in LG 8, the distance between *ec5a7a* and *tg3c1* was 62.3 cM whereas the RF (0.356) was far below 0.4.

Linkage analysis in the present study detected two RFLP loci for the *SLG6* probe, one in LG 9 (*SLG6a*) and another in LG 2 (*SLG6b*). The

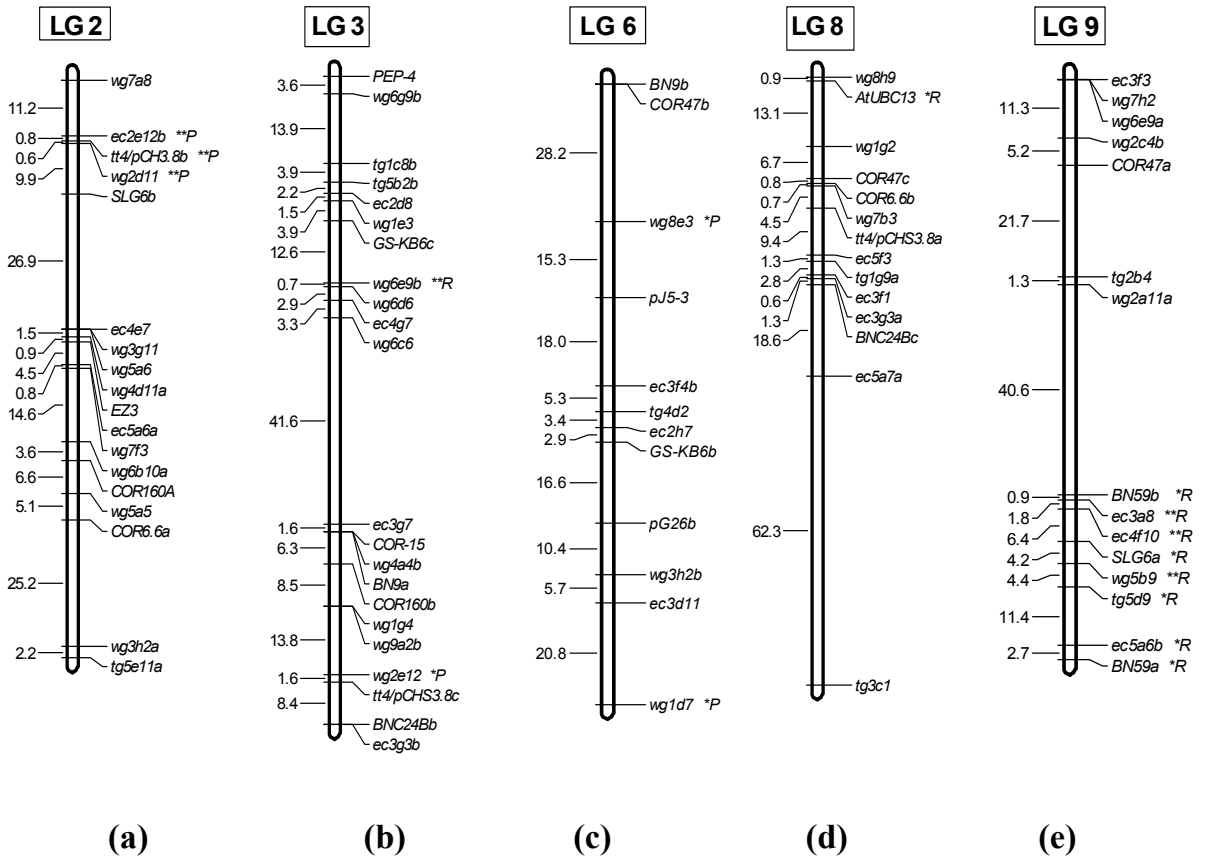

Fig. 1. RFLP linkage maps for five linkage groups containing (a) *SLG6b*, (b) *GS-KB6c*, (c) *COR47b*, *wg8e3*, (d) *COR47c*, *BNC24Bc*, *tg3c1*, and (e) *SLG6a* RFLP loci. Marker loci in italics on the right-hand side; distances in cM on the left-hand side; * and ** deviated significantly from 1:1 segregation ratio at $p < 0.5$ and $p < 0.01$, respectively; suffix of P and R indicate that P/P and R/R genotypes predominated.

Table I. Information on the ten major and two short linkage groups produced from linkage analysis of 151 RFLP loci and four trait loci in the RI population.

Group no.	Marker no.	Map length [cM]	Marker interval [cM]	Log-likelihood [LOD]	LG #[a]
1	13	55.4	4.261	–130.5	5
2	9	44.4	4.933	–97.56	10
3	14	123.1	8.792	–136.62	8
4 and 13	22	130.4	5.927	–205.74	3
5	12	126.7	10.558	–167.46	6
6 and 9	15	112.0	7.467	–160.75	9
7	21	92.5	4.404	–182.76	4
8	18	114.7	6.372	–173.87	2
10	15	86.0	5.733	–173.01	7
11	11	99.1	9.009	–114.01	1
12	3	8.8	2.933	–41.83	A
14	2	0.0	0.0	–0.0	B

[a] Corresponding to the nomenclature of Kole *et al.* (1997a).

Table II. Changes in map length, marker loci number, and marker interval for the linkage groups due to addition of new RFLP loci.

Parameter		LG 2	LG 3	LG 6	LG 8	LG 9	Total [5 LGs]	Whole map
Map length [cM]	Previous	102.4	128.3	105.9	56.7	110.9	504.2	890.3
	Present	114.7	130.4	126.7	123.1	112.0	606.9	993.1
	Change	12.3	2.1	20.8	66.4	1.1	102.7	102.8
Number of marker loci	Previous	17	21	10	11	14	73	147
	Present	18	22	12	14	15	81	155
	Change	1	1	2	3	1	8	8
Marker interval [cM]	Previous	6.02	6.11	10.59	5.16	7.92	6.91	6.06
	Present	6.37	5.93	10.56	8.79	7.47	7.49	6.41
	Change	+0.35	–0.18	–0.03	+3.64	–0.45	0.58	0.35

locus in LG 2 (*SLG6b*) was positioned 9.9 cM apart from *wg2 d11* (Fig. 1a). In LG 9, *SLG6a* was flanked by *ec4f10* and *wg5b9* occurring 6.4 and 4.2 cM apart, respectively (Fig. 1e). This locus, as described previously, exhibited distorted segregation skewed towards the *R500* parent. In fact all the eight marker loci including *SLG6a* in a cluster in this genomic region were skewed towards *R500*.

Three loci were detected by the probe *COR47* in three different linkage groups including LG 9 (*COR47a*), LG 6 (*COR47b*), and LG 8 (*COR47c*) (Figs. 1e, c, d). The locus in LG 9 was reported previously by Kole *et al.* (1997a) as *COR47* using the same RI population and Teutonico and Osborn (1994) as *COR47a* using an F_2 population of the same parentage. The second locus detected by Teutonico and Osborn (1994) as *COR47b* in the same linkage group (LG 9) was not found by Kole *et al.* (1997a). *COR47a* was closely linked to *wg2c4b* (5.2 cM) in LG 9. The locus *COR47b* co-segregated with *BN9b,* a locus detected by the cloned gene encoding stearoyl *ACP* desaturase from *B. napus* in LG 6. The *COR47c* locus was tightly linked to two loci (*COR6.6b*, 0.8 cM apart; *tt4/pCHS3.8a,* 6.0 cM apart) detected by two cloned genes from *Arabidopsis* in LG 8.

Two marker loci were detected for the probe *BNC24B* in two different linkage groups including LG 4 (*BNC24Ba*) and LG 8 (*BNC24Bc*). The first locus was previously detected by Kole *et al.* (1997a). *BNC24Ba* co-segregated with the locus *ec2f1c* and was closely flanked by *wg7e10* (1.5 cM) in LG 4 as reported previously. The new locus *BNC24Bc*, detected in LG 8 in the present study, was closely linked to *ec3 g3a* (1.3 cM) (Fig. 1d). In LG 3, the previous locus *BNC24Bb* co-segregated with *ec3 g3b* and was flanked (8.4 cM) by *tt4/pCHS3.8c*. However, the distance between *BNC24Bb* and *ec3 g3* was resolved for LG 8 and not for LG 3.

By RFLP analysis with the probe *GS-KB6* three loci in three different linkage groups including LG 7 (*GS-KB6a*), LG 6 (*GS-KB6b*), and LG 3 (*GS-KB6c*) were detected. The former two loci were reported previously by Kole *et al.* (1997a). *GS-KB6a*, *GS-KB6b*, and *GS-KB6c* were closely linked to *pC1b* (2.4 cM) in LG 7, *ec2 h7* (2.9 cM) in LG 6 and *wg1e3* (3.9 cM) in LG 3, respectively.

The marker *tg3c1* remained unlinked in the previous mapping by Kole *et al.* (1997a). However, in the present study it could be positioned at the distal end of LG 8, 62.3 cM apart from the locus *ec5a7a* (Fig. 1d). The marker locus *wg8e3* was not mapped previously in the RI population by Kole *et al.* (1997a). However, *wg8e3* locus was detected by Teutonico and Osborn (1994) in LG 9 in their F_2 map. In the present study this locus was detected in LG 6 (Fig. 1c) and was flanked by *BN9b* (28.2 cM) and *pJ5–3* (15.3 cM) detected by stress-related cloned genes from *B. napus* and *Arabidopsis*.

Detection of quantitative trait loci for AC-7

Primary scanning of the linkage groups consisting of 151 RFLP marker loci and four trait loci using IP scores of 77 RI lines as the phenotypic trait data was done with a LOD threshold of 3.0. This scanning resulted in two peaks. The first major QTL was located on LG 4 (Fig. 2b) between trait locus *PUB-1* and the RFLP marker locus *wg6c1a*. However, the peak of the QTL was located on the locus *AC-7*. The LOD value of this

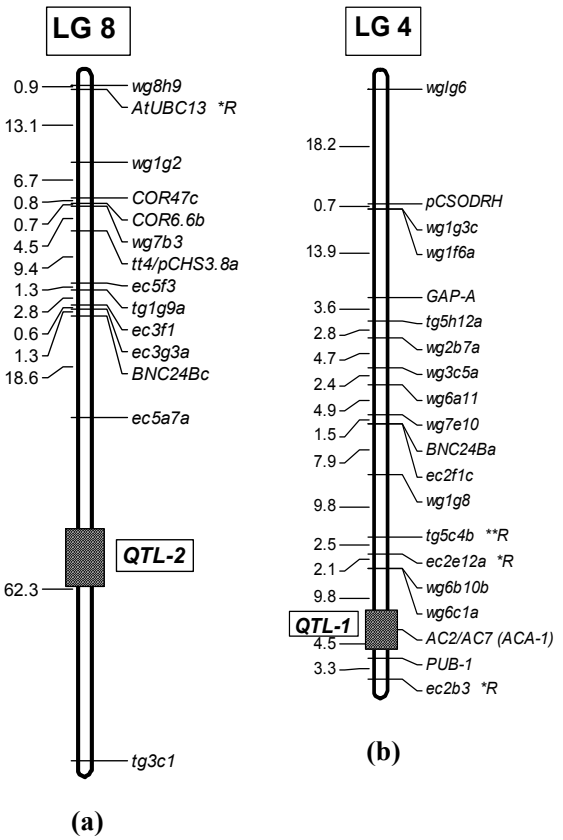

Fig. 2. Genetic map of (a) LG 8 and (b) LG 4 showing the position of two QTLs, QTL-1 and QTL-2, respectively, related to resistance against *A. candida* race 7 (*AC-7*) in *B. rapa*. Distances are given on the left-hand side in cM and the name of RFLP/trait markers are given on the right-hand side. The box indicates the significant QTL effect with 96.8% genetic variation.

QTL was as high as 49.17 and it explains strikingly high proportions (96.3%) of total genetic variation (Table III). The second major QTL was detected on LG-8 and located between RFLP loci *ec5a7a* and *tg3c1* (Fig. 2a). This QTL also had a high LOD value (27.05) explaining spectacularly 96.3% of total variation (Table III). At both the QTL positions, an additional allele from *R-500* contributed 7.89 towards IP scores. Hence, the *PER* allele is the source of resistance for *AC-7*.

Fixation of the above two major QTL and rescanning of the remaining genome did not evidence for any minor QTL. The joint model for these two QTL led to minor changes in the QTL parameters (Table IV). The effects of the *R 500* allele for IP score for first and second QTL are 7.77 and 0.61, respectively. Increase in λ^2 value, LOD and variation explained in the joint model confirmed the presence of both the QTL and evidence for additive gene action.

Discussion

The genus *Brassica* has emerged as one of the front ranking systems for plant genome mapping. It has strikingly high DNA polymorphism among varieties and subspecies within a species that paves the way for construction of highly saturated genetic linkage maps based on DNA markers. Once the genomes were depicted by DNA markers the immediate need was felt for using the information for marker-assisted breeding and detection of chromosomal regions containing the QTL controlling agronomically desirable polygenic characters. This led to dwindling away of the charm of the F_2 or backcross populations which are quickly produced but experience only one cycle of meiosis involving one series of recombination events and constitute all genetically different individuals precluding the scope of replication. RI lines following doubled haploid (DH) lines fitted nicely in this vacuum and emerged as the most favourite mapping population.

RI lines have several advantages over other segregating populations used in linkage mapping. RI lines are nearly homozygous. This allows growing them over multiple seasons, years and locations. Thus it is possible to extricate the precise genetic component of variance for a phenotype that is required for detection of QTL controlling polygenic characters (Burr *et al.*, 1988). Moreover, enrichment of a map with addition of new marker and trait loci can be done infinitely. Besides, several cycles of meiosis pave the way for accurate estimation of the distance between two closely linked markers (Taylor, 1978).

An RI population of *B. rapa* consisting of 87 F_7 families (Kole *et al.*, 1997a) was used for the present study. This population was used several times in the past for different purposes. It was used to detect QTL controlling vernalization responsive flowering time, vernalization independent flowering time, winter survival, freezing tolerance, and resistance to white rust caused by *A. candida* race-2 and race-7 (Osborn *et al.*, 1997; Kole *et al.*, 2001, 2002a, b). It was also used to Mendelize a major vernalization responsive flow-

Table III. Quantitative trait loci controlling the interaction phenotype of 77 recombinant inbred lines of *B. rapa* with *A. candida* race-7 (*AC-7*) on LG 4 and LG 8.

Sl. no.	Parameter	QTL-1 (LG 4)	QTL-2 (LG 8)
1	Confidence interval	*Wg6c1a – Aca1* + 6.0 (LB)[a] *Aca1 – PUB1* + 2.0 (RB)[a]	*ec5a7a – tg3c1* + 22.0 (LB)[a] *ec5a7a – tg3c1* + 32.0 (RB)[a]
2	Marker interval	Aca1 – PUB1	ec5a7a – tg3c1
3	Interval length [cM]	4.5	62.3
4	QTL peak	0.0	28.0
5	Genetics	Free	Free
6	Weight (additive effect)	–7.8938	–7.8938
7	λ^2 Value	226.422	124.556
8	Mean	8.548	8.548
9	Variance	0.595	0.595
10	LOD	49.17	27.05
11	Variation explained	96.3%	96.3%

[a] LB, left boundary; RB, right boundary.

Table IV. Multi-locus model of two QTLs controlling the interaction phenotype of 77 recombinant inbred lines of *B. rapa* with *A. candida* race-7 (*AC-7*) on LG 4 and LG 8.

Sl. no.	Parameter	QTL-1 (LG 4)	QTL-2 (LG 8)
1	Marker interval	*Aca1 – PUB1*	*ec5a7a – tg3c1*
2	Interval length [cM]	4.5	62.3
3	QTL peak [cM]	0.0	11.9
4	Weight (additive effect)	–7.7741	–0.610
5	λ^2 Value	232.565	232.565
6	Mean	8.685	8.685
7	Variance	0.506	0.506
8	LOD	50.50	50.50
9	Variation explained	96.8%	96.8%

ering time QTL (*VFR2*) and its comparative mapping to the flowering time gene 'flowering locus C' (*FLC)* of *Arabidopsis* (Kole *et al*., 2001). The same population was grown in the present study as the mapping population. DNA samples from the 87 RI lines were used for hybridization with four cDNA probes and two gDNA probes to detect 12 RFLP loci. Four of these marker loci were mapped previously. Hence loci for eight RFLPs could be identified for the first time in this population. These new loci were positioned on five linkage groups (LG 2, 3, 6, 8, and 9). This has led to an increase of the total map length by 102.8 cM and of the average marker interval by 0.35 cM. The marker locus *tg3c1* could not be mapped previously. In the present study, it found a place at the distal end of LG 8. Detection of a new locus by the same probe may be due to employment of a different restriction enzyme, improving the clarity of autoradiograms for precise scoring of allelic fragments and induction of new events of recombination. Similarly, Kole *et al*. (1997a) reported on detection of 22 new loci in their RI population for the 96 common probes used by Teutonico and Osborn (1994) for mapping of an F_2 population. Enrichment of genetic maps developed by using a permanent mapping population such as DH has been done in *B. napus* also (Kole, 1997; Osborn *et al*., 1997). A genetic map of 132 RFLP loci (Ferreira *et al*., 1994) was enriched by adding 348 new loci (71 RFLP, 5 isoenzymes, 268 AFLP, and 2 trait loci) that facilitated the detection of QTL controlling winter survival, freezing tolerance, vernalization responsive flowering time, and vernalization independent flowering time besides using the linkage groups in comparative mapping (Osborn *et al*., 1997; Kole *et al*., 2002a, b). Similarly, an RI-based genetic map has been developed in *B. juncea* (Sharma *et al*., 2002). This map has been used for detection of trait loci controlling the resistance to *A. candida* race-2 and oleic acid content.

Interval mapping analysis was employed to scan the enriched map and detect two major QTL governing the resistance to white rust caused by *AC-7* in *B. rapa*. The first major QTL, located on LG 4 between trait locus *PUB-1* and RFLP marker locus *wg6c1a*, coincided with the locus *ACA-1*(*AC-2/AC-7*) detected by the qualitative approach of Kole *et al.* (2002a). The high extent

of the LOD value (49.17), variation explained (96.3%), and λ^2 value (226.42) indicated the resistance to be conferred by single dominant gene from the parent *PER* and obviously not a gene cluster. A Single dominant gene conferring resistance to *AC-2* was reported by Kole *et al.* (1996) in an F_2 population of the same cross used for the present study. Later on, Kole *et al.* (2002a) reported the involvement of the single dominant locus *ACA-1* on LG 4 to govern resistance to both *AC-2* and *AC-7* races. Therefore, it may be concluded that the first QTL under discussion actually designates a qualitative locus controlling the resistance to *AC-7*. The second QTL detected in LG 8 also had a very high LOD value (27.05) explaining high extent of variation (96.3%). The λ^2 value was also strikingly high. One allele of *R-500* could add 7.89 to the IP score, hence, at this QTL position also, the parent *PER* contributed the resistant allele. Fixation of the above two QTL and rescanning of the remaining genome did not evidence for any minor QTL. The joint model for these two QTL led to minor changes in the QTL parameter. Increase in the λ^2 value (232.56), LOD value (50.5), and variation explained (96.8%) in the joint model confirmed the presence of both the QTL and evidenced for additive gene action. Attempts to scan the *B. rapa* genome to detect such QTL were made previously by Kole *et al.* (2002a) without any success. However, addition of markers in the present study facilitated the detection of QTL for *AC-7*. Similarly, Teutonico *et al.* (1995) could not detect any QTL controlling freezing tolerance in *B. napus* using a DH-based RFLP map (Ferreira *et al.*, 1994) consisting of 132 marker loci. Enrichment of this genetic map by adding 348 new loci (71 RFLP, 5 isoenzymes, 268 AFLP, and 2 trait loci) facilitated the detection of QTL controlling winter survival, freezing tolerance, vernalization responsive flowering time, and vernalization independent flowering time besides using the linkage groups in comparative mapping (Osborn *et al.*, 1997; Kole *et al.*, 2002a, b). Similarly, an RI-based genetic map has been developed in *B. juncea* (Sharma *et al.*, 2002). This map has been used for the detection of trait loci controlling the resistance to *A. candida* race-2 and oleic acid content.

Conclusion

This is the first report on the detection of QTL conferring resistance to race *AC-7* of *A. candida* in addition to the major resistance locus. The findings suggested that the *ACA-1* locus on LG 4 contains a major gene that governs the resistance to *AC-7*, and the gene cluster located in LG 8 controls the degree of sporulation of the susceptible genotypes. The major gene *ACA-1* in LG 4 can be introgressed into the susceptible genetic background by using the flanking RFLP marker *wg6c1a* and/or the phenotypic marker *PUB-1*. Similarly, the QTL on LG 8 controlling the degree of resistance can be introgressed into a genetic background by using its flanking markers *ec5a7a* and *tg3c1*. Besides, this QTL can be Mendelized through marker-aided backcross breeding following the same strategy as formulated by Kole *et al.* (2001) employed to Mendelize *VFR-2* controlling vernalization responsive flowering time in this species. Convergence of this QTL to a discrete locus will facilitate its precise monitoring in breeding as well as map-based cloning.

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