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ORIGINAL PAPER

An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers

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Abstract Sequence related amplified polymorphism (SRAP) was used to construct an ultradense genetic recombination map for a doubled haploid (DH) population in *B. napus*. A total of 1,634 primer combinations including 12 fluorescently labeled primers and 442 unlabeled ones produced 13,551 mapped SRAP markers. All these SRAPs were assembled in 1,055 bins that were placed onto 19 linkage groups. Ten of the nineteen linkage groups were assigned to the A genome and the remaining nine to the C genome on the basis of the differential SRAP PCR amplification in two DH lines of *B. rapa* and *B. oleracea*. Furthermore, all 19 linkage groups were assigned to their corresponding N1–N19 groups of *B. napus* by comparison with 55 SSR markers used to construct previous maps in this species. In total, 1,663 crossovers were detected, resulting in a map length span of 1604.8 cM. The marker density is 8.45 SRAPs per cM, and there could be more than one marker in 100 kb physical distance. There are four linkage groups in the A genome with more than 800 SRAP markers each, and three linkage groups in the C genome with more 1,000 SRAP markers each. Our studies suggest that a single SRAP map might be applicable to the three *Brassica* species, *B. napus*, *B. oleracea* and *B. rapa*. The use of this ultra high-density genetic recombination map in marker development and map-based gene cloning is discussed.

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Introduction

Highly saturated genetic maps constructed with molecular markers are basic tools for sequencing a genome, for developing specific markers linked closely to phenotypic traits for marker assisted selection (MAS) in crop breeding, and for cloning of genes controlling the traits of interest through map-based strategies. Molecular markers derive from sequence polymorphism in a genome. On the basis of the whole genome sequence of rice, it has been suggested that thousands or even millions of single nucleotide polymorphim (SNPs) and insertions and deletions (INDELs) could occur in this species (Feltus et al. 2004; Shen et al. 2004). These sequence polymorphisms can be used to develop molecular markers through SNP discovery. However, after the genome sequencing of most major crop plants

is completed, enormous efforts to validate and confirm each individual SNP/INDEL will be required to fully use these markers to construct highly saturated genetic maps through SNP detection. To efficiently and effectively exploit the sequence variation in genomes, exploring other molecular markers that have similar potential to SNP detection is justified.

In potato, van Os et al. (2006) constructed an ultradense genetic recombination map containing 10,000 amplified fragment length polymorphism (AFLP) markers. In other crops, Bowers et al. (2003) constructed in sorghum a map containing 2,512 sequence tagged-site (STS) loci, which can serve as a framework for comparative study of many grains and grasses. In cotton a genetic recombination map with 3,347 STS loci has also been assembled (Rong et al. 2004). There are no ultradense maps available in *Brassica* species. The largest map reported is for the oilseed rape species *B. napus*, consisting of 1,317 RFLP markers, which was successfully used to compare the *Brassica* genomes A and C with the *Arabidopsis* genome (Parkin et al. 2005).

Sequence-related amplified polymorphism (SRAP) is a simple marker detection method that was recently developed (Li and Quiros 2001). It is easily adapted to efficiently perform high throughput data collection from thousands or even millions of individuals, which is critical to any large-scale plant breeding program. It has been successfully applied in several species for different purposes (Budak et al. 2004a, b; Ferriol et al. 2003). Since SRAP can theoretically detect any kind of sequence differences including base changes and INDELs, there are as many of these markers as SNPs in a genome. In contrast to SNPs, a primer pair combination in the SRAP protocol can detect multiple loci in a genome without previous knowledge of sequence information, such as is required for SNP discovery. We report here in an ultradense map for *B. napus* constructed with SRAP markers using a capillary ABI 3100 DNA analyzer (Applied Biosystems, California), which will serve as a platform to perform efficient and effective marker development for many important phenotypic traits in canola and rapeseed. These can be used to implement map-based cloning of Mendelian genes that condition qualitative traits and Mendelized quantitative loci (QTL) among other uses.

Materials and methods

Plant materials

A doubled haploid (DH) population with 58 lines was used to construct the SRAP map. This DH population was produced from a cross of a Canadian spring type canola, ‘Westar’, and a Chinese semi-winter type rapeseed cultivar, ‘Zhongyou 821’. The diverse genetic background of these two parental lines produced a high level of polymorphism among the DH lines. *B. napus* (AC) is a natural allotetraploid that evolved naturally through the interspecific hybridization of *B. rapa* (A) and *B. oleracea* (C) (U 1935). For determining the species origin of the linkage groups on the SRAP map, a *B. rapa* DH line, ‘RI16’, and a *B. oleracea* DH line, ‘B453’ (cauliflower) were included in the analysis. ‘B454’ (broccoli) and ‘B453’ were included to detect polymorphic loci between cauliflower and broccoli with the same primer combinations for the mapping.

Primers

In the original SRAP protocol, PCR products were detected by autoradiography (Li and Quiros 2001). In the present study, SRAP products were separated with an ABI 3100 DNA analyzer, using a five-color fluorescent dye set, including ‘FAM’ (blue), ‘VIC’ (green), ‘NET’ (yellow) and ‘PET’ (red), and ‘LIZ’ (orange), for signal detection. The ‘LIZ’ was used for labeled size standard and the other four fluorescent dye colors for labeled primers. Three primers for each dye color for a total 12 primers were synthesized and labeled by the ABI Company (Foster City, California) (Table 1). Since two primers with different sequences in a SRAP PCR reaction are critical to produce a good SRAP profile, one labeled primer could be easily combined with numerous unlabeled primers having some level of sequence dissimilarity, such as the primers that come from the primer sets in our original protocol (Li and Quiros 2001). In the present study, some of the original primers, including ‘EM1’, ‘EM2’, ‘EM3’, ‘EM5’, ‘EM6’ and ‘EM8’ (the ‘AATT’ set), and ‘ME1’, ‘ME2’, ‘ME3’, ‘ME4’, ‘ME5’, ‘ME6’, ‘ME9’ and ‘ME10’ (the ‘CCGG’ set), were used. In addition to the conventional SRAP primers, new primers used in this paper were designed on the basis of known sequences for cloning *BoGSL-ALK*, *BoGSL-ELONG*, *BoGSL-PRO* involved in the biosynthesis of aliphatic glucosinolates in *B. oleracea* (Li and Quiros 2002, 2003; Li et al. 2003). Although the new primers were not designed as typical SRAP primer sets, the general principles of 35–55% GC content and 17–22 nucleotides in size were followed. In total, 1,634 primer pairs were selected from the 12 labeled and 442 unlabeled primers and used for the map construction (online supplementary data, S1)

Table 1 Summary of labeled primer information for the 12-labeled primers

Primer name	Fluorescent dye	Primer sequence	No. of primer combinations for each labeled primer	Total No. of markers for one labeled primer	Average No. of markers per primer combination
ME2	6-FAM	TGAGTCCAAACCGGAGC	236	1,422	6.03
EM2	NED	GACTGCGTACGAATTCTGC	215	1,210	5.63
DC1	PET	TAAACAATGGCTACTCAAG	184	998	5.42
EM1	VIC	GACTGCGTACGAATTCAAT	263	2,007	7.63
ODD3	6-FAM	CCAAAACCTAAAACCAGGA	70	635	9.07
SA12	NED	TTCTAGGTAATCCAACAACA	77	568	7.38
ODD20	PET	TCGTTGTTATGGCTGGAGA	76	533	7.01
GA3	VIC	TCATCTCAAACCATCTACAC	76	646	8.50
FC1	6-FAM	TCAAGGGCAGGTAAGAACAA	107	1,284	12.00
BG23	NED	ATTCAAGGAGAGTGCGTGG	111	1,430	12.88
PM88	PET	CGAAACCTCACCTCTCTCA	103	941	9.14
SA7	VIC	CGCAAGACCCACCACAA	116	1,877	16.18
Total			1634	13,551	8.29

DNA extraction protocol and PCR amplification program

DNA was extracted with 2× CTAB buffer as previously described (Li and Quiros 2001), but with the following major modifications. After incubation of the samples at 65°C for 90 min, an equal volume of chloroform was added to the eppendorf tubes containing the samples, and the tubes were thoroughly vortexed. Another modification was the precipitation of DNA with a reduced amount of iso-propanol, 0.5–0.55 volume of the supernatant, and reduced centrifuge speed to 5,000 rpm for 3 min. The PCR amplification program was the same as reported in the original SRAP protocol (Li et al. 2001).

Marker detection with ABI 3100 DNA analyzer

All PCR reactions were set up with 10 μl volume in 384-well plates using the same components reported by Li et al. (2001). Normally, all samples in one plate had the same labeled primer, and samples from four different color labeled primers in four plates were pooled together after running PCR reactions in a thermocycler. 2.5 μl of the pooled samples was added to a 5.5 μl mixture of formamide and 500-LIZ size standard (ABI), and then denatured at 95°C for 3 min. The plates containing the samples were then loaded into the auto sampler of the ABI 3100 DNA analyzer, and the SRAP products were separated using 36-cm 16-channel arrays with a 40 min running time.

Data analysis

After data were collected by the ABI 3100 DNA analyzer, they were analyzed with Genscan software (ABI). Then the previously analyzed data were exported and loaded into another program, 'Genographer' (available at http://www.hordeum.oscs.montana.edu/genographer), to produce an image that looked like a gel picture. 'Genographer' allowed scoring each polymorphic locus by drawing a double-line window, and the scores were then converted directly into MAPMARKER compatible data, which were copied and pasted into a Microsoft Excel sheet. Each band showing polymorphism was scored as a dominant marker.

Map construction

MAPMAKER v2.0 running on a Macintosh computer was used to construct the genetic recombination map (software available online at http://www.linkage.rockefeller.edu/soft/mapmaker/). First, we divided more than 15,000 scored SRAPs into 15 subsets of which each contained about 1,000 markers since MAPMAKER v2.0 can only accept 1,020 markers at once. Second, a LOD value of 6.0 was used to group each data subset, and three to five markers from each group were selected to form the next data set. After the first round of grouping and selection, the selected markers was reduced to approximately 2,000 for the whole data set, and they were run in two data subsets to get new groups for these 2,000 markers. This procedure was performed through several rounds of grouping and selection until the whole data set was divided into less than 30 groups. Third, each group containing 300–1,500 markers were grouped with a LOD value of 15, and with this high LOD value, the markers in the same group not showing any recombination among these 58 DH lines, were assigned into a bin. Next, one marker was selected from each bin, and approximately 1,000 selected markers from the whole

marker pool were used to assemble a framework map containing 19 linkage groups that would represent the 19 chromosomes of *B. napus*. Finally, all markers were assigned into these 19 linkage groups based on the grouping information in the previous steps.

Recombination points in each DH line were checked carefully in a Microsoft Excel spreadsheet using the sorting function in the Excel software. The recombination points were searched manually and the minimum recombination events were determined with the Excel spreadsheet through reordering of the adjacent markers and removing the singletons that were attributed to the missing data and scoring errors. The final version of the genetic recombination map was calculated with Mapmaker Macintosh v2.0.

Alignment to other maps

Ninety of the simple sequence repeats (SSR) markers reported by Piquemal et al. (2005) were tested in our mapping population to permit alignment of both maps. Additionally one new SSR primer pair, ′AGGGTTTG AGATATAGTGT′, ′TGGCGATGAATTTGCAA GG′, based on the sequence of the BAC clone (AC152123) was also used to align one of our SRAP linkage groups to N15, since the SSR markers on that group were not polymorphic in our mapping population. The SSR primer sequences were taken from the electronic supplementary material by Piquemal et al. (2005) and the M13 sequence, (CACGACGTTGTAAAACG AC), was put at the beginning of each forward primer for the detection of PCR products. The M13 primer was labeled by the ABI Company and added to the PCR reaction mix. The components in PCR reactions and the PCR amplification program were the same as described by Piquemal et al. (2005). The PCR products were separated with an ABI 3100 DNA analyzer. After data collection and analysis, the SSR mapping data were run in Mapmaker together with 1,055 SRAP markers, of which one marker was taken from each bin on the SRAP map. All SSR markers were integrated into the corresponding SRAP marker bins on the SRAP map. The N1–N19 linkage group nomenclature on the SSR map constructed by Piquemal et al. (2005) was used to name the linkage groups on our ultradense SRAP *B. napus* map.

Results

Recombination map

A total of 1,634 primer combinations produced over 15,000 bands scored which showed polymorphism. All SRAP markers were scored as dominant. Table 1 illustrates the mapped SRAP markers that were inventoried on the basis of the 12-labeled primers as explained in Materials and methods. The average number of mapped primers per primer combination is 8.29. The best labeled primer, 'SA7', was used in 116 primer combinations, producing 16.18 markers per primer combination on average (Table 1).

With more than 15,000 recorded SRAP markers, and through grouping and regrouping of the whole data and reordering of adjacent markers by minimizing the recombination events and removing 1,005 singletons, 13,551 SRAP markers were assembled into 19 linkage groups to form a genetic recombination map. All markers were listed with their marker name and size, their bin position on the map, and the primer pairs used for producing the markers (online supplementary data, S2). Nine of these 19 linkage groups are presumed to represent the nine chromosomes of the C genome in *B. napus* genome, based on the alignment of these linkage groups with the published N11–N19 groups cytologically assigned to their corresponding chromosomes (Howell et al. 2002).

Assigning linkage groups into the A and C genomes

The following banding patterns were detected in *B. napus* and the two diploid species. First, a group of bands that were amplified in *B. napus*, but not in *B. rapa* or *B. oleracea*, indicating that sequence divergence occured between *B. napus* and its diploid parents. Second, some bands appeared in all three species, representing the highly conserved sequences in the A, C and AC genomes. The third group of the bands was amplified in *B. napus* and *B. rapa*, but not in *B. oleracea*, most of which would likely represent A-genome specific amplifications. Finally the last group of the bands was amplified in B. *napus* and *B. oleracea*, but not in *B. rapa*, thus being most likely C-genome specific. With these preliminary testing results, 5,527 mapped SRAP markers in the *B. napus* DH population were also tested in the DH B. *rapa* and B. *oleracea* lines for comparison of the bands from the A, C and AC genomes. As described previously, all bands tested could be placed into one of these four groups when the band distribution on the same linkage group were checked and counted separately (Table 2). A total of 2,566 (46.4%) of the 5,527 mapped marker loci only appeared in *B. napus*. On the other hand, 575 (10.4%) loci were found in all three species. Some of 1,132 (20.5 %) loci appeared only in *B. rapa* and *B. napus*, and 1,253 (22.7 %), only in *B. oleracea* and *B. napus*. Those last two groups with 2,385 loci that appeared in only

Table 2 Distribution of mapped SRAP markers with the corresponding bands detected in *B. rapa* and *B. oleracea* for assigning linkage groups to the A and C genomes in *B. napus*

Linkage group	Grouping of mapped SRAP markers and percentage of SRAPs in one of two diploid species						
	BB	AA	BA	AB	BA + AB	BA/(BA + AB)*100	AB/(BA + AB)*100
N01	127	17	95	22	117	81.2	18.8
N02	157	22	98	27	125	78.4	21.6
N03	137	12	69	24	93	74.2	25.8
N04	134	16	81	20	101	80.2	19.8
N05	201	54	120	33	153	78.4	21.6
N06	229	27	111	38	149	74.5	25.5
N07	169	35	99	71	170	58.2	41.8
N08	138	30	76	33	109	69.7	30.3
N09	68	109	74	64	138	53.6	46.4
N10	101	13	81	15	96	84.4	15.6
A genome	1,461	335	904	347	1,251	72.3	27.7
N11	119	24	27	101	128	21.1	78.9
N12	243	34	31	193	224	13.8	86.2
N13	189	45	40	168	208	19.2	80.8
N14	127	19	16	108	124	12.9	87.1
N15	51	24	24	41	65	36.9	63.1
N16	23	18	11	21	32	34.4	65.6
N17	114	22	16	88	104	15.4	84.6
N18	38	7	17	43	60	28.3	71.7
N19	201	47	46	143	189	24.3	75.7
C genome	1,105	240	228	906	1,134	20.1	79.9
Total	2,566	575	1,132	1,253	2,385	47.5	52.5

BB number of mapped SRAP markers without counterparts in diploid species, *AA* number of mapped SRAP markers with counterparts in both diploid species, *BA* number of mapped SRAP makers with counterparts only in *B. rapa*, *AB* number of mapped SRAP markers with counterparts only in *B. oleracea*

one of the two diploid genomes allowed us to assign the 19 linkage groups into their corresponding genomes. Eventually, when the bands amplified in the A and AC or in the C and AC genomes on each of the 19 linkage groups were counted, 10 linkage groups have more bands (53.6–84.4%) that amplified only in the A and AC genomes of *B. rapa* and *B. napus* so these were assigned into the A genomes. On the remaining nine linkage groups, more bands (63.1–87.1%) were found only in the C and AC genomes, and these were assigned into the C genome.

Alignment of the SRAP map with other maps

The 19 linkage groups that were previously assigned into the A and C genomes were further analyzed with SSR markers including 90 of the markers on the SSR map of Piquemal et al. (2005). With these 90 SSR primer pairs, 79 SSR loci were integrated on the SRAP map. Since most SSR primer pairs produce multiple marker loci, every mapped SSR marker on the SRAP map was checked carefully and only those mapped SSR markers on the SRAP map showing similar size to that obtained by Piquemal et al. (2005) were used to assign the SRAP linkage groups. Others, which showed different sizes were listed as New marker loci (Table 3). Fifteen out of the nineteen linkage groups were easily assigned into the same N number linkage groups as those on the SSR map, but there was some discrepancy for three groups; N4, N14 and N19. Six SSR markers on the Piquemal N4 linkage group mapped onto a SRAP linkage group that was assigned into the C genome, based on the differential SRAP PCR amplification in *B. rapa* and *B. oleracea*. Since N4 is a linkage group assigned to the A genome and the N4 and N14 (C genome) share a high level similarity on the RFLP map of Parkin et al. (2005), the SRAP linkage group that was integrated with the six SSR markers on the N4 linkage group of the SSR map was assigned as the N14 linkage group on the current SRAP map. However, four SSR markers on the Piquemal N14 linkage map were integrated onto another SRAP linkage group. According to the mapped markers available at http://www.brassica.bbsrc.ac.uk/IMSORB and the mapped SSR markers at Huazhong Agricultural University (Drs Tu and Fu, personal communication), two of these four SSR markers, Na12-G04 and Ra2-F11, were mapped on the N19 linkage group by two different labs. Therefore the SRAP linkage group which has the corresponding SSR markers on the Piquemal N14 map was assigned instead to the N19 linkage group. Unfortunately, none of the SSR markers on the Piquemal

Table 3 Distribution of integrated SSR markers on the SRAP map for assigning the SRAP linkage groups into N1–N19 linkage groups

Primer name	Size of PCR products[a]	Linkage group and bin position	Map position (cM) on the SRAP map	Linkage group on the SSR map	Map position (cM) on the SSR map[b]	Markers with the same order on both maps
CB10099	217	N01–11	17.4	N01	39.7	Yes
BRAS041	236	N01–16	25.7	N01	65.6	Yes
BRAS026a	198	N01–27	48	N01	96.4	Yes
BRAS083a	250	N02–17	25.6	N02	186	Yes
Na14-H11	131	N02–18	26.5	N02	175	Yes
BRAS011	222	N02–32	48.5	New		
BRAS002a	230	N03–02	0.9	*n19	34.9	Yes
CB10034	230	N03–02	0.9	New		
Na10-b11	221	N03–02	0.9	*n19	45.4	Yes
CB10036a	137	N03–35	63.1	N03	30.8	Yes
NA12-E02a	136	N03–43	74.1	N03	60.2	Yes
CB10347	214	N04–25	24.3	*n9	19.9	
CB10493a	174	N04–48	55.5	N04	170	
CB10545	98	N05–01	0	N05	5.7	Yes
BRAS002b	202	N05–21	21.6	New		
BRAS026b	179	N05–30	29.7	New		
CB10080	163	N05–44	45.1	N05	86.8	Yes
CB10229a	271	N05–59	60.5	New		
CB10229b	268	N05–64	67.8	New		
CB10487	268	N05–64	67.8	New		
Na12-D08	142	N06–11	17.5	N06	121	
BRAS026c	173	N06–46	59.2	New		
Na12-A02a	211	N07–47	75.4	New		
Na10-C06	225	N07–57	94.6	*n16	85.3	
Na12-A02b	181	N07–78	132.6	N07	98.7	
NI4-D09a	223	N08–14	15.4	*n24	20	
Ra2-E12	224	N08–27	71.2	N08	58.6	Yes
CB10364	252	N08–29	73.9	N08	18.1	Yes
CB10124a	170	N09–11	20.4	*n10	109	
NA10-A08	170	N09–20	28.5	N09	119	
CB10124b	164	N09–24	26.7	*n15	0	
CB10199a	173	N09–59	43.9	New		
Na12-H04	113	N10–01	0	N10	98.7	
CB10124c	173	N10–02	0.9	N10	109	Yes
MR156	209	N10–10	8.9	N10	76.6	Yes
CB10524	244	N10–25	25.2	N10	54	Yes
CB10079	189	N10–38	48.2	N10	6.9	Yes
Na14-F11	275	N11–08	10	New		
Ra2-F11a	285	N11–11	16.5	New		
CB10277	236	N11–30	40.4	N11	40.9	
BRAS083b	270	N12–06	7.3	New		
CB10316a	239	N12–06	7.3	N12	6.6	Yes
BRAS083c	176	N12–08	9.1	*n2	190	
CB10526	151	N12–16	18.2	New		
OI13-G05	135	N12–21	27.4	N12	28.2	Yes
BRAS123a	262	N12–48	81.3	New		
Na12-E03	281	N12–60	98.7	New		
CB10036b	143	N13–14	27.6	New		
Na14-E02	183	N13–16	33	New		
Na12-E02b	110	N13–19	36.8	N13	87.5	Yes
BRAS120a	240	N13–20	37.7	N13	89.2	Yes
BRAS120b	154	N13–21	38.6	New		
BRAS005	218	N13–31	51.1	N13	131	Yes
BRAS051	163	N13–31	51.1	N13	131	Yes
Na10-c01a	171	N13–62	100.2	N13	266	Yes
MR049	206	N13–66	105.6	N13	267	Yes
CB10316b	266	N14–14	15.3	*n4	48.1	Yes
MR036	145	N14–15	20.3	*n4	3	
BRAS123b	240	N14–20	26.7	*n4	54.8	Yes
BRAS021	222	N14–28	38.6	*n4	95.4	Yes

Table 3 continued

Primer name	Size of PCR products[a]	Linkage group and bin position	Map position (cM) on the SRAP map	Linkage group on the SSR map	Map position (cM) on the SSR map[b]	Markers with the same order on both maps
Na10-c01b	296	N14–34	44.9	*n4	102	Yes
CB10493b	220	N14–47	75.5	*n4	170	Yes
BGSSR2	265	N15–86	34.8	New		
CB10526a	314	N15–88	49.8	New		
Na12-A02c	193	N16–01	0	N16	59.9	Yes
CB10316c	452	N16–13	19.2	New		
CB10526b	339	N16–13	19.8	N16	26.5	Yes
Na10-c01c	224	N17–26	35.9	N17	8.7	
BRAS026d	365	N17–32	43	New		
CB10528	301	N17–33	44.8	N17	5.6	
BRAS019	176	N17–40	54.7	N17	6.7	
CB10028	186	N18–05	7.4	N18	14.4	Yes
CB10092	228	N18–08	11	N18	25.7	Yes
NI4-D09b	189	N18–18	24.6	N18	46.9	Yes
BRAS050	247	N19–03	1.8	New		
CB10199b	197	N19–18	38.9	New		
OI10-D08	196	N19–29	51.5	*n14	35.5	Yes
Ra2-F11b	212	N19–29	51.5	*n14	35.5	Yes
OI10-A09	101	N19–31	53.3	*n14	13.2	
Na12-g04	184	N19–48	72.2	*n14	72.1	Yes

[a] PCR products were separated with ABI 3100 DNA analyzer

[b] SSR map is the map of Piquemal et al (2005)

N15 were polymorphic in our mapping population so we were not be able to used them to assign it to its corresponding SRAP linkage group instead we used a sequence in the *B. oleracea* BAC clone (Gao et al. 2006) harboring the *BoGSL-PRO* gene involved in the biosynthesis of aliphatic glucosinolate to generate a SSR marker BGSSR2. The genes on this BAC clone have their counterparts on the top of the chromosome 1 in *Arabidopsis*. On the basis of the comparative maps of Parkin et al. (2005), this chromosome 1 region has two corresponding parts on the N15 and N18 of the C genome on the *B. napus* RFLP map. Since the N18 was assigned with the SSR markers of Piquemal et al. (2005), to a different linkage group, the only alternative was that our unassigned SRAP linkage group corresponds to N15. Furthermore, the *BoGSL-PRO* gene on this BAC clone has been mapped on the O5 linkage group in *B. oleracea* (Gao et al. 2006), which is equivalent to the N15 linkage group in *B. napus.* Consequently this further supports the assignment to the N15 linkage group. When the order of the integrated SSR markers on both the SRAP map and the SSR map of Piquemal et al. (2005) was compared, 25 of 27 SSR markers that were located on six linkage groups with more than three SSR markers each showed the same order and relatively similar map length, which suggested the current SRAP map share the similar marker order as that on the SSR map of Piquemal et al. (2005).

Detection of transpositions

After all 19 SRAP linkage groups were assigned into the N1–N19 linkage group, all mapped SRAP markers which showed differential amplification in *B. rapa* and *B. oleracea* were revisited and in total five transpositions between the A and C genomes were found judged by the differential amplification in these two diploid species. The N7 linkage group contains a region from N07-62 to the end of the linkage group, spanning a genetic distance of 35.4 cM, where 29 of 32 mapped markers have their corresponding loci only in *B. oleracea*, suggesting that this region consists of one third of the N7 linkage group that originally comes from the C genome. On the N16 linkage group, the region covering the bins from N16–01 to N16–16 spanning a genetic distance of 25.4 cM, contains more SRAPs markers (10 of 18) that could be amplified only in *B. rapa*. These two regions on the N7 and the N16 linkage groups are presumed to correspond to the homeologous reciprocal transposition reported by Osborn et al. (2003). Additionally with regards to the differential amplification of the mapped SRAP markers only in *B. rapa* or only in *B. oleracea*, two transpositions located on the N9 linkage groups that originally come from the C genome and another one on the N19 linkage group, from the A genome, respectively, were identified (Table 4).

Table 4 Summary of transpositions detected by the differential amplification of mapped SRAP markers in the two diploid species *B. rapa* and *B. oleracea*

Name of linkage group	Bins of transposition on the linkage group	Map length of transposition	SRAP bands amplified in *B. oleracea*, but not in *B. rapa*	SRAP bands amplified in *B. rapa* , but not in *B. oleracea*
N07	62–80	35.3	29	3
N09	37–42	10.4	8	3
N09	48–59	10.8	8	5
N16	1–16	25.4	8	10
N19	5–21	34.2	7	9

Recombination events and mapping

Every recombination event was checked and counted in each of the 58 DH lines on each of the 19 linkage groups (online supplementary data, S3). In total, 1,663 crossovers were detected according to the marker arrangement in each individual DH line on a linkage group, and the average number of recombination events per linkage group was 1.4. Since each DH line derived from a single gamete, then there are average 28.7 inferred crossovers occurring among the 58 gametes that produced the 58 DH lines for the map. Among these lines, there were five individuals that had less than one crossover, and eight others that had more than two crossovers on average on a single linkage group. When the counting was done on the basis of individual groups, there were two linkage groups that had less than one recombination event and two others with more than two recombination events on average. If the previously assigned linkage groups of the A genome and the C genome are counted separately, there are 904 crossovers in the A genome and 759 in the C genome. There is a considerable discrepancy between the genome size and the number of crossovers in a genome, considering that the genome size of the A genome is ~500 Mb and that, of the C genome ~600 Mb.

The total map length for the *B. napus* genome is 1604.8 cM, which includes 829.1 cM on the ten linkage groups of the A genome (N1–N10) and 775.7 cM on the nine linkage groups of the C genome (N11–N19) (Table 5; Fig. 1). The largest linkage group, N7, in the A genome spans 134.4 cM distance, and the smallest one, N18, in the C genome was 34.5 cM in size. On the basis of the mapping population with 58 DH lines, one crossover is approximately equal to 0.9 cM of genetic distance and this is the minimum size required to separate two marker bins. On all 19 linkage groups, all 13,551 SRAP markers are assigned into 1,055 marker bins, among which there are seven bins with 100 to 188 markers. Table 5 illustrates the numbers of bins and markers on all 19 linkage groups, of which there are four linkage groups with 76 to 88 bins and seven linkage groups with more than 800 markers per group. There are 7,120 SRAP markers on the 10 linkage groups of the A genome, and 6,221 of the C genome.

Table 5 Summary of 13351 SRAP markers assigned into bins on N1 to N19 linkage groups and their respective map length

Name of linkage group and genomes	No. of bins on linkage group	No. of markers on linkage group	Map length (cM)
N01	42	586	78.0
N02	78	824	127.3
N03	49	525	79.5
N04	51	630	60.0
N05	64	989	67.8
N06	58	930	73.6
N07	80	967	134.4
N08	32	609	76.7
N09	62	768	76.3
N10	42	508	55.5
A genome	558	7,336	829.1
N11	44	624	66.6
N12	65	1,124	110.3
N13	76	1,059	119.2
N14	48	602	78.3
N15	88	634	129.5
N16	31	157	56.2
N17	54	635	81.4
N18	27	307	34.5
N19	64	1,073	99.7
C genome	497	6,215	775.7
Total	1,055	1,3551	1604.8

Cross species testing

Besides the map construction in *B. napus*, SRAP data were collected from two DH lines of *B. oleracea*, 'B453' (a cauliflower DH line) and 'B454' (a broccoli DH line), with the same primer combinations as used in the 58 DH lines of *B. napus*. Among the 13,551 SRAP markers on the high density genetic recombination map in *B. napus*, 6,828 were developed together with the *B. oleracea* DH lines. 2,438 (35.6%) of those 6,828 markers have corresponding bands judged by the band size in both DH lines or in just one of the two DH lines

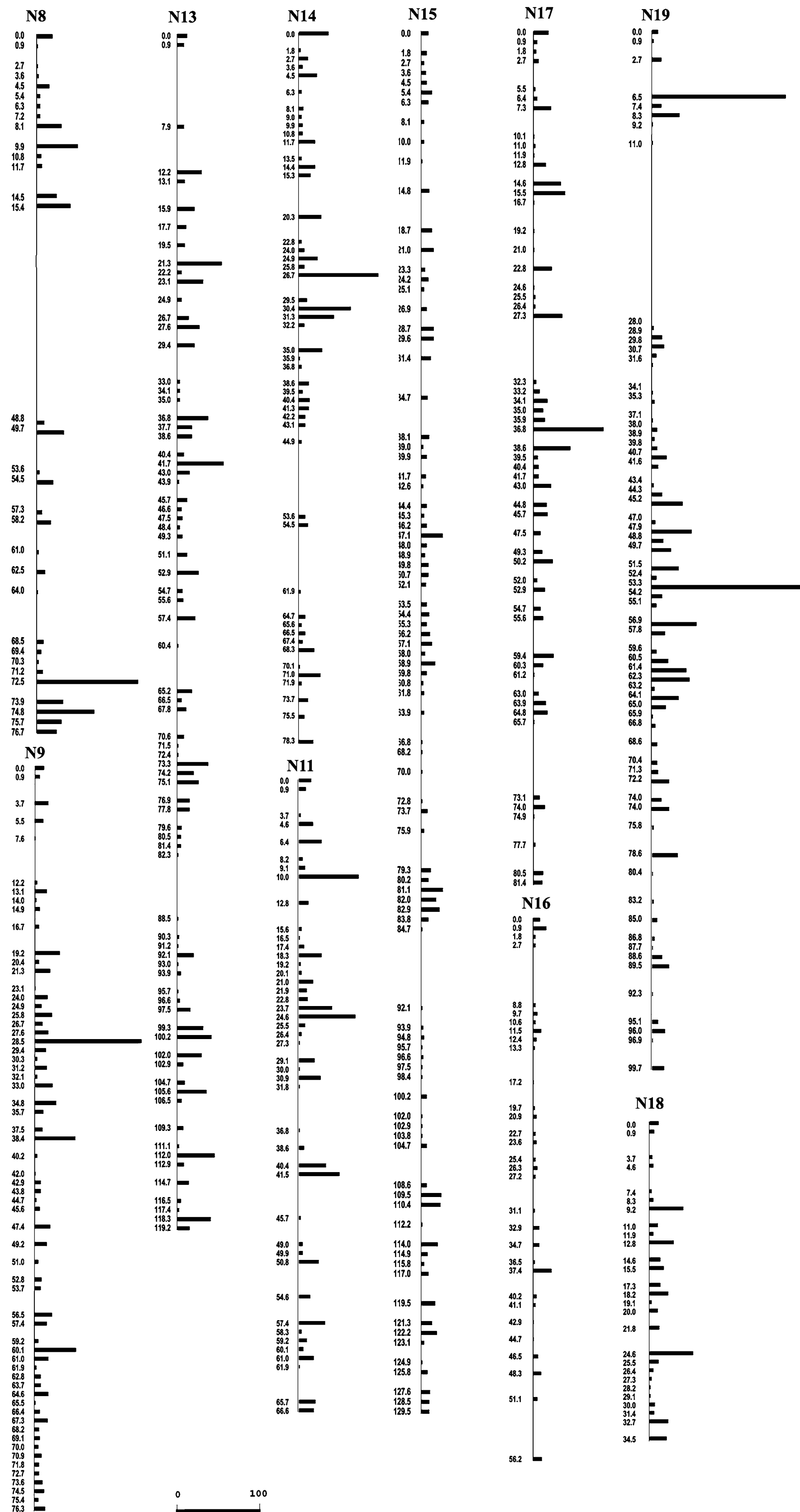
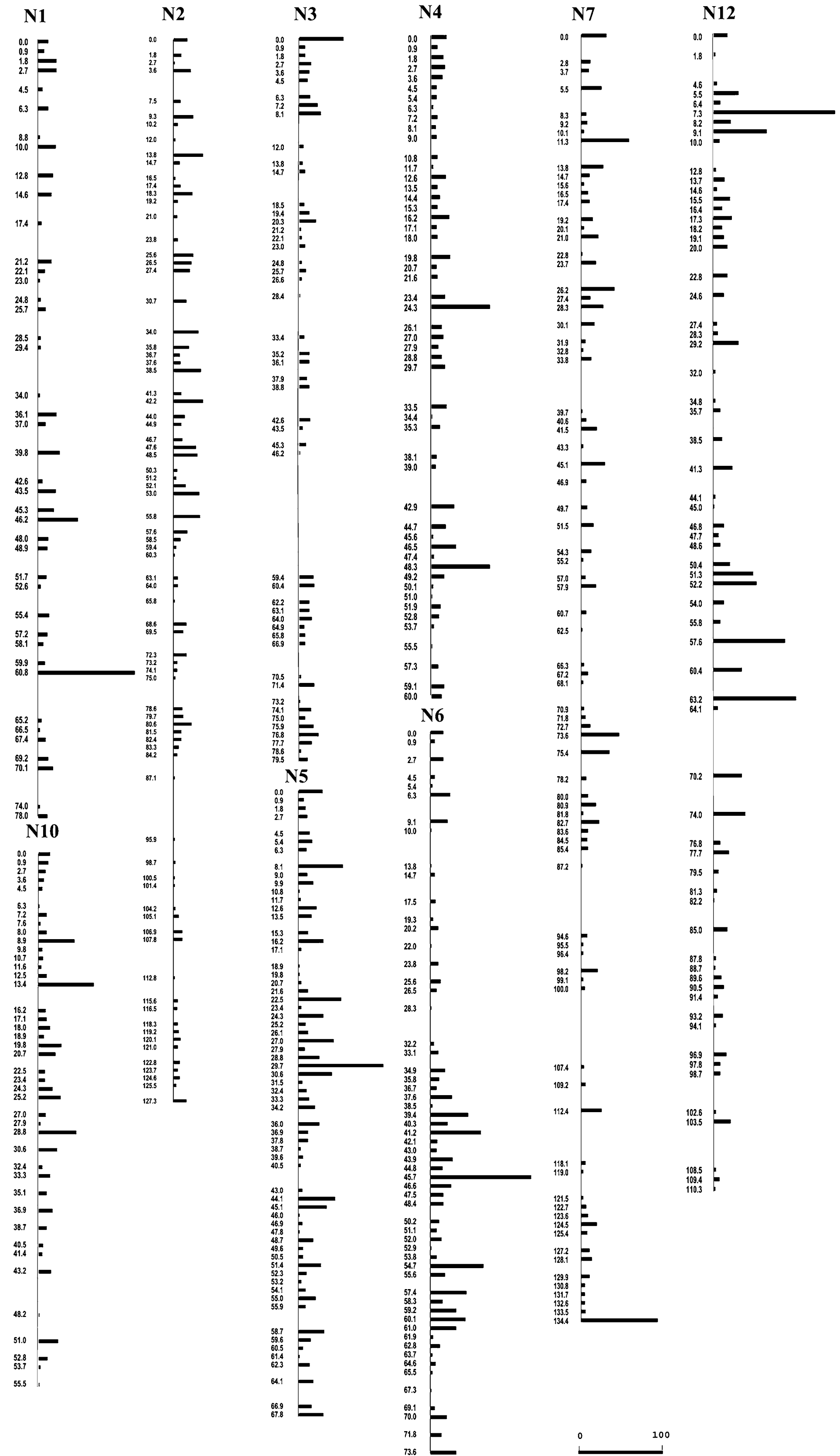


Fig. 1 Genetic recombination bin map constructed with a DH line population from a cross of 'Westar' and 'Zhongyou 821'. A total of 13,351 SRAP markers are assigned into 1,055 bins on N1-N19 linkage groups. The horizontal bar indicates the number of SRAP markers in a bin

Fig. 1 continued



in *B. oleracea*, which share the similar frequency for the previously described 5,527 SRAPs that were used to assign the 19 linkage groups into the A and C genomes. In the previous data set, there were 1,707 mapped SRAP markers (30.9 %) in *B. napus* that showed bands of the same size in *B. oleracea*. Among the 2,438 markers that have their counterparts in the C genome, there were 1,405 markers that were amplified in one of these two DH lines in *B. oleracea*, indicating that they could be applied directly as usable molecular markers in *B. oleracea* crops. Therefore, many of the SRAP markers on this high density genetic recombination map in *B. napus* may be also applicable to *B. rapa* and *B. oleracea*.

Discussion

Potential marker abundance in a genome, marker availability and high throughput detection at a reasonable cost are major factors that determine the usefulness of a molecular marker system. Although there are several kinds of molecular marker systems, such as RFLP, AFLP, RAPD, SSR, and SNP available, each method has unique advantages and disadvantages, which have been discussed elsewhere (Peters et al. 2003). The advantages of the SRAP molecular marker detection protocol have been clearly demonstrated through construction of an ultradense genetic recombination map in *B. napus*. This is the most saturated genetic recombination map reported in *Brassica* species to date.

The crossovers inferred from the current data display a similar range to those reported in sorghum. In a genetic recombination map for sorghum constructed with 2,512 loci, 1,376 crossovers were detected, covering a map length of 1059.2 cM (Bowers et al. 2003). Similarly, with over 13,500 SRAP markers, 26.05 crossovers in a gamete and 0.39–2.41 crossovers per linkage group on average in *B. napus* were inferred and a genetic recombination map with a length of 1604.8 cM was constructed. In general, the length of a recombination map become smaller than a genetic map since the high marker density allows the feasible identification of recombination points and removal of the singletons that come out of missing data and scoring errors. For instance, the genetic recombination map for sorghum is shortened from 1445 cM to 1059.2 cM in size. In line with the results in other crop plant species, the length of the current genetic recombination map is reduced compared with other published *B. napus* genetic maps that have a range of 1,968 to 2,619 cM.

The alignment of genetic maps generated by different laboratories is highly beneficial to the *Brassica* community. However, there is no generally accepted method that is applicable to align different genetic maps constructed with different kinds of markers and populations. The RFLP method is often used for the alignment of different maps (Parkin et al. 2005; Piquemal et al. 2005). There are, however, some limitations with respect to this approach. The method per se is time consuming and RFLP detection is too difficult to become a universal method. Additionally there are no standard band patterns for the probes that are available publicly. One RFLP probe generally produces multiple bands representing different loci in the allotetraploid *B. napus* species. Without a standard band pattern for a probe, it is not easy to make a reliable alignment of linkage groups from different genetic maps. Here we used SSR markers to align our SRAP map with the SSR map of Piquemal et al. (2005), which is considered the consensus map for this species. Although we could easily align 15 of the 19 linkage groups of the SRAP map with the SSR map, we had difficulty with group N15 because of lack of polymorphism for those markers in our mapping population. This problem could prove to be an obstacle for putting the maps from different laboratories together. With the SRAP markers, we could detect the genomic origin of a marker in *B. napus* through the differential amplification of *B. rapa* and *B. oleracea*. This allowed us to identify syntenic differences for linkage groups N4, N14 and N19 when constructed by SSRs versus SRAP markers. These could be real differences reflecting structural chromosomal changes in the different *B. napus* stocks used in the two mapping populations. However, it is also possible that the RFLP markers used for assigning the Piquemal's map did not disclose the correct synteny of these groups. Additionally, we also detected some transpositions between the A and C genomes in *B. napus* representing genomic rearrangements during the evolution of this species.

The marker bin method was used for the construction of the current ultradense recombination map. This strategy was also used quite effectively for the construction of the AFLP map in potato (van Os et al. 2006). Due to the small size of the mapping population and limited number of crossovers occurring in the genome, the SRAP markers were organized into bins, most of which have more than one marker, and a few bins have over 100 markers. To circumvent the limitations of Mapmarker software in dealing with thousands or even millions of markers on a map, a bin map strategy can be implemented to assign New markers into existing bins.

The current genetic recombination map provides a platform for map-based gene cloning, and this has been intensively implemented in the cloning of several genes

conditioning important traits, such as resistance to blackleg disease, yellow seed coat, and male sterility in *B. napus* and *B. rapa* in our lab (unpublished data). For the location of known genes from other related species, those known genes can be first incorporated onto the genetic recombination map. Then co-segregation analysis will allow the confirmation of the known genes involving the trait of interest. For instance, the *BoGSL-ALK*, *BoGSL-ELONG* and *BoGSL-PRO* genes in the aliphatic glucosinolate pathway that were identified in *B. oleracea* (Li and Quiros 2002, 2003; Li et al. 2003) have been placed on the current genetic recombination map of *B. napus*. The flanking markers of these genes will allow us to find the relationship between the known genes and glucosinolate content, and the SRAP markers will be further used for MAS in canola/rapeseed breeding.

Using the current genetic recombination map, a resistance gene to blackleg disease in cultivar, 'Surpass 400', introgressed from a wild *B. rapa ssp. sylvetris*, is being targeted. Since the population used for cloning the resistance gene is different from the mapping population, a marker was first found in the segregating population of 'Westar' and 'Surpass 400'. Then the marker was integrated on the genetic recombination map, and finally a dozen SRAP markers that were closely linked to the resistance gene were identified (unpublished data).

Construction of a single map for three *Brassica* species is another goal of the current recombination map. Since there is a close relationship of *B. napus*, *B. rapa* and *B. oleracea*, the SRAP method detects corresponding markers simultaneously in those three species. As the data illustrates, approximately 20% of the SRAP markers on the *B. napus* map are also polymorphic in *B. oleracea*, suggesting that approximately another 20% may be polymorphic in *B. rapa*. For example, when a marker for yellow-seeded *B. napus* was integrated on the genetic recombination map, the flanking SRAP markers that co-segregated with a yellow seed trait in a *B. rapa* segregating population were found. This was expected since a yellow-seed *B. rapa* line per se was the original parent for the development of the yellow-seeded *B. napus* (unpublished data). Finally, these markers could be used for anchoring physical and genetic maps when genomic libraries, such as BAC libraries, are available. Since it is easy to sequence most SRAP markers and approximately 50% of SRAP markers locate in gene regions, the sequence information allows the identification of the corresponding regions in *Arabidopsis* via BLASTn analysis. This certainly will expedite gene cloning by saturating the genome with marker regions harboring candidate genes.

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