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ORIGINAL PAPER

Comparative mapping of *Raphanus sativus* genome using *Brassica* markers and quantitative trait loci analysis for the Fusarium wilt resistance trait

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Abstract Fusarium wilt (FW), caused by the soil-borne fungal pathogen *Fusarium oxysporum* is a serious disease in cruciferous plants, including the radish (*Raphanus sativus*). To identify quantitative trait loci (QTL) or gene(s) conferring resistance to FW, we constructed a genetic map of *R. sativus* using an F_2 mapping population derived by crossing the inbred lines '835' (susceptible) and 'B2' (resistant). A total of 220 markers distributed in 9 linkage groups (LGs) were mapped in the *Raphanus* genome, covering a distance of 1,041.5 cM with an average distance between adjacent markers of 4.7 cM. Comparative analysis of the *R. sativus* genome with that of *Arabidopsis thaliana* and *Brassica rapa* revealed 21 and 22 conserved syntenic regions, respectively. QTL mapping detected a total of 8 loci conferring FW resistance that were distributed on 4 LGs, namely, 2, 3, 6, and 7 of the *Raphanus* genome. Of the detected QTL, 3 QTLs (2 on LG 3 and 1 on LG 7) were constitutively detected throughout the 2-year experiment. QTL analysis of LG 3, flanked by ACMP0609 and cnu_mBRPGM0085, showed a comparatively higher logarithm of the odds (LOD) value and percentage of phenotypic variation. Synteny analysis using the linked markers to this QTL showed homology to *A. thaliana* chromosome 3, which contains disease-resistance gene clusters, suggesting conservation of resistance genes between them.

X. Yu and S. R. Choi contributed equally to this work.

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Introduction

Radish, *Raphanus sativus* (2n = 18), belonging to the Brassicaceae family, is cultivated worldwide for producing leafy and root vegetables. Although an economically important crop, the genetics of yield and quality traits, biotic and abiotic stresses are not well-studied in this crop. Since *R. sativus* belongs to the same family as well-studied *Brassica* species, the genome of this crop is reported to be closely related to A and C genomes of *Brassica rapa*, *B. oleracea*, and *B. napus* (Warwick and Black 1991). Therefore, the first genetic map of *R. sativus*, based on restriction fragment length polymorphism (RFLP) markers of *Brassica*, was developed using a mapping population derived by crossing between *R. sativus* and *R. raphanistrum* since no available marker system was developed in this crop (Bett and Lydiate 2003). This was followed by the development of an intraspecific genetic map in *R. sativus*, where amplified fragment length polymorphism (AFLP)

and *Brassica*-simple sequence repeat (SSR) markers were used (Tsuro et al. 2005). Subsequently, development of many genetic maps and quantitative trait loci (QTL) analyse has been conducted for many traits, including root shape, red pigmentation (Tsuro et al. 2008), and resistance to beet cyst nematodes (Budahn et al. 2009) and clubroot diseases (Kamei et al. 2010). Recently, expressed sequence tag derived SSRs (EST-SSRs) and single nucleotide polymorphism (SNP) markers were used for the development of linkage maps in *R. sativus*, and genome wide comparative analysis was conducted with the model plants *Arabidopsis thaliana* and *B. rapa*, where segmental conservation and diversification of the *R. sativus* genome were reported and compared to already known genomes of the same family (Shirasawa et al. 2011; Li et al. 2011).

Fusarium wilt (FW), caused by the soil-borne fungal pathogen *Fusarium oxysporum*, is a serious disease in cruciferous plants (Armstrong and Armstrong 1952; Bosland and Williams 1987; Garibaldi et al. 2006). This pathogen can survive for long periods in the absence of a suitable host plant. Thus, the production of radishes is also adversely affected by FW disease caused by *F. oxysporum* f. sp. *raphani*. Diseased plants show yellowing, dropping of the leaves, development of a dark brown and vascular discoloration, severe stunting, and eventually death, resulting in serious crop yield loss and reduced commercial value (Kendrick and Snyder 1942). Therefore, the breeding of radish cultivars resistant to FW will provide great advantages to farmers, reducing millions of dollars of losses due to this disease.

The inheritance and genetics of this disease have been studied in *A. thaliana* (Diener and Ausubel 2005) and *B. oleracea* (Pu et al. 2011). QTL mapping identified a dominant-resistance QTL/gene, *Foc-Bol*, in linkage group 7 (O7) of *B. oleracea* (Pu et al. 2011); *RFO1*, another dominant-resistance gene for *F. oxysporum*, is found on chromosome 1 of *Arabidopsis*. *RFO1* induces a hypersensitive reaction in resistant lines (Diener and Ausubel 2005). In radish, Kaneko et al. (2007) identified a QTL resistance to Fusarium wilt which linked to the OPJ14 locus on LG1. Furthermore, Shirasawa et al. (2011) predicted this locus was corresponding to the long arm of *A. thaliana* Chr1 by comparative map between *R. sativus* and *A. thaliana*.

In the present study, we report the development of a genetic map in *R. sativus* using *Brassica* markers, including *B. rapa* chromosome-specific bacterial artificial chromosomes (BACs) and EST-derived SSR markers, intron polymorphic markers, and some radish reference markers from already published works (Panjabi et al. 2008; Ramchiary et al. 2011; Shirasawa et al. 2011). Comparison of radish map with Brassica maps was done in this study, Furthermore, we studied the detailed genetics of FW and performed QTL mapping to identify resistant loci/QTL. The present study is report of molecular mapping of a new major QTL region conferring to FW resistance in *R. sativus*.

Materials and methods

Plant materials

The $F_{2:3}$ mapping population of *R. sativus* was developed by crossing 2 inbred lines showing contrasting phenotypes for FW disease. The inbred lines were '835' (P1), which shows severe symptoms (and hence was used as the susceptible parent), and 'B2' (P2), which shows strong resistance phenotype to FW. An F_1 hybrid generated by crossing between '835' and 'B2' was self-pollinated to produce the F_2 generation. A total of 222 F_2 plants were selected at random for construction of genetic linkage maps. For the production of F_3 lines, single seeds from each F_2 were selfed to produce F_3 lines, which were later used to inoculate with fungal spores for phenotypic screening in the green house of Chungnam National University in 2011 and 2012.

DNA extraction, marker development and genotyping

DNA was extracted from young expanded leaves of greenhouse-grown plants following the CTAB method (Murray and Thompson 1980). The following markers were used for a polymorphism survey between the parental lines ('835' and 'B2'): 189 SSRs previously mapped in *B. rapa* (Kim et al. 2009; prefixed cnu and nia), 528 SSRs developed from BAC end sequences for *B.rapa* (Li et al. 2010; prefixed BRPGM), 272 A genome-specific *B. juncea* IP markers developed by Panjabi et al. (2008), 707 ACMP EST-SSR markers of *B. rapa* (Ramchiary et al. 2011), 245 radish EST-SSRs (Shirasawa et al. 2011), 210 BRMS SSR markers (Suwabe et al. 2006) and 10 markers adjacent to the *Foc-bo1* QTL of *B. oleracea* (Pu et al. 2011). The SSR polymorphism survey, genotyping, and scoring were performed following the methods of Ramchiary et al. (2011).

Genetic map construction, comparative mapping, and QTL analysis

The *R. sativus* genetic map was constructed using JoinMap version 4.0 (Van Ooijen 2006). Logarithms of odds (LOD) scores 4.0–7.0 were used to assign markers to the linkage groups, and Kosambi's mapping function was used to convert the recombination value into the map distance. Recombination frequencies >0.25 and LODs >2.0 were set to arrange the markers in order. Since the genomic locations of the markers used in the present study are already known in *B. rapa* (http://brassicadb.org/brad/) and *A. thaliana*

(http://www.arabidopsis.org/) genomes, we compared the present *Raphanus* genetic map with those species. Synteny analysis and identification of homologous chromosomal segments and crucifer building blocks proposed by Schranz et al. (2006) were conducted following the methods of Ramchiary et al. (2011). Regions with three or more markers showing continuous collinearity between *R. sativus* and *A.thaliana* or *B.rapa* genomes were referred to synteny regions. WinQTL cartographer (Zeng 1994; Wang et al. 2006) version 2.5 was used to perform QTL analysis using the composite interval mapping function as described by Li et al. (2013).

Pathogen inoculation

The FW pathogen (*F. oxysporum* f. sp. *raphani*) was obtained from symptomatic root tissues collected from two different local fields (Syngenta Co., Korea). To produce inoculums, spore suspensions were prepared by flooding colonized 2-week-old cultures on potato dextrose agar liquid medium. The density of Fusarium spore suspensions was adjusted to 10^6 per mm^2 concentration using a hemocytometer. Disease resistance was evaluated by inoculating F_3 lines derived from each F_2 line in a glasshouse in September of 2011 and July of 2012. Five F_3 seeds from each F_2 family were grown in five pots, i.e., 1 seed per pot, for evaluation of disease resistance. In 2011, the seedlings were inoculated with pathogen spores using two different methods, i.e., the root-cut dipping method (Smith et al. 1981) and the dropping method. For the root-cut dipping method, the roots of 2-week-old seedlings were rinsed with tap water, and their secondary roots were cut to 2–3 cm in length. They were then immediately dipped into an aqueous suspension solution containing Fusarium spores for 1 min. Seedlings were then transplanted into 50-cell plug trays containing autoclaved soil. The temperature of the glasshouse was maintained between 25 and 29 °C. Sixteen days after inoculation, disease symptoms were recorded in response to FW, and the disease index (DI) was calculated as described below. For the dropping method, pathogen suspensions were inoculated by injection of 2-week-old seedlings growing in 50-cell plug trays along the root direction. Thirty-one days after inoculation, the DI was assayed. In 2012, experiments were performed in a 25 °C chamber room and repeated using the root-cut dipping method.

Disease symptoms were used to calculate the DI, which was divided into 1–7 grades (Fig. 1), depending on the severity of the symptoms, as follows: grade 1, healthy and no symptoms; grade 2, wilting in cotyledon and slightly dwarfed; grade 3, <25 % of the plant exhibited disease symptoms; grade 4, 25–50 % of the plant exhibited disease symptoms; grade 5, 51–90 % of the plant exhibited disease symptoms; grade 6, plant wilting and defoliated; grade 7, plants died.

Results

Development of the linkage map for *R. sativus*

Although we screened 2,161 markers for polymorphisms between the parental lines to construct a genetic map of *R. sativus*, we were unable to obtain many polymorphic markers that showed relative transferability from *Brassica* markers. Of the 717 cnu/nia and BRPGM SSRs of *B. rapa*, only 112 (15.62 %) showed polymorphisms between the parental lines; similarly, only 23 of 272 (8.46 %) A genome-specific *B. juncea* IP markers, 57 of 707 (8.06 %) ACMP EST-SSRs and 39 of 245 (15.92 %) *R. sativus* EST-SSRs, 4 of 210 (1.90 %) BRMS SSR markers and 2 of 10 (20 %) markers adjacent to the FW-resistant QTL *Foc-bo1* of *B. oleracea* were polymorphic between the parental lines, giving a total of 237 polymorphic marker loci between the parental lines. Of these markers, four did not group with the others. After scoring the segregation of molecular markers, each locus was tested in JoinMap software by Chi square test; we removed nine markers non significant deviation from the expected 3:1 or 1:2:1 ratio. Four markers were removed where genotyping information of more than 20 % individuals in 222 F_2 plants were

Fig. 1 The symptoms of *Raphanus sativus* plant after infection with fusarium wilt (FW). The plants are arranged from *left* to *right* according to the degree of wilted plant growth. The FW disease rating *scale ranges* between 1 (*left* resistant) and 7 (*right* fully susceptible)

found missing and with non clear bands. Therefore, the final map consisted of 220 loci mapped into nine linkage groups. The nine linkage groups were named LG 1–LG 9 following Shirasawa's map (2011) based on the common markers located on each chromosome. The genetic linkage map covered a total distance of 1,041.6 cM, and an average distance between the adjacent loci was 4.7 cM. Details, including the length of each linkage group, number of markers in each linkage group, and other characteristics of the present *R. sativus* map, are provided in Table 1.

Comparative mapping of *R. sativus* with *B. rapa* and *A. thaliana*

Of the 220 markers mapped in present study, BLASTN was used to check for genes homologous to those in the *A. thaliana* genome, a total of 139 loci which consist of 21 conserved syntenic regions corresponding in the *A. thaliana* genome were identified (Supplementary Table 1). Ancestral karyotype genomic blocks (Schranz et al. 2006), were also identified and the result was shown in Fig. 2. All markers in the present map were derived from *B. rapa*, with the

Table 1 Characteristics of *R. sativus* linkage map

Linkage group	Length (cM)	Number of markers							
		cnu/nia[a]	BRPGM[b]	ACMP[c]	IP[d]	EST-SSR[e]	BRMS[f]	KBRS[g]	Total
1	113.8	0	10	9	2	3	0	0	24
2	135.0	0	25	8	2	8	1	0	44
3	140.9	2	13	9	2	15	1	0	42
4	166.9	0	10	12	6	3	1	2	34
5	70.6	0	5	4	0	2	0	0	11
6	125.6	1	10	4	2	5	0	0	22
7	96.2	1	8	2	1	0	0	0	12
8	85.4	2	5	2	3	1	1	0	14
9	107.1	0	10	5	0	2		0	17
Total	1,041.5	6	96	55	18	39	4	2	220

[a] Cited from Kim et al. (2009)

[b] Cited from Li et al. (2010) and Ramchiary et al. (2011)

[c] Cited from Ramchiary et al. (2011)

[d] Cited from Panjabi et al. (2008)

[e] Cited from Shirasawa et al. (2011)

[f] Cited from Suwabe et al. (2006)

[g] Cited from Pu et al. (2011)

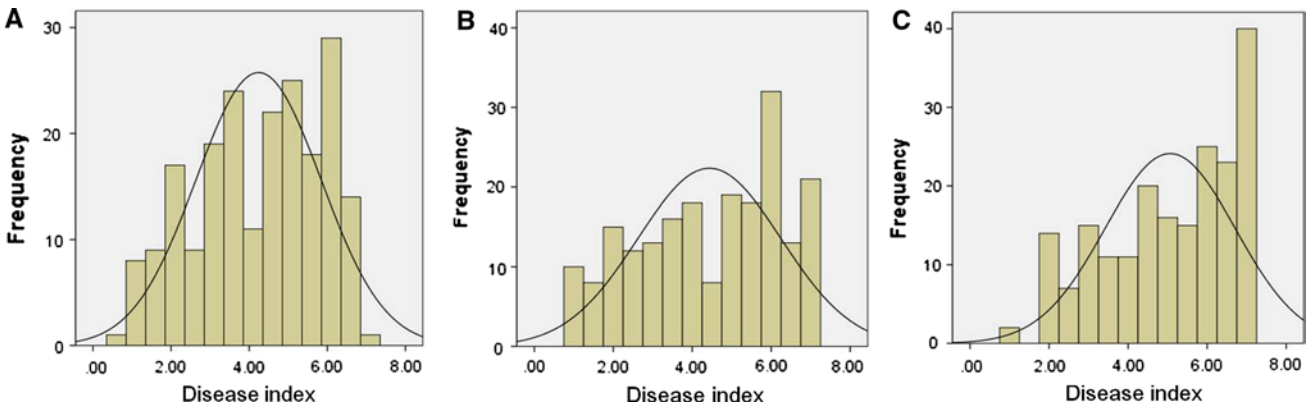

Fig. 2 Frequency distributions of the fusarium wilt disease traits of $F_{2:3}$. **a**, **b**, **c** represent the frequency 2011 dropping, 2011 root-cut dipping and 2012 root-cut dipping method respectively, the curve is normal distribution line

exception of the *R. sativus* EST-RSS and IP markers, which helped to identify colinearity between the *R. sativus* LGs and *B. rapa* chromosomes. The 10 chromosomes of *B. rapa* were divided into many segments along the *R. sativus* map which revealed 22 syntenic regions (Fig. 2; Supplementary Table 1). The LG 2 was divided into many segments of *B. rapa* (A01, A02, A05, A07, A09, and A10). LG 7 was the least with only one large region (A01), followed by LG 5, with two regions (A01 and A02). Homologous segments A06 in LG 9 were interrupted with segments homologous to A08. Subsequently, the genomic region of *B. rapa* was observed in linkage groups LG 1 (consisting of A07 and A10), LG 3 (composed of A03, A05, A06, and A09), and LG 4 (composed of A03, A04, and A06).

QTL mapping for FW resistance

The parental lines showed contrasting characteristics for disease resistance (mean parental disease scores were 6.45 ± 1.57 for '835' and 2.57 ± 1.83 for 'B2'), the F_1 was susceptible to FW (the mean disease score of F1 was 5.95 ± 1.16), indicating that resistance is likely governed by recessive gene(s). The DI in $F_{2:3}$ lines segregated continuously and exhibited a bimodal distribution in all environments, suggesting that both major and minor QTL were involved in *R. sativus* resistance to FW (Fig. 3). Using the developed radish map in the present study, composite interval mapping was conducted to detect resistance QTL. Eight different QTL associated with FW resistance were detected in five linkage groups, LG 2, LG 3, LG 4 (2 QTL each), LG 6, and LG 7 (1 QTL each) by confirming our phenotypic observation (Table 2). The LOD values for the 8 QTL ranged from 2.50 to 10.08, and the R^2 ranged from 0.49 to 14.63 %. Of these many QTL, 3 QTL, i.e., *qFW3*, *qFW4*, and *qFW8*, were detected in all three experiments conducted over 2 years, suggesting these three loci may be the important loci for conferring FW resistance traits. Among these, one major locus, *qFW4*, explained most of the phenotypic variations (R^2) 14.63 % in the 2011 root-cut dipping method. This locus was mapped to the marker interval between ACMP0606 and cnu_mBRPGM0085 on LG3 and exhibited a relatively high LOD value ranging from 4.34 to 8.84 with an additive effect from 0.41 to 0.52. This QTL was named Fusarium wilt Resistance locus 1 (*Fwr1*). In addition, *qFW1* was detected only in 2011 for both methods, but not in 2012 test and was located at LG 2. *qFW2*, *qFW5*, and *qFW6* were detected only in 2012.

Synteny analysis and identification of crucifer blocks containing major FW resistance QTL

We further investigated in which crucifer blocks our FW resistance QTL was located. Since synteny analysis was already conducted for the *Raphanus* genome in the present study with the *B. rapa* and *A. thaliana* genomes, we could identify crucifer blocks harboring major and minor FW resistance QTL. Of the many blocks identified, we detected four major crucifer blocks (F, N, U, X) to be more important with respect to disease-resistance loci (Table 2). These were the F block in LG 3 (which contained constitutive QTL *qFW3*), N blocks in LG 3 (*qFW4*, major QTL) and LG 4 (*qFW5*), U blocks in LG 2 (*qFW1*), LG 4 (*qFW6*) and LG 7 (*qFW8*, constitutive QTL), and the X block in LG 6 (*qFW7*). Comparative genome analysis revealed extensive synteny between LG 3 of the *Raphanus* genome (which harbors *qFW3* and *qFW4*, major QTL) and *A. thaliana* chromosome 3. The flanking markers of *qFW4* showed homology to the central region of *A. thaliana* chromosome 3, in which the physical distance ranges from 17.7 to 22.2 Mb and contains 272 orthologous genes, including 11 genes associated with disease resistance (Fig. 4, Supplementary Table 2). *qFW4* was involved in the *Foc-Bol* region of *B. oleracea* (Pu et al. 2011), which aligned with the corresponding region between chromosomes 3 and 4 of *A. thaliana* in a previous study. The markers KBrB089H07N1 and KBrB084F01N1 around *Foc-Bol* were syntenic to *A. thaliana* chromosome 3 and were near the top of the synteny region with the marker ACMP0606 in *R. sativus* (Fig. 4).

Discussion

In *R. sativus*, linkage maps have been constructed by RFLP (Bett and Lydiate 2003), RAPD and AFLP (Tsuro et al. 2005; Xu et al. 2012), and *Brassica*-SSR (Tsuro et al. 2005; Kamei et al. 2010), EST-SSR (Shirasawa et al. 2011), and SNP (Li et al. 2011) markers contributed to various genetic analyses. In the present study, a genetic linkage map was developed using 112 SSRs and 55 EST-SSRs of *B. rapa* BAC-derived markers, 18 genome-specific *B. juncea* IP markers, and 39 *R. sativus* EST-based markers using 222 F_2 populations. The map covered 1,041.5 cM, with an average distance between adjacent markers of 4.7 cM and comprised nine linkage groups. Compared to all published linkage maps of *Raphanus* species, this map is the most extensive available, with markers from the *B. rapa* genome, which was fully sequenced in 2011 (Wang et al. 2011). Hence, we used this well established and characterized *B. rapa* resource for mapping and map-based cloning.

Brassica species and *Arabidopsis* belong to the same family, Brassicaceae. *Brassica* and *Arabidopsis* lineages diverged from *Brassica* about 14.5–20.4 million years ago (Yang et al. 1999). In this study, we used a BLAST search to find that 63.2 % of marker loci corresponded to 139 orthologous genes, covering almost the entire *A. thaliana*

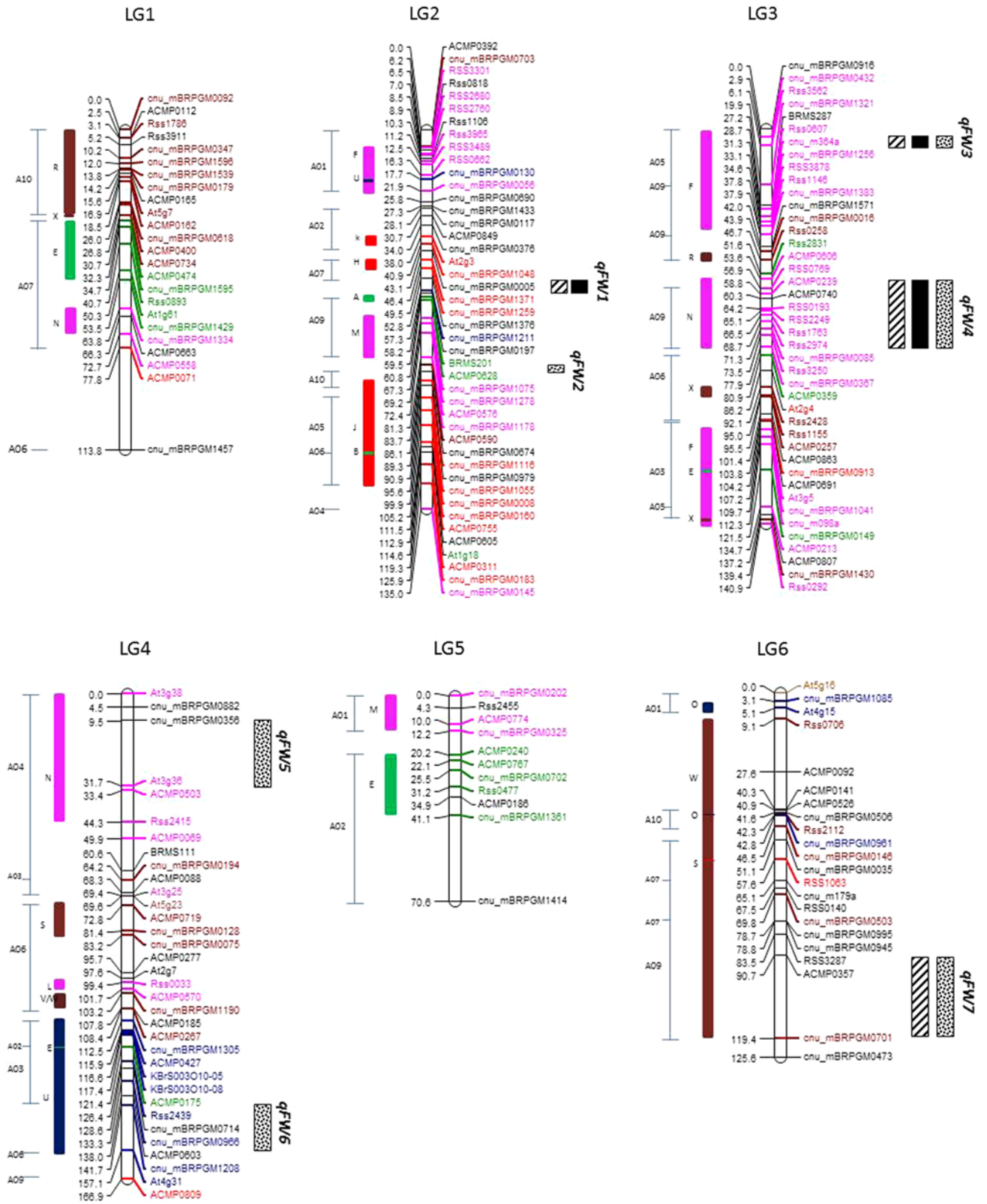

Fig. 3 Genetic map of radish shows nine linkage groups. The *different color* indicates homology to 5 chromosomes of *A. thaliana*. The *rectangular bars* on the *left* of each linkage group indicate the crucifer blocks (Schranz et al. 2006) identified in the present sudy in *R.sativus* genome. *Vertical lines* next to the *rectangular bars* show the chromosome number of *Brassica rapa.* Detected QTL was indicated by *rectangular bars* of different shades in the *right* side of the linkage groups with followed by QTL name

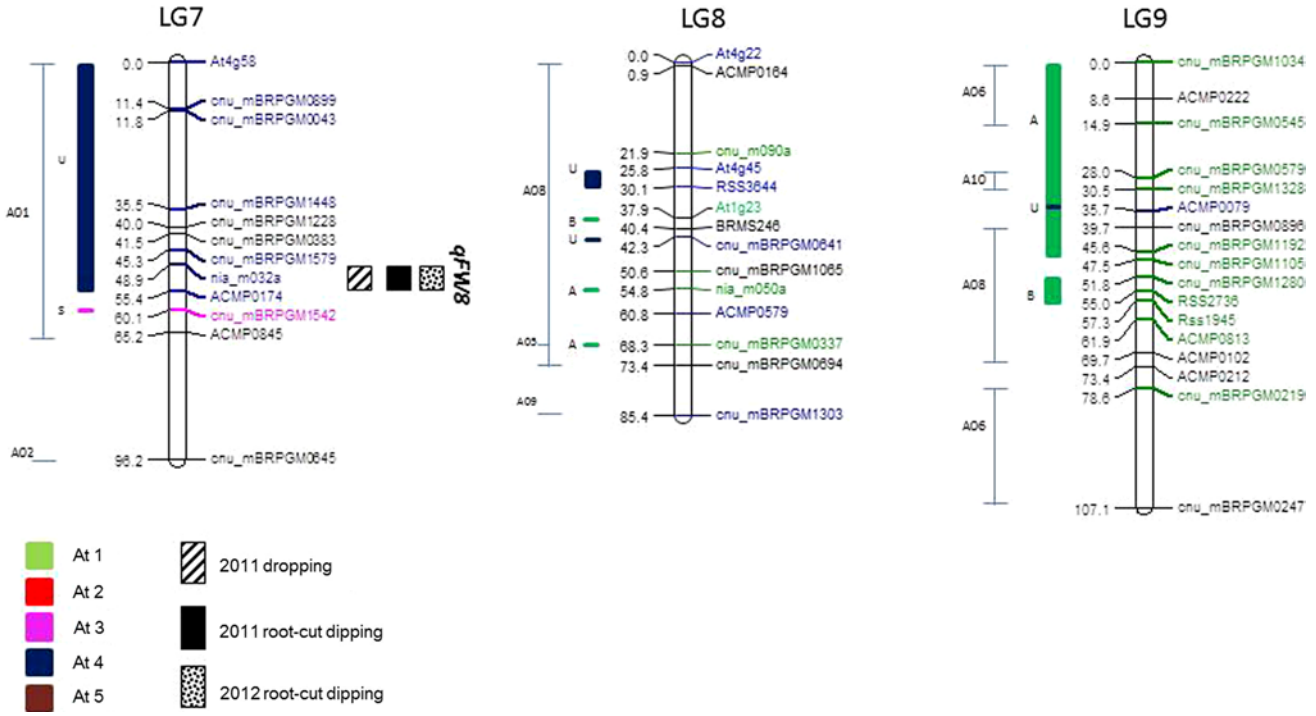

Fig. 3 continued

genome. For example, almost the entire length of LG 3 was homologous to chromosome 3 of *A. thaliana*; this conserved synteny allowed us to capitalize on the genomic information available for *A. thaliana* in our study of *R. sativus*. However, the *Raphanus* genome is much larger than that of *Arabidopsis* (Meyerowitz 1992), and the frequent observation of rearrangements of the *A. thaliana* genome in *Brassica* crops is expected in accordance with previous studies (Suwabe et al. 2006; Wang et al. 2011). We also confirmed the genome structure of *R. sativus* by 24 genomic blocks within the hypothetical ancestral karyotype (Schranz et al. 2006). Except for D, G, P, and I blocks, all the blocks were confirmed to be present in the *R. sativus* genome. The absence of these four blocks in the present map was perhaps due to the short centromere or the proximity to the centromere in the hypothetical ancestral karyotype. Most blocks were doubled or tripled, as reported in *B. rapa* (Lagercrantz 1998; Parkin et al. 2005; Li et al. 2010). Some adjacent block (e.g., A/B and V/W) in *R. sativus* were detected as continuous distributions in the genome of the ancestral karyotype. These A, and B blocks were completely conserved in LG 9 of *R. sativus*, as shown in previous research (Li et al. 2011). The *Brassica* genomes were tripled often corresponded to homologous segments of *A. thaliana* (O'Neill and Bancroft 2000; Lysak et al. 2005). These occurrences suggested the hypothesis that the *R. sativus* genome structure arose from rearrangement and divergence from a common ancestor with *A. thaliana* (Yang et al. 1999; Koch et al. 2000; Shirasawa et al. 2011). In the present study, large syntenic regions of *B. rapa* covered the *R. sativus* genome, indicating that 22 segments in the *R. sativus* and *B. rapa* genomes were conserved. Moreover, the majority of the chromosome region of the *B. rapa* genome was replicated, giving two or three copies for different linkage groups, especially in chromosome A08, which had been further replicated (Mun et al. 2008). The observed 216 (98.2 %) loci conservation colinearity regions, exhibiting relative high conservation in the *R. sativus* genome, contained potential candidate genes for disease resistance.

The parents for the mapping population had visibly different phenotypes in terms of resistance to FW: '835' was a susceptible cultivar, and 'B2' was a resistant cultivar. The DI in $F_{2:3}$ lines segregated continuously, and a bimodal distribution was observed in all environments, our results agree with that both major and minor QTL were involved in *R. sativus* resistance to FW. In addition, the F_1 plants, which showed obvious susceptible phenotype, support the presence of a recessive gene effect in this population. Analysis of the disease phenotype in $F_{2:3}$ lines did not give clear-cut gene segregation ratios, indicating the possible involvement of many genes in governing FW disease-resistance traits. A major locus for FW resistance, *qFW4* (Fwr1), was flanked by ACMP0606

Table 2 Position and effect of QTL for fusarium resistance in *R. sativus*

Trait	Linkage group	Marker interval	Block region[a]	2011 Dropping method				2011 Root-cut dipping method				2012 Root-cut dipping method			
				LOD	R^2 (%)	Additive effects[b]	Dominance effects	LOD	R^2 (%)	Additive effects[b]	Dominance effects	LOD	R^2 (%)	Additive effects[b]	Dominance effects
qFW1	LG2	cnu_mBRPGM1376-cnu_mBRPGM1211	U	3.72	3.80	0.01	1.18	3.29	1.01	0.02	1.24				
qFW2	LG2	ACMP0590-cnu_mBRPGM0674	X									5.62	1.40	−0.03	−4.04
qFW3	LG3	cnu_mBRPGM0432-RSS3562	F	3.76	10.91	0.20	0.98	4.84	12.50	0.20	1.74	4.23	6.72	0.24	1.14
qFW4	LG3	ACMP0606-cnu_mBRPGM0085	N	7.94	13.32	0.47	0.08	8.84	14.63	0.52	0.02	4.34	9.11	0.41	−0.08
qFW5	LG4	cnu_mBRPGM0356-At3g36	N									8.57	6.42	−0.16	−3.38
qFW6	LG4	cnu_mBRPGM-At4g31	U									9.42	3.60	0.14	−4.20
qFW7	LG6	ACMP0357-cnu_mBRPGM0701	X	3.2	1.79	0.03	−2.86					10.08	1.57	0.14	−4.00
qFW8	LG7	nia032a-cnu_mBRPGM1542	U	2.5	2.21	−0.28	0.77	3.31	0.49	−0.37	1.15	3.03	4.49	−0.35	0.89

[a] Blocks expanded compared to the previous reports (Schranz et al. 2006)

[b] Positive values indicate that alleles are inherited from parent "835" and the negative values indicate that alleles are from parent "B2"

and cnu_mBRPGM0085, with a high LOD value of 8.75, and explained the 14.63 % proportion that had a positive additive effect. Therefore, this genomic location represents a potential beneficial candidate for marker-assisted selection (MAS) for FW disease resistance. In the present study, disease infection was investigated according to two factors: (1) the effects of different methods and (2) the effects of different environments. However, the methods effect showed no significant QTL × environment interactions, while the environment effect, with different temperatures observed between the 2 years test, resulted in higher LOD values in 2011 than in 2012, probably because FW disease incidence increases when the temperature is raised (Farnham et al. 2001). Minor QTL for resistance to FW were located on different linkage groups in different environments; *qFW3* and *qFW8* were identified in all tests, *qFW1* was detected only in 2011, and *qFW2*, *qFW5*, and *qFW6* were detected only in 2012 tests. Moreover, an interaction effect of the environment was also observed because the FW resistance of the $F_{2:3}$ population evaluated in different environments. This reports the novel QTL conferring FW resistance in *R. sativus*, and these data may play an important role in MAS breeding.

In *A. thaliana*, *RFO1* (on chromosome 1) confers resistance to *F. oxysporum* f. sp. *matthioli*, f. sp. *conglutinans*, and f. sp. *raphani*, which are causal agents of FW in Brassicaceae (Diener and Ausubel 2005). However, in this study, we did not detect a QTL/gene that were homologous in *A. thaliana* chromosome 1. We detected the major QTL region corresponding to chromosome 3 of *A. thaliana*, while the central QTL region of *B. oleracea* O7 (Pu et al. 2011) was aligned with the corresponding region of chromosomes 3 and 4 of *A. thaliana*. This suggested that (1) new resistance gene(s) exist or the resistant genes for FW are distributed into different genomic regions after rearrangement occurred in the chromosome; or (2) diversity of the evolution of the resistance mechanism may have occurred in *Brassica* species (Suwabe et al. 2006). Furthermore, the central region of *Foc-Bol* in *B. oleracea* corresponded to the homology region of chromosome 4 of *A. thaliana*, which contains a disease-resistance gene cluster (Mayer et al. 1999; Young 2000). The syntenic region of *qFW4* (*Fwr1*), located in *A. thaliana* chromosome 3, was also identified as a disease-resistant gene cluster (Holub 1997). Eleven disease-resistant orthologous genes that contained gene-mediated disease resistance in *A. thaliana*, disease-resistance protein (TIR-NBS-LRR class) family, and others related to ATP binding and defense responses were identified (Supplementary Table 2). Such a resistance gene cluster represents a significant chance to identify genes with resistance functions in this QTL region.

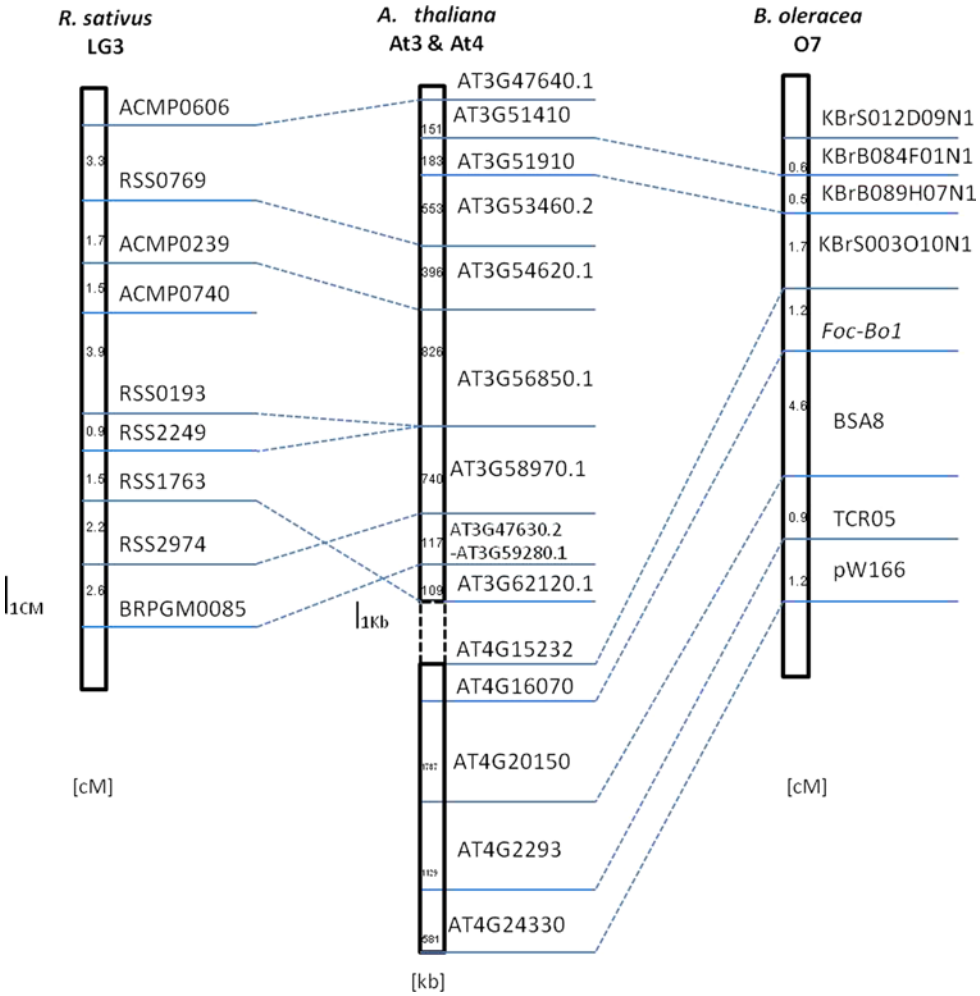

Fig. 4 *R. sativus* genomic region around the major fusarium wilt resistant QTL Locus *Fwr1* shows synteny with the *A. thaliana* chromosome 3, 4 and *B. oleracea* O7. The *markers* of radish that are homologous with *A. thaliana* genes or *B. oleracea Foc-Bol* gene are connected by *broken lines*

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