Development of a high density integrated reference genetic linkage map for the multinational *Brassica rapa* Genome Sequencing Project[1]

Xiaonan Li, Nirala Ramchiary, Su Ryun Choi, Dan Van Nguyen, Md. Jamil Hossain, Hyeon Kook Yang, and Yong Pyo Lim

Abstract: We constructed a high-density *Brassica rapa* integrated linkage map by combining a reference genetic map of 78 doubled haploid lines derived from Chiifu-401-42 × Kenshin (CKDH) and a new map of 190 F_2 lines derived from Chiifu-401-42 × rapid cycling *B. rapa* (CRF_2). The integrated map contains 1017 markers and covers 1262.0 cM of the *B. rapa* genome, with an average interlocus distance of 1.24 cM. High similarity of marker order and position was observed among the linkage groups of the maps with few short-distance inversions. In total, 155 simple sequence repeat (SSR) markers, anchored to 102 new bacterial artificial chromosomes (BACs) and 146 intron polymorphic (IP) markers were mapped in the integrated map, which would be helpful to align the sequenced BACs in the ongoing multinational *Brassica rapa* Genome Sequencing Project (BrGSP). Further, comparison of the *B. rapa* consensus map with the 10 *B. juncea* A-genome linkage groups by using 98 common IP markers showed high-degree colinearity between the A-genome linkage groups, except for few markers showing inversion or translocation. Suggesting that chromosomes are highly conserved between these *Brassica* species, although they evolved independently after divergence. The sequence information coming out of BrGSP would be useful for *B. juncea* breeding. and the identified *Arabidopsis* chromosomal blocks and known quantitative trait loci (QTL) information of *B. juncea* could be applied to improve other *Brassica* crops including *B. rapa*.

Key words: bacterial artificial chromosome (BAC), *Brassica rapa*, intron polymorphic (IP) marker, linkage map, genome sequencing, simple sequence repeats (SSRs).

Résumé : Les auteurs ont produit une carte génétique intégrée à haute densité chez le *Brassica rapa* en combinant une carte de référence fondée sur une population de 78 lignées haploïdes doublées dérivées du croisement Chiifu-401-42 × Kenshin (CKDH) et une nouvelle carte basée sur 190 lignées F_2 dérivées du croisement Chiifu-401-42 × *B. rapa* à cycle court (CRF2). La carte intégrée compte 1017 marqueurs et couvre 1262,0 cM du génome du *B. rapa*, pour une distance moyenne entre les marqueurs de 1,24 cM. Une grande similitude dans l'ordre et la position des marqueurs a été observée parmi les groupes de liaison des cartes si ce n'est de quelques petites inversions. Au total, 155 marqueurs microsatellites (SSR), provenant de 102 clones de chromosomes bactériens artificiels (BAC), et 146 marqueurs de polymorphisme d'introns (IP) ont été situés sur la carte intégrée. Cela facilitera l'alignement des clones BAC séquencés dans le cadre du projet multinational de séquençage du génome du *Brassica rapa* (BrGSP). De plus, une comparaison de la carte consensus du *B. rapa* avec les 10 groupes de liaison du génome A du *B. juncea* à l'aide de 98 marqueurs IP partagés a montré une grande colinéarité entre les groupes de liaison des génomes A, à l'exception de quelques marqueurs au sein d'inversions ou de translocations. Cela suggère que les chromosomes sont largement conservés entre ces deux espèces de *Brassica* bien qu'elles aient évolué séparément depuis leur divergence. L'information sur la séquence génomique émanant du BrGSP sera utile pour l'amélioration génétique chez le *B. juncea*. Pareillement, les blocs chromosomiques identifiés chez l'Arabidopsis de même que les QTL identifiés chez le *B. juncea* pourront contribuer aux efforts de sélection chez d'autres espèces de *Brassica* incluant le *B. rapa*.

Mots-clés : chromosome bactérien artificiel (BAC), *Brassica rapa*, marqueur de polymorphisme d'introns (IP), carte génétique, séquençage génomique, microsatellites (SSR).

[Traduit par la Rédaction]

Received 24 March 2010. Accepted 13 June 2010. Published on the NRC Research Press Web site at genome.nrc.ca on 5 November 2010.

Corresponding Editor: B. Golding.

X. Li,[2] N. Ramchiary,[2] S.R. Choi,[2] D. VanNguyen, M.J. Hossain, H.K. Yang, and Y.P. Lim.[3] Molecular Genetics and Genomics Lab, Department of Horticulture, Chungnam National University, Gung-Dong, Yuseong-Gu, Daejeon 305 764, Republic of Korea.

[1]This article is one of a selection of papers from the conference "Exploiting Genome-wide Association in Oilseed Brassicas: a model for genetic improvement of major OECD crops for sustainable farming".

[2]These authors contributed equally to this work.
[3]Corresponding author (e-mail: yplim@cnu.ac.kr).

Introduction

Brassica species are one of the world's most economically important plant groups. The presence of wide genetic and morphological diversity and the immense economic importance has led to extensive morphological, genetic, and genomic studies on different cultivated *Brassica* crops. In the past two decades, studies using molecular markers in economically important vegetable and oilseed *Brassica* crops have helped researchers to map, tag, and clone important quantitative trait loci (QTL) or genes in addition to marker-assisted breeding and gene pyramiding (Teutonico and Osborn 1994; Nozaki et al. 1997; Snowdon and Friedt 2004; Suwabe et al. 2006; Ramchiary et al. 2007; Zhang et al. 2009). Further, the organization and evolution of different *Brassica* genomes and the syntenic relationships among themselves and with the model plant *Arabidopsis thaliana*, their closest relative, are well established (Lagercrantz and Lydiate 1996; Lagercrantz 1998; Parkin et al. 2005; Suwabe et al. 2006; Panjabi et al. 2008). Although much structural and functional genomic information on *A. thaliana* could be utilized for *Brassica*, the complexity of the genome arrangement and the presence of an average of three copies of an *Arabidopsis* chromosomal segment in *Brassica* genomes (Lagercrantz 1998; Parkin et al. 2005; Panjabi et al. 2008) led to the initiation of the multinational *Brassica* Genome Project (MBGP) with the aim to sequence the whole genome of *Brassica* (Yang et al. 2005).

Brassica rapa subsp. *pekinensis* 'Chiifu-401-42' (hereafter Chiifu), a Chinese cabbage inbred line with comparatively smaller genome size among the cultivated brassicas, was selected as the first representative model for *Brassica* A-genome sequencing by the MBGP in 2003 (Yang et al. 2005). The ongoing efforts of the multinational *Brassica rapa* Genome Sequencing Project (BrGSP) have generated enormous DNA sequence information from *B. rapa* "seed bacterial artificial chromosomes (BACs)" with a good opportunity to identify, develop, and map BAC-specific simple sequence repeat (SSR) markers in the whole genome and an excellent opportunity to develop integrated genetic, physical, and sequence-based maps of *B. rapa*. Choi et al. (2007) constructed the first (version I) reference genetic map of *B. rapa* with doubled haploid mapping populations derived from a cross involving Chiifu and Kenshin (CKDH) for the BrGSP. Kim et al. (2009) updated the version I reference genetic map (as version II) and aligned 188 seed BACs in the 10 *B. rapa* linkage groups using BAC-anchored SSR markers. Mun et al. (2008) published the first physical map of *B. rapa*, in which they have accommodated 242 anchored contigs of the 1428 contigs identified in the 10 linkage groups of *B. rapa*. Mun et al. (2009) have also constructed two *B. rapa* maps, JWF_3 and VCS, using BAC-derived SSR markers (Table S1[4] and www.brassica-rapa.org). However, the number of BAC-anchored SSR markers in all the maps is too low to cover the whole genome of *B. rapa* for the BrGSP.

Therefore, in this study, we developed and mapped additional BAC-derived SSR markers and constructed a high-density integrated linkage map of *B. rapa* for the BrGSP using two populations with Chiifu as the common parent. Furthermore, we used intron polymorphic (IP) markers developed and mapped by Panjabi et al. (2008) to the *B. juncea* genome for comparative mapping of the *B. rapa* linkage groups with the 10 A-genome linkage groups of *B. juncea*.

Materials and methods

Plant materials

Two mapping populations from the Korea *Brassica* Genome Resource Bank were used in the study. CKDH, consisting of 78 doubled haploid lines, was derived from microspore culture of the F_1 plant of a cross between diverse Chinese cabbage inbred lines Chiifu and Kenshin. The second population (CRF_2) comprised 190 F_2 lines derived from a cross between Chiifu and IBM 218 DH rcb, a rapid cycling *B. rapa* line (hereafter RCBr) kindly gifted by Professor Tom Osborn (University of Wisconsin, Madison, Wis., USA).

DNA extraction, marker development, and genotyping

DNA was extracted from young expanded leaves of greenhouse-grown plants by using an RBC genomic DNA extraction kit (Real Biotech Corporation, Taipei, Taiwan). Seven hundred forty-nine previously developed SSR markers (Kim et al. 2009; prefixed cnu and nia) and 528 SSR markers (prefixed brpgm) newly developed from BAC-end sequences were used for a polymorphism survey between Chiifu and RCBr. The SSR polymorphism survey, genotyping, and scoring were conducted following Kim et al. (2009). Two hundred seventy-two A genome-specific *B. juncea* IP markers developed by Panjabi et al. (2008) were custom-synthesized and used to screen polymorphism among Chiifu, Kenshin, and RCBr. The PCR condition used for the IP markers was as follows: initial denaturation at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min; final extension at 72 °C for 7 min. The amplified PCR products were analyzed on 1.5%–2% (*w*/*v*) agarose gel to score polymorphism on the basis of either length difference or presence/absence (dominant) of PCR products. The nonpolymorphic IP markers on 1.5%–2% agarose gel were further screened on 6% polyacrylamide gel for parental polymorphism. Multiple or duplicated loci amplified by the same primer pairs were designated by lowercase letters (a, b, c, etc.) starting from the larger fragment to the smaller fragment after the primer name.

Map construction

The CKDH and CRF_2 genetic maps were constructed as described by Kim et al. (2009) using JoinMap version 4 (Stam 1993; Van Ooijen and Voorrips 2001). Logarithm of odds (LOD) scores 4.0–8.0 were used to assign markers to the linkage groups, and Kosambi's (1944) mapping function was used to convert the recombination value into the map distance. A recombination frequency smaller than 0.4 and an LOD larger than 1.0 were set to arrange the marker order. The *B. juncea* map constructed by Panjabi et al. (2008) was used for comparative mapping with the *B. rapa* consen-

[4] Supplementary data for this article are available on the journal Web site (genome.nrc.ca).

sus map developed in this study. To integrate the two maps, the "Combine the Groups for Map Integration" function was used. The linkage maps were drawn by the MapChart program (Voorrips 2002).

Results

Construction of the CKDH linkage map

The version II CKDH reference genetic map contains 719 markers covering a total distance of 1123.3 cM. We screened 272 *B. juncea* A genome-specific IP markers developed and mapped by Panjabi et al. (2008) for a parental polymorphism survey between Chiifu and Kenshin. Of the 272 markers, 85 IP markers (including 40 markers on 2% agarose gel and 45 markers on 4% polyacrylamide gel) showed polymorphism with 99 marker loci between the parents. However, only 95 polymorphic loci could be mapped to the 10 linkage groups of *B. rapa.* Most of the previously mapped markers have remained in the same order, although few markers showed inversion within a 5 cM interval. The number of mapped IP markers ranges from 5 in linkage group A4 to 21 in linkage group A9 (Table S1). The addition of IP markers to the version II reference genetic map increased the marker density and map length of all the linkage groups except A8, showing a decrease in length of 2 cM. The updated map shows an increased length of 51.8 cM, resulting in total genome coverage of 1175.1 cM. The length of the linkage groups was enlarged at the bottom (A2, A3, A9, and A10), at the top (A7), and on both sides (A5, A6, and A8) by inserting 1–5 loci. The map now comprises 813 marker loci, including 267 AFLP, 410 SSR, 24 RAPD, 8 STS, 7 ESTP, 2 CAPS, and 95 IP markers. The map length ranges from 91.9 cM in linkage group A8 to 155.2 cM in linkage group A6, with an average of one marker in every 1.45 cM.

Construction of the CRF_2 linkage map

In the polymorphism survey between Chiifu and RCBr, by using the 749 previously developed SSR markers and the 528 newly developed SSR markers, polymorphic loci were identified from 502 and 71 SSR markers, respectively. However, for genotyping the F_2 population, only 280 good, reproducible, and clear band-producing SSR markers comprising 43 nia_ssr, 166 cnu_ssr, and 71 brpgm_ssr were used, resulting in the identification of 285 marker loci including eight dominant loci. The polymorphism survey of 272 IP markers between the parents initially showed 65 polymorphic loci on 2% agarose gel. Further screening of the nonpolymorphic IP markers on 6% polyacrylamide gel led to the identification of 12 other polymorphic loci, reaching a total of 77 polymorphic loci (65 codominant loci and 12 dominant loci) from 62 IP markers. Six IP markers gave two polymorphic loci each, and one IP marker gave three polymorphic loci.

The CRF_2 genetic map was constructed by using the genotyping data of 190 F_2 individuals derived by crossing Chiifu and RCBr, and 362 polymorphic marker loci comprising 285 SSR and 77 IP markers. Of these markers, 349 were included in the 10 linkage groups, but 13 markers could not be added in any linkage group. Further, we removed 31 non-BAC-anchored SSR markers showing distorted segregation, missing genotypic information, and insufficiently clear bands; therefore, the final map consists of 318 markers including 250 SSR and 68 IP markers. The 10 linkage groups were designated A1–A10 on the basis of the common markers located on each chromosome in the version II reference genetic map. Random distribution of all the mapped SSR and IP markers was found in the 10 linkage groups, although the distribution is not uniform in few regions. The genetic map covers a length of 1105 cM with an average of one marker in every 3.47 cM. The length of the linkage groups ranges from 76.1 cM in A6 to 162.7 cM in A9, and the number of markers per linkage group varies from 14 in A6 to 64 in A9 (Table S1). The number of BAC-derived SSR loci in this map is 250, whereas the version II reference genetic map has 191 loci. The number of BACs anchoring SSRs varies from 10 in linkage groups A6 and A10 to 39 in linkage group A9; further, the number of new BACs mapped to the *B. rapa* genome ranges from 3 in linkage group A6 to 15 in linkage group A9. The order and positions of the BAC-anchored SSRs and IP markers is the same, although few changes were observed in some linkage groups. Moreover, the marker order in linkage groups A2, A4, A8, and A9 is the same, with one or no change in marker order. The remaining linkage groups show changes in the order of few markers, mostly within the range of 5 cM, although inversions within 10–15 cM were observed for two or more markers (in A3 and A5).

Construction of the *B. rapa* consensus map

After identifying the linkage groups having common markers, the CKDH and CRF_2 maps were integrated. In total, 112 (95 SSR and 17 IP) common marker loci were identified between the maps and used as bridge markers during the integration. SSR and IP markers with more than one polymorphic locus in the same linkage groups were correctly identified for size and order before designating the common loci between the maps. The number of common markers between the maps ranged from 5 (A4 and A8) to 23 (A9). The integrated map now has 1017 markers comprising 267 AFLP, 24 RAPD, 563 SSR, 146 IP, 8 STS, 7 ESTP, and 2 CAPS markers (Fig. 1 and Table S1) and covers a total distance of 1262 cM, which is greater than the 1175.1 cM and 1105 cM length of the CKDH and CRF_2 maps, respectively. The number of markers in the integrated map ranged from 61 in linkage group A4 to 151 in linkage group A9, and the length of the linkage groups varied from 92.6 cM in A10 to 158.5 cM in A6. The length of the integrated linkage groups is about the same as the corresponding longest linkage groups of the component maps with a slight increase in map length except for linkage group A1, in which a decrease of approximately 10 cM was observed compared with the maximal length of the individual maps. Large gaps (>10 cM) in few linkage groups of the CKDH and CRF_2 maps were reduced during the integration. For example, of the two gaps observed in linkage group A2 of the CKDH map (Kim et al. 2009), one was filled at the bottom by six IP and two SSR markers from the CRF_2 map upon integration. In the CRF_2 map, we observed 12 large gaps (>10 cM) in eight chromosomes except in linkage groups A7 and A8. However, upon integration, the gaps decreased in length or were saturated by the markers from the CKDH map. There-

Fig. 1. Comparative map between the A genome chromosomes of *Brassica rapa* (Integ/A1–Integ/A10) and *B. juncea* (J1–J10). Cumulative recombination distances are shown to the left and marker loci to the right of the linkage groups. The colored bars to the left of the *B. rapa* linkage groups (Integ/A1–Integ/A10) represents crucifer genome blocks (proposed by Schranz et al. 2006) synteny to *Arabidopsis* chromosomes (chromosome 1, light blue; chromosome 2, yellow; chromosome 3, dark blue; chromosome 4, green; and chromosome 5, red). The crucifer blocks identified in *B. juncea* A genome chromosomes by Panjabi et al. (2008), but not in the integrated *B. rapa* linkage groups are indicated by colored bars left of the *B. juncea* linkage groups (J1–J10). SSR markers and IP markers containing representative blocks are indicated by bold strokes. Single and double asterisk (*, **) indicates marker loci derived from multicopy *Arabidopsis thaliana* genes (Panjabi et al. 2008; http://www.arabidopsis.org/) and SSR loci that were not anchored to BACs, respectively. Common markers are linked by lines indicating colinearity between the species. The single letter marker assay type designators were used for different types of markers i.e.,: a, AFLP; e, ESTP; f, RFLP; m, SSR; p, CAPS; r, RAPD; t, STS; and At, IP markers. (Figure 1 continues on pages 944 and 945.)

fore, the average interlocus interval decreased from 1.45 cM and 3.47 cM in the CKDH and CRF_2 maps, respectively, to 1.24 cM in the integrated map along with an increase in marker density. The CRF_2 map allowed us to anchor 155 new SSR markers derived from 102 seed BACs and 61 new gene-specific IP markers to the integrated map, as more polymorphic BAC-derived SSR markers were found between Chiifu and RCBr than between Chiifu and Kenshin. Linkage groups A2 and A10 in the CKDH map contained only five and nine SSRs, respectively, to which 12 and 5 new SSR markers derived from BAC-end sequences of the CRF_2 map were added in the integrated map. A2 and A10 were further strengthened by the addition of 10 and 6 other PCR-based IP markers, respectively. The integrated consensus map increased the length of four linkage groups at the end. The top region of A7 and A8 was enlarged by the addition of one SSR locus (A7) and two SSR loci (A8) as well as one IP locus each from the CRF_2 map. The bottom of A10 was enlarged by the addition of three SSR loci from BAC-end sequences and three IP loci compared with the CKDH map. The length of A4 was increased toward the top by the addition of three SSR loci from BAC-end sequences and two IP loci; toward the bottom, the addition of two SSR loci from BAC-end sequences increased the length compared with the CKDH map.

Comparison of the *B. rapa* consensus map with the *B. juncea* map

The high-density *B. rapa* integrated map contains 146 gene-specific IP markers spread over the 10 linkage groups, with the number of marker loci varying from 10 in A4, A6, and A10 to 28 in A3 and A9. Of these markers, 98 were common to those mapped to the 10 homologous A-genome linkage groups of *B. juncea* (Panjabi et al. 2008). The number of marker loci between the homologous linkage groups of *B. rapa* and *B. juncea* vary from 4 in A5 to 22 in A9 (Fig. 1). The other markers were multiple copies mapped to the same region of the same block in other chromosomes. The position and order of most of the IP markers in the homologous linkage groups were colinear between the genomes. However, one to three marker loci inversions (change of order) were observed within a 5 cM interval (mostly within 1–3 cM) in most of the homologous linkage groups of *B. rapa* compared with *B. juncea* (Fig. 1). More than 10 cM inversions with one or two marker loci were also observed in linkage groups A9 and A10 of *B. rapa* compared with *B. juncea*. The IP markers covered most of the *B. rapa* linkage groups although the marker density was less. Further, we identified some BAC-derived SSR markers in the *B. rapa* linkage groups that could be mapped to the corresponding large gap in the *B. juncea* linkage groups, especially in J1, J4, J5, J7, and J10 (Panjabi et al. 2008).

Mapping of the 146 original IP loci and the 155 new BAC-derived SSR markers to the *B. rapa* genome helped us to identify 24 Brassicaceae genomic blocks (A–X) proposed by Schranz et al. (2006) more accurately. For genomic block identification with SSR markers, SSR sequences were aligned with *A. thaliana* DNA sequences as described previously by Kim et al. (2009). We identified 25 new genomic blocks in the *B. rapa* genome (Fig. S1[4]): five blocks each in A2 (W, E, K, L, and Q), A3 (W, I, O, H, and N), and A9 (O, Q, H, P, and A); two blocks each in A5 (B and C), A6 (Q and V), A7 (H and G), and A8 (C and T); and one block each in A4 (S), and A10 (W). However, we could not identify any new block in A1. The identified blocks showed the same orientation as the A-genome chromosomes in *B. juncea* (Panjabi et al. 2008) and *B. napus* (Parkin et al. 2005) (Fig. S1). In addition, the integration of the *B. rapa* and *B. juncea* linkage maps using IP markers allowed us to identify conserved regions of the A-genome chromosomes of *B. rapa* in *A. thaliana* chromosomal segments.

Discussion

The main objectives of this study were to develop a high-density integrated linkage map of *B. rapa* by using codominant BAC-derived SSR markers and align additional seed BACs in the *B. rapa* genome for the BrGSP. In the version II CKDH reference genetic map, only 188 seed BACs anchored to 191 SSR marker loci were mapped. Further mapping of BAC-anchored SSRs could not be performed owing to the lack of polymorphism between the parental inbred lines (Chiifu and Kenshin). Many gaps (5–10 cM) were found in few linkage groups. By the present study, we have added 95 additional PCR-based gene-specific IP markers that were mapped to the 10 A genome-specific linkage groups of *B. juncea* (Panjabi et al. 2008) to the version II CKDH reference genetic map of Kim et al. (2009). Further, we constructed a CRF_2 map based on genetically diverse Chinese cabbage inbred line Chiifu and RCBr. We found additional polymorphic BAC-derived SSR markers between these lines, allowing us to map 155 new codominant SSR markers derived from 102 seed BACs and 61 new gene-specific IP markers to the *B. rapa* genome compared with the CKDH map.

We also developed an integrated high-density *B. rapa* genetic map by combining the two individual maps. In the integrated map, we mapped and aligned 102 new *B. rapa* seed BACs in addition to the 146 gene-specific IP markers,

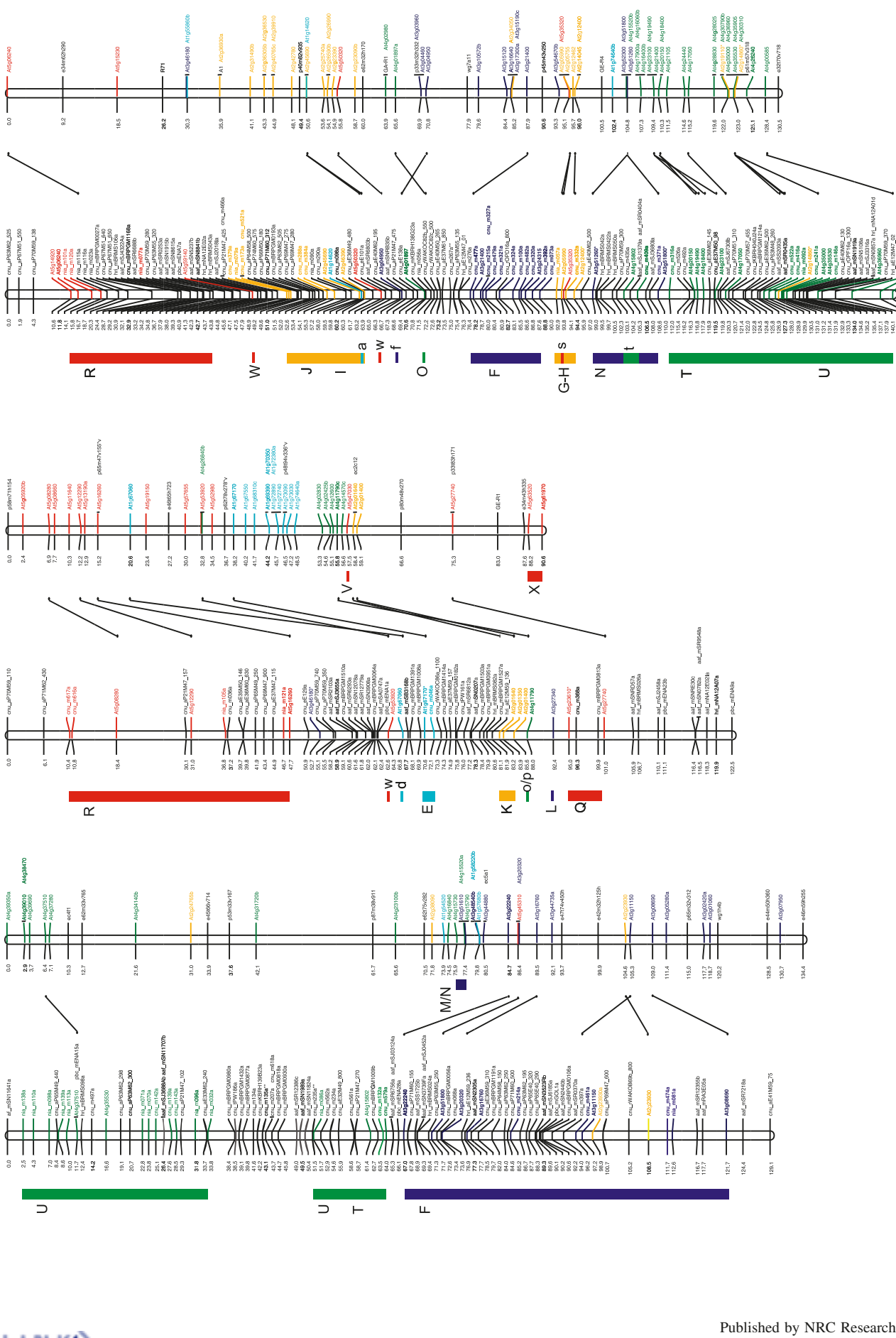


Fig. 1 (*continued*).

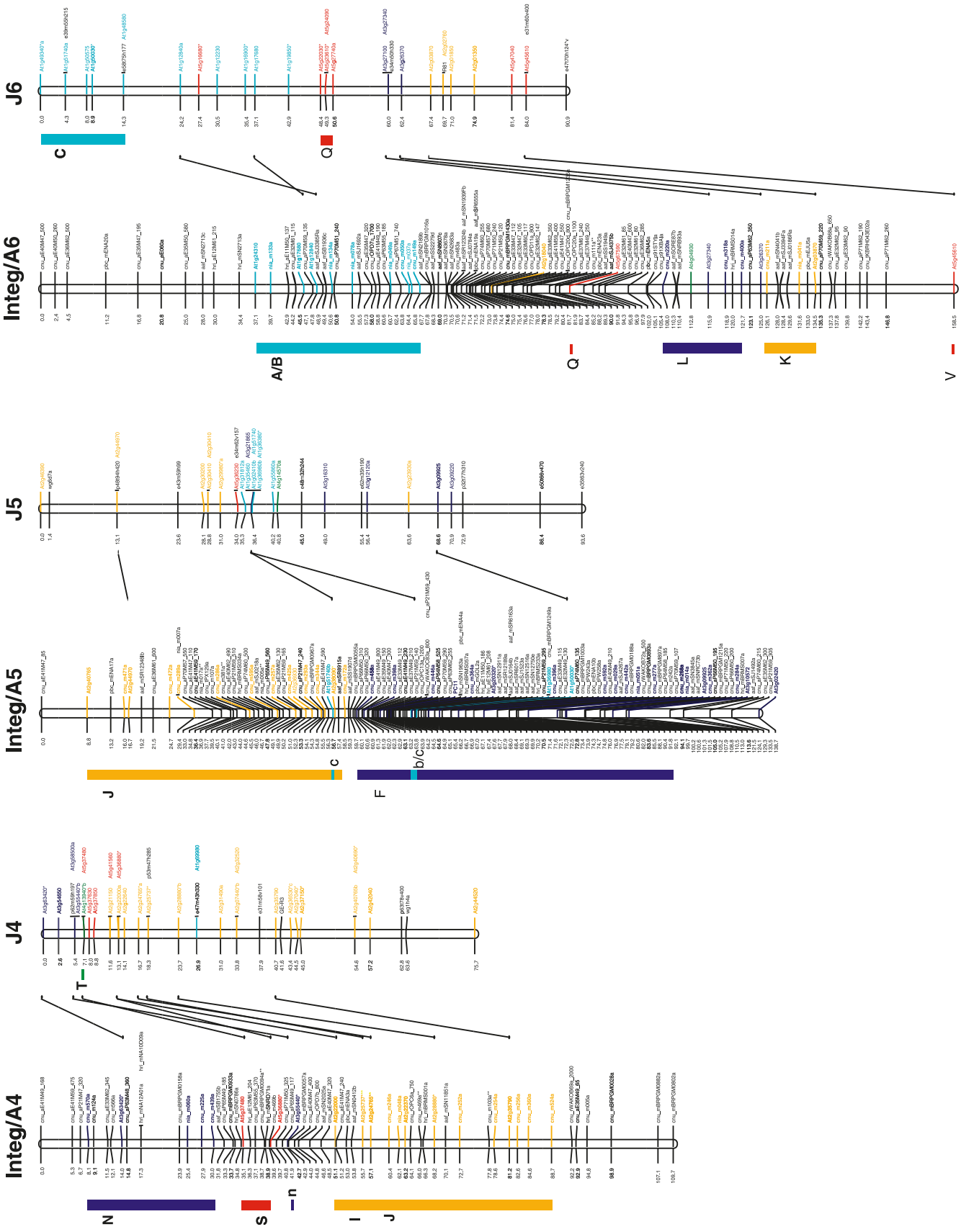
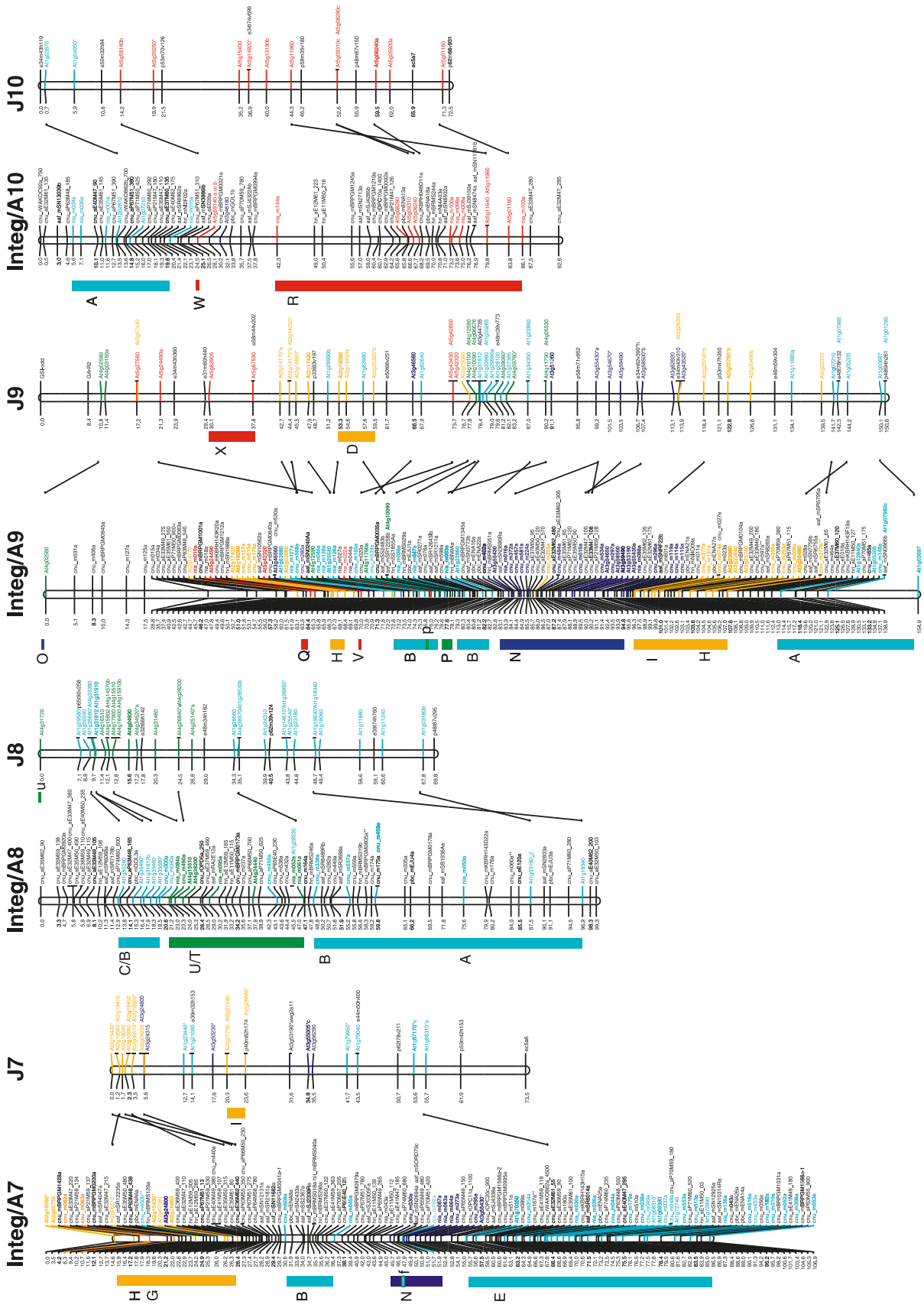


Fig. 1 (*concluded*).



which helped not only to increase the marker density by filling large gaps in both the individual maps, but also to enlarge the genome coverage of *B. rapa*, although some large intervals still exist (in A2, A4, A6, A9, and A10) (Fig. 1). The number of BACs per linkage group in the integrated map ranged from 14 in A10 to 50 in A9. The work of Mun et al. (2008), who constructed the first BAC-based physical map of *B. rapa*, serves as the point for bidirectional chromosomal extension in genome sequencing. Our high-density integrated map would supplement the correct alignment of seed BACs in the 10 chromosomes of *B. rapa*. In addition, the sequenced BAC clones anchored on the integrated genetic map could serve as the basis for integration with the physical map of *B. rapa.*

The overall order and positions of the markers remained the same in the CKDH, CRF_2, and integrated maps despite minor local inversions within a 5 cM distance in many linkage groups; few markers showed longer distance inversions (in A2, A3, A4, and A9). Such inversions have been frequently observed during map integration in *Arabidopsis* (Hauge et al. 1993), loblolly pine (Sewell et al. 1999), *B. olearcea* (Sebastian et al. 2000), lettuce (Jeuken et al. 2001), rape seed (Lombard and Delourme 2001), sugi (Tani et al. 2003), soybean (Hwang et al. 2009), rye (Gustafson et al. 2009), sorghum (Mace et al. 2009), and pepper (Lee et al. 2009). The reasons for inversions during map integration include small discrepancies in marker order due to mapping imprecision in small mapping populations (Lombard and Delourme 2001), closely spaced markers in one population (Mace et al. 2009), distorted segregation of markers, use of different mapping software (Wu et al. 2008), and real inversions. The first three reasons might explain the inversions in this study, because we found a high degree of colinearity in marker order with the homologous A-genome linkage groups of *B. juncea* despite inversions among the CKDH, CRF_2, and integrated maps.

The increased number of PCR-based IP markers in our map allowed us to compare the homologous A genome linkage groups between the *B. rapa* genome and the *B. juncea* genome which showed conservation of order and position of most markers, except for the markers showing inversion. This finding suggests that although *B. juncea* and *B. rapa* evolved independently, their A-genome chromosomes are still conserved at the macro level. Previous mapping studies also reported conservation of the A-genome linkage groups between *B. juncea* and *B. rapa*; and *B. napus* and *B. rapa*, respectively (Axelsson et al. 2000; Suwabe et al. 2008). Many studies have also shown conservation of chromosomes between *Brassica* species and with *A. thaliana* at the micro or macro level (O'Neill and Bancroft 2000; Rana et al. 2004; Parkin et al. 2005; Panjabi et al. 2008; Kim et al. 2009). By comparing the linkage groups of *B. rapa* and *B. juncea*, we confirmed previously reported crucifer genomic blocks (Parkin et al. 2005; Schranz et al. 2006; Panjabi et al. 2008; Kim et al. 2009) in our map. Panjabi et al. (2008) used IP markers to identify and establish interrelationships between the A, B, and C genomes of *Brassica* species, and identified the 24 crucifer building blocks (A–X) reported by Schranz et al. (2006) through comparative mapping of *A. thaliana* with the three genomes of *Brassica* species. With our high-density *B. rapa* integrated map, we could identify genomic blocks more accurately in all the 10 linkage groups: 25 previously unidentified blocks were identified in nine linkage groups of *B. rapa*. All the genomic blocks in A8 and A10 have been identified. However, 14 other genomic blocks in eight linkage groups of *B. rapa* compared with *B. napus* (Parkin et al. 2005) and *B. juncea* (Panjabi et al. 2008) still need to be identified (Fig. S1). We believe that the addition of IP markers in these regions would help to identify the unidentified blocks in the *B. rapa* genome.

Ramchiary et al. (2007) mapped many yield-related QTL in linkage groups J10 and J7 of *B. juncea* using AFLP and RFLP markers. Their map was later completely updated with PCR-based IP markers by Panjabi et al. (2008). Comparative mapping between the BAC-anchored high-density *B. rapa* map in the present study and the *B. juncea* map of Panjabi et al. (2008) would help to apply the BrGSP sequence information for efficient breeding of *B. juncea*. In addition, the transfer of information between the *B. rapa* and *B. juncea* genomes and the demarcation of *Arabidopsis* chromosomal blocks in the *B. rapa* genome would facilitate the identification and isolation of candidate genes contributing to traits of agronomic value in *B. juncea* and other *Brassica* crops.

Acknowledgements

The authors are highly thankful to Deepak Pental and Akshay K. Pradhan, Department of Genetics, University of Delhi South Campus, New Delhi, India, for kindly providing the *B. juncea* map used in this study. This work was supported by grants for the Technology Development Program for Agriculture and Forestry (Grant No. 607002-05), Ministry of Agriculture, Forestry and Fisheries, Republic of Korea.

References

Axelsson, T., Bowman, C.M., Sharpe, A.G., Lydiate, D.J., and Lagercrantz, U. 2000. Amphidiploid *Brassica juncea* contains conserved progenitor genomes. Genome, **43**(4): 679–688. doi:10.1139/gen-43-4-679. PMID:10984181.

Choi, S.R., Teakle, G.R., Plaha, P., Kim, J.H., Allender, C.J., Beynon, E., et al. 2007. The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. Theor. Appl. Genet. **115**(6): 777–792. doi:10.1007/s00122-007-0608-z. PMID:17646962.

Gustafson, J.P., Ma, X.F., Korzun, V., and Snape, J.W. 2009. A consensus map of rye integrating mapping data from five mapping populations. Theor. Appl. Genet. **118**(4): 793–800. doi:10.1007/s00122-008-0939-4. PMID:19066841.

Hauge, B.M., Hanley, S.M., Cartinhour, S., Cherry, J.M., Goodman, H.M. , Koornneef, M., et al. 1993. An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. **3**(5): 745–754. doi:10.1111/j.1365-313X.1993.00745.x.

Hwang, T.Y., Sayama, T., Takahashi, M., Takada, Y., Nakamoto, Y., Funatsuki, H., et al. 2009. High-density integrated linkage map based on SSR markers in soybean. DNA Res. **16**(4): 213–225. doi:10.1093/dnares/dsp010. PMID:19531560.

Jeuken, M., Wijk, R., Peleman, J., and Lindhout, P. 2001. An integrated interspecific AFLP map of lettuce (*Lactuca*) based on two *L. sativa* × *L. saligna* F_2 populations. Theor. Appl. Genet. **103**: 638–647. doi:10.1007/s001220100657.

Kim, H., Choi, S.R., Bae, J., Hong, C.P., Lee, S.Y., Hossain, M.J.,

et al. 2009. Sequenced BAC anchored reference genetic map that reconciles the ten individual chromosomes of *Brassica rapa*. BMC Genomics, **10**(1): 432. doi:10.1186/1471-2164-10-432. PMID:19751531.

Kosambi, D.D. 1944. The estimation of map distance from recombination values. Ann. Eugen. **12**: 172–175.

Lagercrantz, U. 1998. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics, **150**(3): 1217–1228. PMID:9799273.

Lagercrantz, U., and Lydiate, D.J. 1996. Comparative genome mapping in *Brassica*. Genetics, **144**(4): 1903–1910. PMID: 8978073.

Lee, H.R., Bae, I.H., Park, S.W., Kim, H.J., Min, W.K., Han, J.H., et al. 2009. Construction of an integrated pepper map using RFLP, SSR, CAPS, AFLP, WRKY, rRAMP, and BAC end sequences. Mol. Cells, **27**(1): 21–37. doi:10.1007/s10059-009-0002-6. PMID:19214431.

Lombard, V., and Delourme, R. 2001. A consensus linkage map for rapeseed (*Brassica napus* L.): construction and integration of three individual maps from DH populations. Theor. Appl. Genet. **103**(4): 491–507. doi:10.1007/s001220100560.

Mace, E.S., Rami, J.-F., Bouchet, S., Klein, P.E., Klein, R.R., Kilian, A., et al. 2009. A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. BMC Plant Biol. **9**(1): 13. doi:10.1186/1471-2229-9-13. PMID:19171067.

Mun, J.H., Kwon, S.J., Yang, T.J., Kim, H.S., Choi, B.S., Baek, S., et al. 2008. The first generation of a BAC-based physical map of *Brassica rapa*. BMC Genomics, **9**(1): 280. doi:10.1186/1471-2164-9-280. PMID:18549474.

Mun, J.H., Kwon, S.J., Yang, T.J., Seol, Y.J., Jin, M., Kim, J.A., et al. 2009. Genome-wide comparative analysis of the *Brassica rapa* gene space reveals genome shrinkage and differential loss of duplicated genes after whole genome triplication. Genome Biol. **10**(10): R111. doi:10.1186/gb-2009-10-10-r111. PMID: 19821981.

Nozaki, T., Kumazaki, A., Koba, T., Ishikawa, K., and Ikehashi, H. 1997. Linkage analysis among loci for RAPDs, isozyme and some agronomic traits in *Brassica campestris* L. Euphytica, **95**(1): 115–123. doi:10.1023/A:1002981208509.

O'Neill, C.M., and Bancroft, I. 2000. Comparative physical mapping of segments of the genome of *Brassica oleracea* var. *alboglabra* that are homoeologous to sequenced regions of chromosomes 4 and 5 of *Arabidopsis thaliana*. Plant J. **23**(2): 233–243. doi:10.1046/j.1365-313x.2000.00781.x. PMID: 10929117.

Panjabi, P., Jagannath, A., Bisht, N.C., Padmaja, K.L., Sharma, S., Gupta, V., et al. 2008. Comparative mapping of *Brassica juncea* and *Arabidopsis thaliana* using Intron Polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C *Brassica* genomes. BMC Genomics, **9**(1): 113. doi:10.1186/1471-2164-9-113. PMID:18315867.

Parkin, I.A.P., Gulden, S.M., Sharpe, A.G., Lukens, L., Trick, M., Osborn, T.C., and Lydiate, D.J. 2005. Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics, **171**(2): 765–781. doi:10.1534/genetics.105.042093. PMID:16020789.

Ramchiary, N., Padmaja, K.L., Sharma, S., Gupta, V., Sodhi, Y.S., Mukhopadhyay, A., et al. 2007. Mapping of yield influencing QTL in *Brassica juncea*: implications for breeding of a major oilseed crop of dryland areas. Theor. Appl. Genet. **115**(6): 807–817. doi:10.1007/s00122-007-0610-5. PMID:17646960.

Rana, D., van den Boogaart, T., O'Neill, C.M., Hynes, L., Bent, E., Macpherson, L., et al. 2004. Conservation of the microstructure of genome segments in *Brassica napus* and its diploid relatives. Plant J. **40**(5): 725–733. doi:10.1111/j.1365-313X.2004.02244.x. PMID:15546355.

Schranz, M.E., Lysak, M.A., and Mitchell-Olds, T. 2006. The ABC's of comparative genomics in the Brassicaceae: building blocks of crucifer genomes. Trends Plant Sci. **11**(11): 535–542. doi:10.1016/j.tplants.2006.09.002. PMID:17029932.

Sebastian, R.L., Howell, E.C., King, G.J., Marshall, D.F., and Kearsey, M.J. 2000. An integrated AFLP and RFLP *Brassica oleracea* linkage map from two morphologically distinct doubled-haploid mapping populations. Theor. Appl. Genet. **100**(1): 75–81. doi:10.1007/s001220050011.

Sewell, M.M., Sherman, B.K., and Neale, D.B. 1999. A consensus map for loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics, **151**(1): 321–330. PMID:9872970.

Snowdon, R.J., and Friedt, W. 2004. Molecular markers in *Brassica* oilseed breeding: current status and future possibilities. Plant Breed. **123**(1): 1–8. doi:10.1111/j.1439-0523.2003.00968.x.

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. **3**(5): 739–744. doi:10.1111/j.1365-313X.1993.00739.x.

Suwabe, K., Tsukazaki, H., Iketani, H., Hatakeyama, K., Kondo, M., Fujimura, M., et al. 2006. Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics, **173**(1): 309–319. doi:10.1534/genetics.104.038968. PMID: 16723420.

Suwabe, K., Morgan, C., and Bancroft, I. 2008. Integration of *Brassica* A genome genetic linkage map between *Brassica napus* and *B. rapa*. Genome, **51**(3): 169–176. doi:10.1139/G07-113. PMID:18356952.

Tani, N., Takahashi, T., Iwata, H., Mukai, Y., Ujino-Ihara, T., Matsumoto, A., et al. 2003. A consensus linkage map for sugi (*Cryptomeria japonica*) from two pedigrees, based on microsatellites and expressed sequence tags. Genetics, **165**(3): 1551–1568. PMID:14668402.

Teutonico, R.A., and Osborn, T.C. 1994. Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus*, *B. oleracea*, and *Arabidopsis thaliana*. Theor. Appl. Genet. **89**(7–8): 885–894. doi:10.1007/BF00224514.

Van Ooijen, J.W., and Voorrips, R.E. 2001. JoinMap. Version 4.0 [computer program]. Plant Research International, Wageningen, the Netherlands.

Voorrips, R.E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. **93**(1): 77–78. doi:10.1093/jhered/93.1.77. PMID:12011185.

Wu, Y.H., Close, T.J., and Lonardi, S. 2008. On the accurate construction of consensus genetic maps. Comput. Syst. Bioinformatics Conf. **7**: 285–296. PMID:19642288.

Yang, T.-J., Kim, J.-S., Lim, K.-B., Kwon, S.-J., Kim, J.A., Jin, M., et al. 2005. The Korea *Brassica* Genome Project: a glimpse of the *Brassica* genome based on comparative genome analysis with *Arabidopsis*. Comp. Funct. Genomics, **6**(3): 138–146. doi:10.1002/cfg.465. PMID:18629219.

Zhang, J., Lu, Y., Yuan, Y., Zhang, X., Geng, J., Chen, Y., et al. 2009. Map-based cloning and characterization of a gene controlling hairiness and seed coat color traits in *Brassica rapa*. Plant Mol. Biol. **69**(5): 553–563. doi:10.1007/s11103-008-9437-y. PMID:19039665.