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An Integrated Genetic Map for *Brassica napus* Derived from Double Haploid and Recombinant Inbred Populations

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Abstract

A hybrid developed from a cross between two diverse *Brassica napus* cultivars ("Polo" and "Topas") was used to produce a microspore derived double haploid (DH) population and a single seed descent derived recombinant inbred (RI) population for genetic mapping. Each of the two populations consisting of 190 DH lines and 94 RI lines was characterized for various types (SSR, SRAP, ISSR, SCAR) of polymorphic molecular markers. The DH population was scored for 620 molecular markers while the RI population was scored for 349 molecular markers to construct two independent genetic maps. In both genetic maps, all of the molecular markers were found to cluster in 19 linkage groups (LGs) covered a total genome length of 2244.1 cM and 1649.1 cM for the DH and RI maps, respectively. The data from the two genetic maps was used to construct a consensus integrated genetic map covering a total genome length of 2464.9 cM. Previously published *Brassica* reference genetic maps were used to assign each of the nineteen LGs to corresponding *Brassica napus* chromosomes named N01 to N19. To our knowledge, this is the first integrated genetic map based on DH and RI populations developed from the same cross in *Brassica napus*.

Keywords: *Brassica napus*; Integrated genetic map

Introduction

Genetic maps of crop plants are now considered standard tools or even "road maps" [1], not only to understand genome structure and organization but also to tag economically important traits or genes. Such maps are developed by following the inheritance of detectable markers or genes in segregating populations derived from crosses of diverse parents. Rapid development in the field of molecular biology has allowed the use of molecular markers for the construction of high density genetic maps by exploiting variations (polymorphism) at the DNA level. Since the first use of restriction fragment length polymorphism (RFLP) as molecular markers followed by several other types of first generation markers, more than 30 types of 2nd and 3rd generation molecular markers are now used for the construction of genetic maps [2]. Among different types of 2nd generation molecular markers, SSRs (simple sequence repeats) are becoming the preferred markers of choice for construction of genetic maps, tagging genes and assessing genetic diversity. This is largely due to the many useful features of SSRs such as co-dominant inheritance, multi-allelic nature with high polymorphism, abundance and even distribution in genomes, the low amount of DNA required for their detection by Polymerase Chain Reaction (PCR) and their suitablity for high-throughput analysis [3]. The SSRs are also ideal for anchoring molecular linkage maps since they are readily transferable among mapping populations [4]. This unique feature has been exploited to anchor genetic maps to physical maps in many important crop plants such as barley [5], cotton [6], Brassica *rapa* [7], and melon [8]. Sequence-related amplified polymorphism (SRAP), intersimple sequence repeat (ISSR) and sequence-characterized amplified region (SCAR) are some of the new generation markers which are getting increased usage in the construction of genetic maps due to various desirable features [9-15].

Availability of various types of useful molecular markers as described above and several efficient methods now available for the development of segregating populations (Double Haploid, Recombinant Inbreds etc.) has provided opportunities to construct integrated genetic maps using multiple types of segregating populations and molecular markers. This approach is becoming popular in map construction since a large number of potentially useful markers can be mapped and validated in various genetic backgrounds. Consquently, greater genome coverage is obtained [16]. A number of integrated genetic maps using multiple segregating populations and multiple types of molecular markers were constructed in sorghum [4,17-21], red clover pepper soybean ryegrass and common bean.

Brassica napus is the second most important oilseed crop in the world after soybean [22], and there is tremendous interest to understand the genetic structure and genome organization of this plant species including the construction of genetic maps. Many genetic maps of *Brassica* species have been published in recent years which are mainly based on a single type of population [23-29]. A number of studies [7,10,16,30-35], have also reported genetic maps based on multiple molecular markers and population types.

In this study we have attempted to construct genetic maps of *Brassica napus* using two types of mapping populations (a DH and a RI) segregating for various types of molecular markers. These two maps were further combined into an integrated genetic map. The genetic maps are mainly populated with previously published SSR markers [16,34,36-38]. However, the map was also saturated using newly developed SSR markers designed from the information of SSR sequences in the gene bank and by using other marker types such as SRAP, ISSR, EST-SSR and SCAR. These genetic maps will be a useful addition in understanding the *Brassica napus* genome and tagging the economically important genes in this important oil seed crop species.

Plant Materials and DNA Extraction

More than two hundred DH lines were developed by microspore

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culture from an F_1 hybrid generated from a cross between two Canadian canola (*B. napus*) cultivars called *Polo* and *Topas*. Randomly selected 190 fertile DH lines and two parents were used for the construction of a genetic map. One hundred and thirty-six F5 RI lines were developed through continuous self-pollination from the same cross between Polo and *Topas*. Randomly selected 94 RI lines and two parents were used for the construction of a second genetic map. Considering the differences in parental cultivars, populations were found to be segregating for various agronomic traits such as plant height, yield and oil content. Approximately 0.7 gram of young leaves from each greenhouse grown DH and RI lines were collected for genomic DNA extraction using a modified CTAB method [9,10].

Molecular markers

Simple Sequence Repeats (SSR): The sequences of 387 public SSR primer pairs were obtained from published papers [16,34,37-39]. In addition, 130 unpublished sequences for SSR primer pairs, named SR+ hereafter, were kindly provided by the Molecular Genetics Laboratory at the University of Manitoba. Morever, new SSRs were also developed in this study. For this purpose, an online SSR identification tool called SSRIT (http://www.gramene.org/db/markers/ssrtool) was applied to detect the di-, tri-, tetra- SSR sequences in 4563 *Brassica* genome survey sequences downloaded from the NCBI website. The SSR primers were then designed using a Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) program with options to generate primers with a length of 18-22 bp, GC content of 45-55%, and the predicted PCR products ranging from 100 to 500 bp. The newly developed SSR markers were named RaAC (represents *Brassica rapa* AC library), OlBH (reprents *Brassica oleracea* BH library) and NaJB (reprents *Brassica napus* JBnB BAC). Using the same tool, EST-SSR primers were detected and designed from sequences of *Brassica* ESTs downloaded from NCBI. The sequence information of SSR and EST-SSR is available in supplementary materials.

Sequence-Related Amplified Polymorphism (SRAP): The protocols to develop SRAP markers and sequences for primer pairs have been described previously [9,10]. The primers were kindly provided by the Molecular Genetics Laboratory at the University of Manitoba.

Intersimple Sequence Repeats (ISSR): The ISSRs are semi-arbitrary markers developed by Zietkiewicz et al. [11], and consist of PCR amplification of DNA sequences delimited by two inserted microsatellites. The PCR amplifications are performed with only one primer composed of SSR units, with or without an anchoring end. Seventy seven ISSR primers were obtained from Nanjing Sunshine Biotechnology Co., Ltd, China while 116 ISSR primers were designed in our lab (details in supplementary materials).

Sequence-Characterized Amplified Region (SCAR): The published sequences of full length *B. napus* genes which are involved in fatty acid biosynthesis were used to design gene specific primers using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The remaining sixteen primer sequences have been reported previously [40].

Genotyping for molecular markers

General Protocol: The DNA from individual lines in each of the mapping populations described earlier was used as a template to amplify the regions corresponding to various types of primers as described above. In order to separate the PCR products on the ABI 3100 Genetic analyzer, a common 19-base sequence of M13 (CACGACGTTGTAAAACGAC) was added before the 5' end of forward primers. Four-color fluorescent dyed M13 primers (FAM-blue, VIC-green, NET-yellow PET-red) were synthesized by ABI company (Foster City, California). The PCR reactions were performed in 384-well plates where each well contained 10 µl of mixture containing 20 ng of template DNA, 0.05 mM forward primer, 0.15 mM reverse primer, 0.1 mM labed-M13 primer, 0.15mM dNTPs, 2.0 mM $MgCl_2$, 1× PCR buffer, and 0.5 Units of Taq DNA polymerase. The PCR cycling was programmed as i) 94°C for 5 min; ii) 5 cycles of 94°C for 50 s, 56°C for 50 s, 72°C for 1min, with a 0.8°C decrease in annealing temperature at each cycle; iii) 30 cycles of 94°C for 50 s, 51°C for 50 s, 72°C for 1min; iv) an elongation step of 7 min at 72°C. Samples from four different color (FAM-blue, VIC-green, NET-yellow PET-red) labeled primers were pooled together after running PCR reactions and 2.5 µl of the pooled samples was added to a 5.5 µl mixture of formamide and 500-LIZ size standard (Applied Biosystems, Foster City, California) and denatured at 95°C for 5 minutes. The plates containing the samples were loaded into the auto sampler of the ABI 3100 Genetic analyzer equipped with 36 cm 16-channel arrays with a 40 min running time. The array profiles were analyzed with Genscan (ABI) software and specific polymorphic loci were scored with Genographer software (). The ABI files were converted to gel-like images and scoring of polymorphic loci by the software was confirmed visually. Each of the polymorphic loci was scored as a dominant marker (only one band was scored for codominant bands based on the maternal parent). The final molecular markers were scored as a primer name followed by the size of the amplified DNA fragment or visual band on the gel image. For example, OlBH001-234 represents the primer OlBH001 which amplified a 234 base pair DNA fragment.

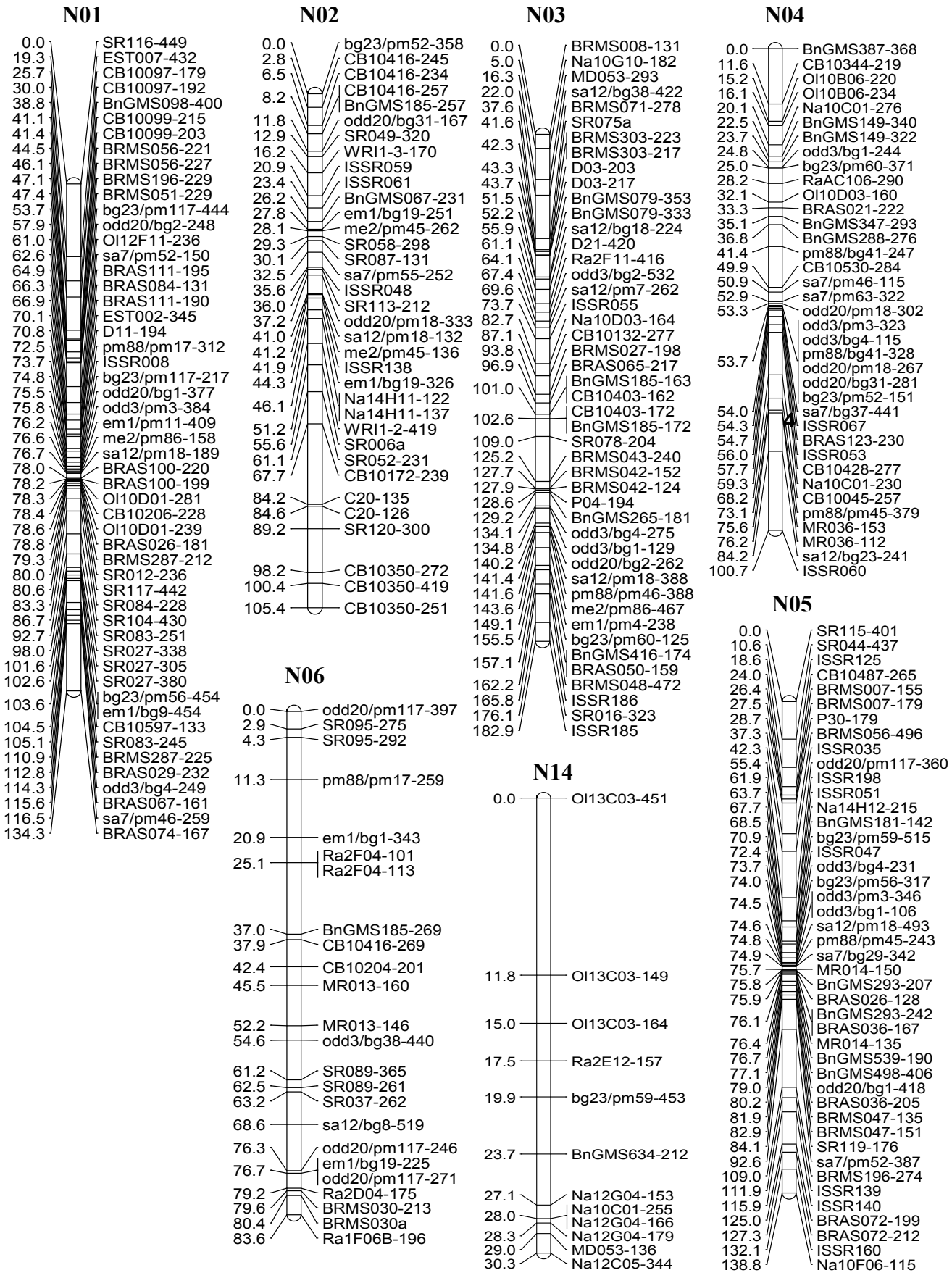
Segregation analysis and map construction

Markers that were reproducibly polymorphic between the parental lines were scored in the DH and RI populations. Goodness of fit to expected ratios (1:1) of segregation of the markers was estimated by chi-square test (χ^2 test) at significance $P < 0.05$. Markers that deviated from the expected ratios were also incorporated into the linkage analysis. Linkage analysis and map construction were performed by using Joinmap 3.0 software [41], to assemble 19 linkage groups. The *Kosambi* map function [42], was used for converting recombination frequency into genetic distance with LOD values of 6 to 12 and a recombination rate of 0.4. Linkage groups containing more than two common markers in each map were selected and integrated using the 'Combine the Groups for Map Integration' function.

Results

Segregation analysis of molecular markers

Segregation analysis of molecular markers showing detectable allelic variation or polymorphism between parents is an inevitable step in map construction. A total of 1013 primer pairs or primers, corresponding to various types (SSR, SRAP, ISSR and SCAR) of molecular markers were used in this study. Of these primers, 698 (68.9%) amplified successfully, and were further used to screen polymorphism between parents. A total of 716 and 533 polymorphic bands were collected to construct genetic maps for the DH and RI populations, respectively. A test of goodness of fit to 1:1 (χ^2 test) revealed that 362 (50.6%) and 174 (32.6%) molecular markers (distorted markers) violated the expected Mendelian segregation ratio of 1:1 for the DH and RI populations,



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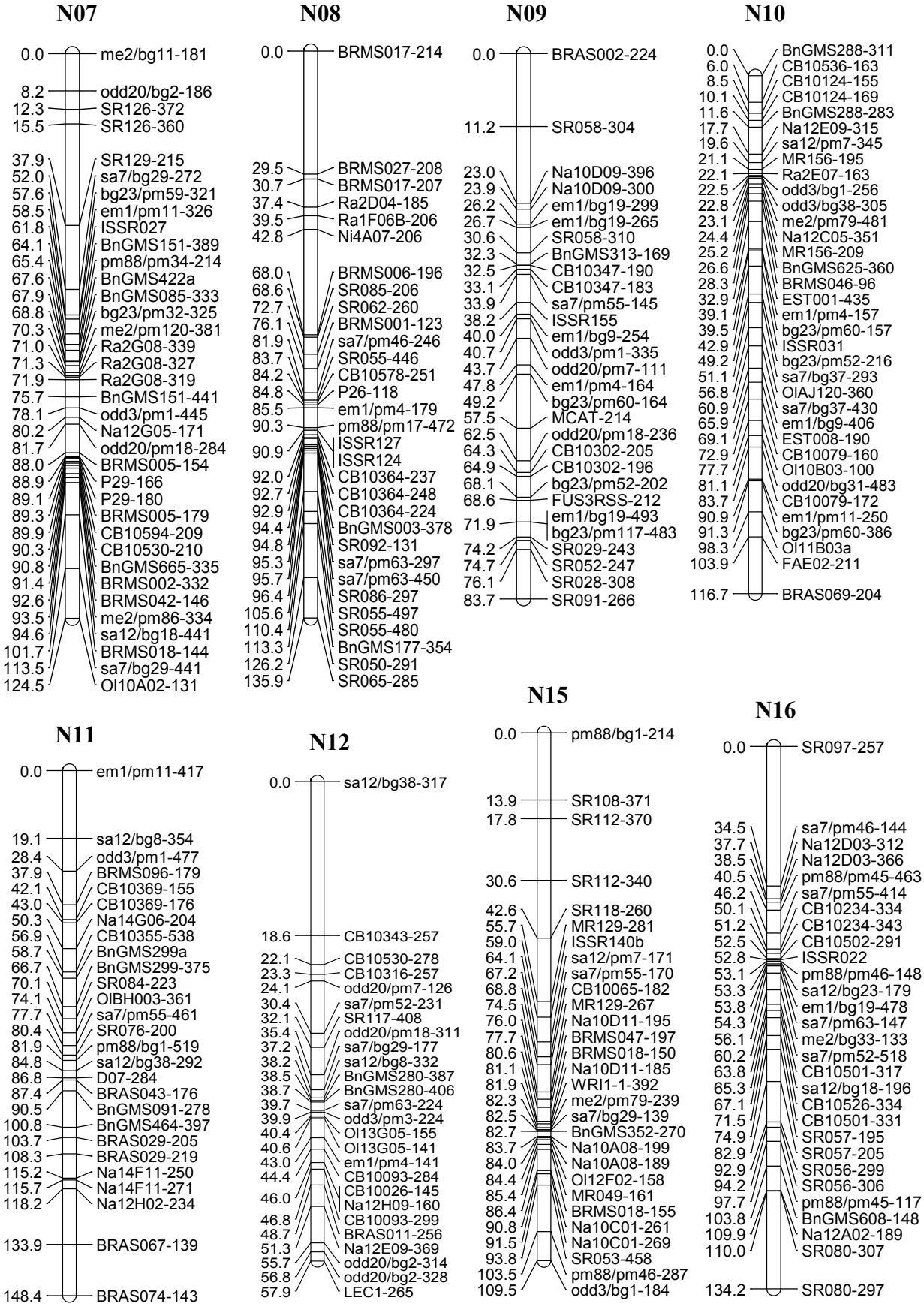

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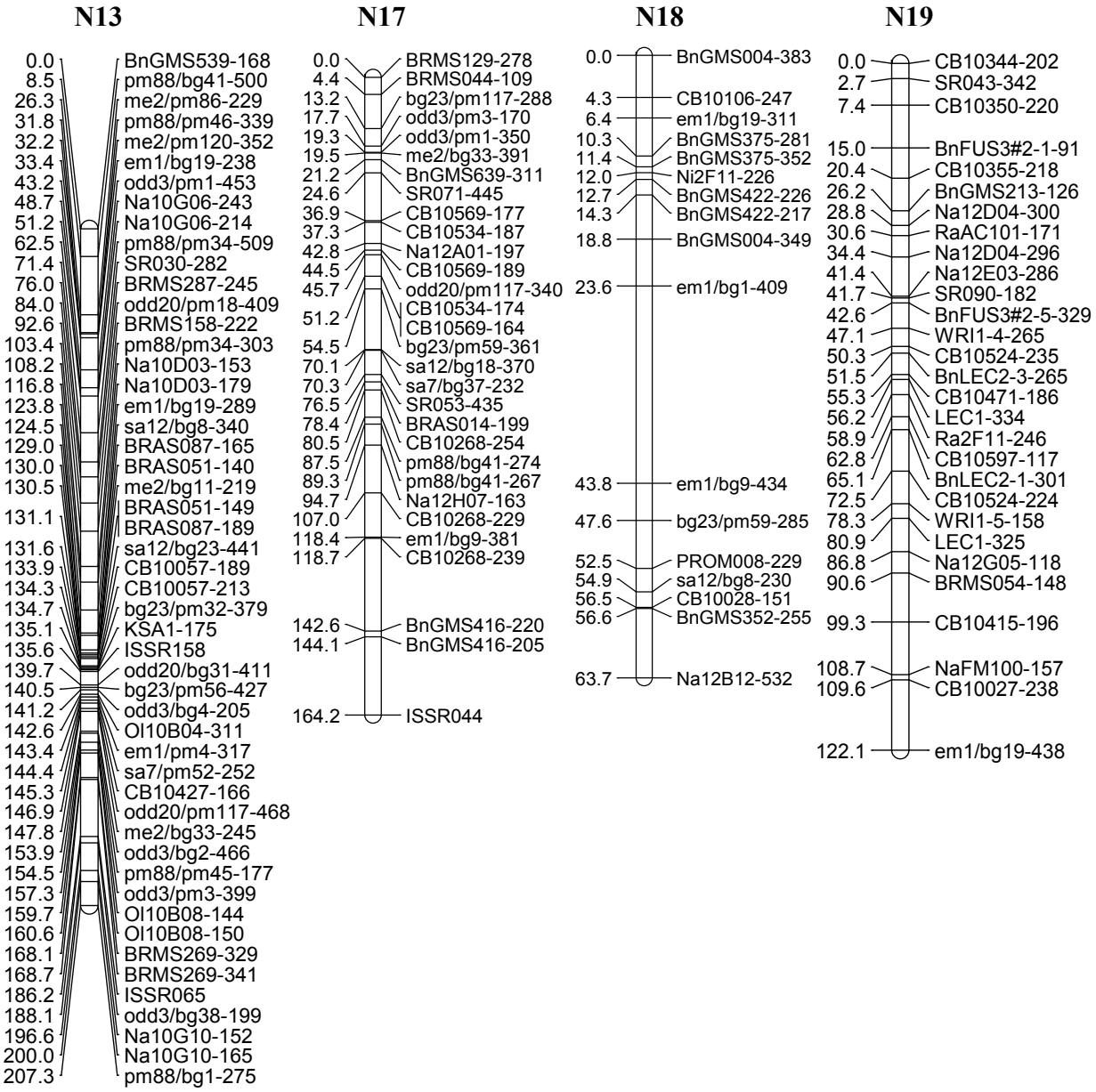


Figure 1: A DH-based genetic map for *Brassica napus*.

respectively (details in supplementary materials). Among these 362 distorted markers in the DH population, 175 (48.3%) markers biased to the maternal parent *Polo*, and 187 (51.7%) markers showed bias to the paternal parent *Topas*, however, the second χ^2 test on distorted markers resulted in a perfect fit to a Mendelian segregation ratio of 1:1, which indicates that the DH population was a nomal population. Whereas, among the 174 distorted markers of the RI population, 113 (64.9%) markers biased to the maternal parent *Polo*, and 71 (35.1%) markers biased to the paternal parent *Topas*, the result of second χ^2 test showed a significant violation to Mendelian segregation ratio of 1:1.

Construction of individual genetic maps

The data collected from the genotyping for all the polymorphic molecular markers were used for linkage analysis and map construction. Out of 716 molecular markers in the DH population, a total of 620 (87.0%) molecular markers, including 383 SSR, 191 SRAP, 29 ISSR and 17 SCAR markers were assigned onto 19 linkage groups (LGs). Using common SSR markers from existing *Brassica* reference linkage maps (16,34) the LGs were anchored to chromosomes named N01 to N19 (Figure 1). This map covered 2244.1cM with an average marker spacing of 3.6 cM. The length of each LG ranged from 30.3 cM to 207.3 cM for N14 and N13, respectively, the average marker spacing ranged from 2.2 to 5.5 cM for N12 and N11, respectively. The overall number of markers on each chromosome ranged from 12 to 53 for N14 and N01, respectively. Individually, SSR markers ranged from 11 to 38, SRAP markers ranged from 1 to 26, 29 ISSR markers were mapped onto 13 chromosomes, ranged from 1 to 8 and 17 SCAR markers were mapped onto 8 chromosomes , ranged from 1 to 8 (Table 1).

LGs	Total Markers	SSR	ISSR	SRAP	SCAR	Length (cM)	Marker density (cM/marker)
N01	53	38	1	14	-	134.3	2.5
N02	35	20	4	9	2	105.4	3.0
N03	46	31	3	12	-	182.9	4.0
N04	37	19	3	15	-	100.7	2.7
N05	44	25	8	11	-	138.8	3.2
N06	24	16	-	8	-	83.6	3.5
N07	36	22	1	13	-	124.5	3.5
N08	31	24	2	5	-	135.9	4.4
N09	29	14	1	12	2	83.7	2.9
N10	35	20	1	13	1	116.7	3.3
N11	27	21	-	6	-	148.4	5.5
N12	26	14	-	11	1	57.9	2.2
N13	51	22	2	26	1	207.3	4.1
N14	12	11	-	1	-	30.3	2.5
N15	29	20	1	7	1	109.5	3.8
N16	29	17	1	11	-	134.2	4.6
N17	30	18	1	11	-	164.2	5.5
N18	17	11	-	5	1	63.7	3.7
N19	29	20	-	1	8	122.1	4.2
Total	620	383	29	191	17	2244.1	3.7
Average	32.6	20.2	2.2	10.1	0.9	118.1	3.7

Table 1: Main characteristics of DH-based genetic map for *Brassica napus*.

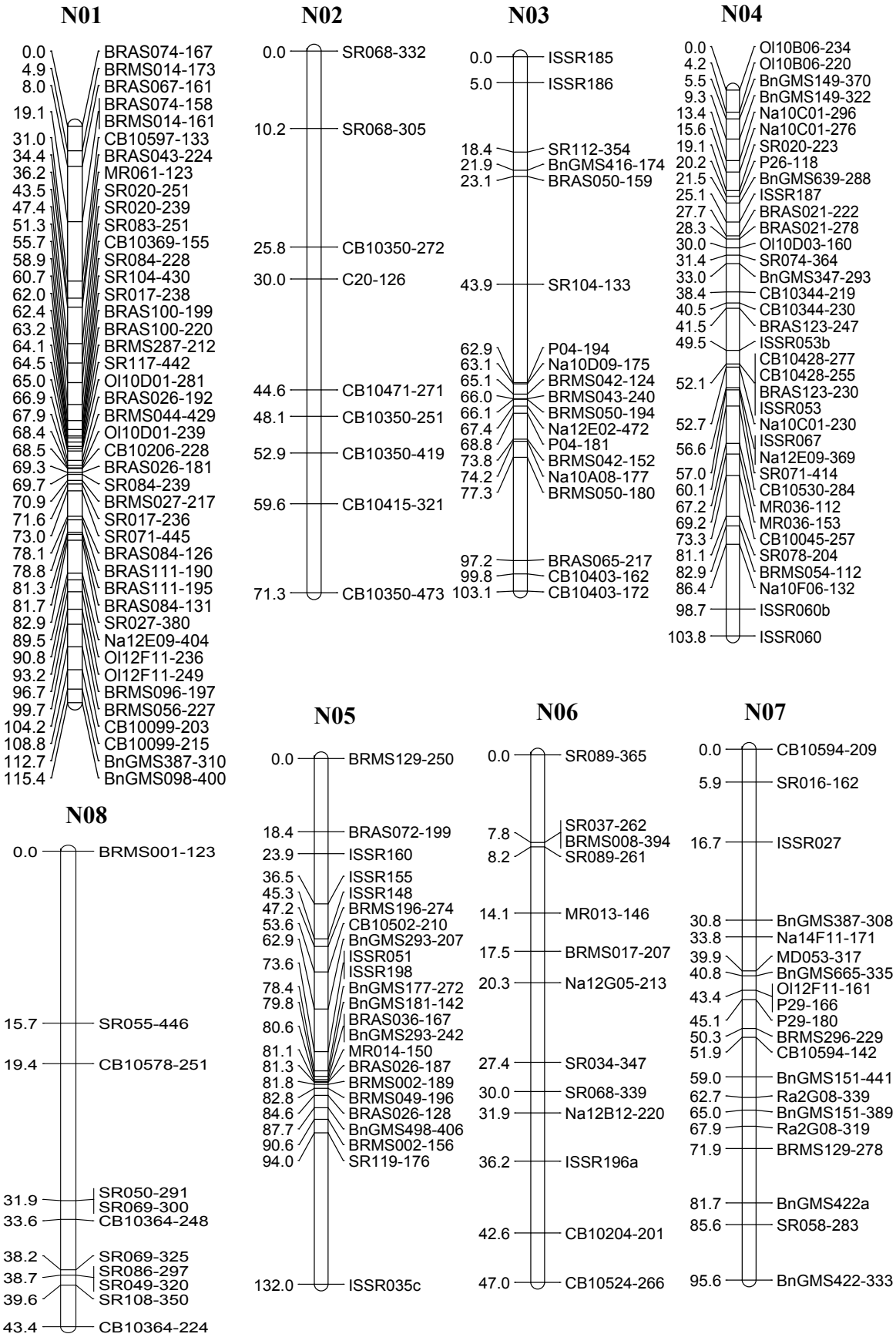
LGs	Total markers	SSR	ISSR	SCAR	Length (cM)	Marker density (cM/marker)
N01	43	43	-	-	115.4	2.7
N02	9	9	-	-	71.3	7.9
N03	19	17	2	-	103.1	5.4
N04	36	30	6	-	103.8	2.9
N05	23	17	6	-	132.0	5.7
N06	13	12	1	-	47.0	3.6
N07	20	19	1	-	95.6	4.8
N08	11	11	-	-	43.4	3.9
N09	17	13	1	3	95.1	5.6
N10	10	10	-	-	52.8	5.3
N11	21	19	2	-	108.3	5.2
N12	19	15	2	2	112.9	5.9
N13	25	22	2	1	107.8	4.3
N14	15	14	1	-	97.1	6.5
N15	17	16	1	-	53.2	3.1
N16	18	16	2	-	54.2	3.0
N17	12	12	-	-	112.6	9.4
N18	13	13	-	-	82.0	6.3
N19	8	8	-	-	62.6	7.8
Total	349	316	27	6	1650.2	4.7
Average	18.4	16.6	1.4	0.3	86.9	4.7

Table 2: Main characteristics of RI-based genetic map for *Brassica napus*.

For the RI population, a total of 349 (65.5%) molecular markers, including 316 SSR, 27 ISSR and 6 SCAR markers were assembled onto 19 LGs, and similar public SSR markers as for the DH population was used to anchor LGs to chromosomes (Figure 2). The average distance between markers and the total genome coverage were 4.7 cM and 1649.1 cM, respectively. The length of each LG ranged from 43.4 cM to 131.9 cM for N08 and N05, respectively, the average marker spacing ranged from 2.7 to 9.4 cM for N01 and N17, respectively. The total number of markers on each chromosome ranged from 8 to 43 for N19 and N01, repectively. Individually, SSR markers ranged from 8 to 43, ISSR markers were mapped onto 12 chromosomes, ranged from 1 to 6 and SCAR markers were mapped onto N09, N11 and N12 with numbers of 3, 2 and 1, respectively (Table 2).

Construction of an integrated genetic map

Allele data sets related to the same LGs with at least two loci in common were integrated into one data set by applying Joinmap software. Common markers, ranging from 2 to 23 with an average of 9.6 were detected in the same linkage groups of the DH and RI populations, which allowed the construction of an integrated genetic map. A total number of 796 markers, including 539 SSR, 193 SRAP, 45 ISSR and 19 SCAR markers were combined into an integrated genetic map. This integrated genetic map comprised of 19 LGs and covered 2464.9 cM with a marker density of 3.1 cM per marker (Figure 3). The length of each LG ranged from 83.7 cM to 209.4 cM for N14 and N13, respectively. The number of markers on each LG ranged from18 to 73



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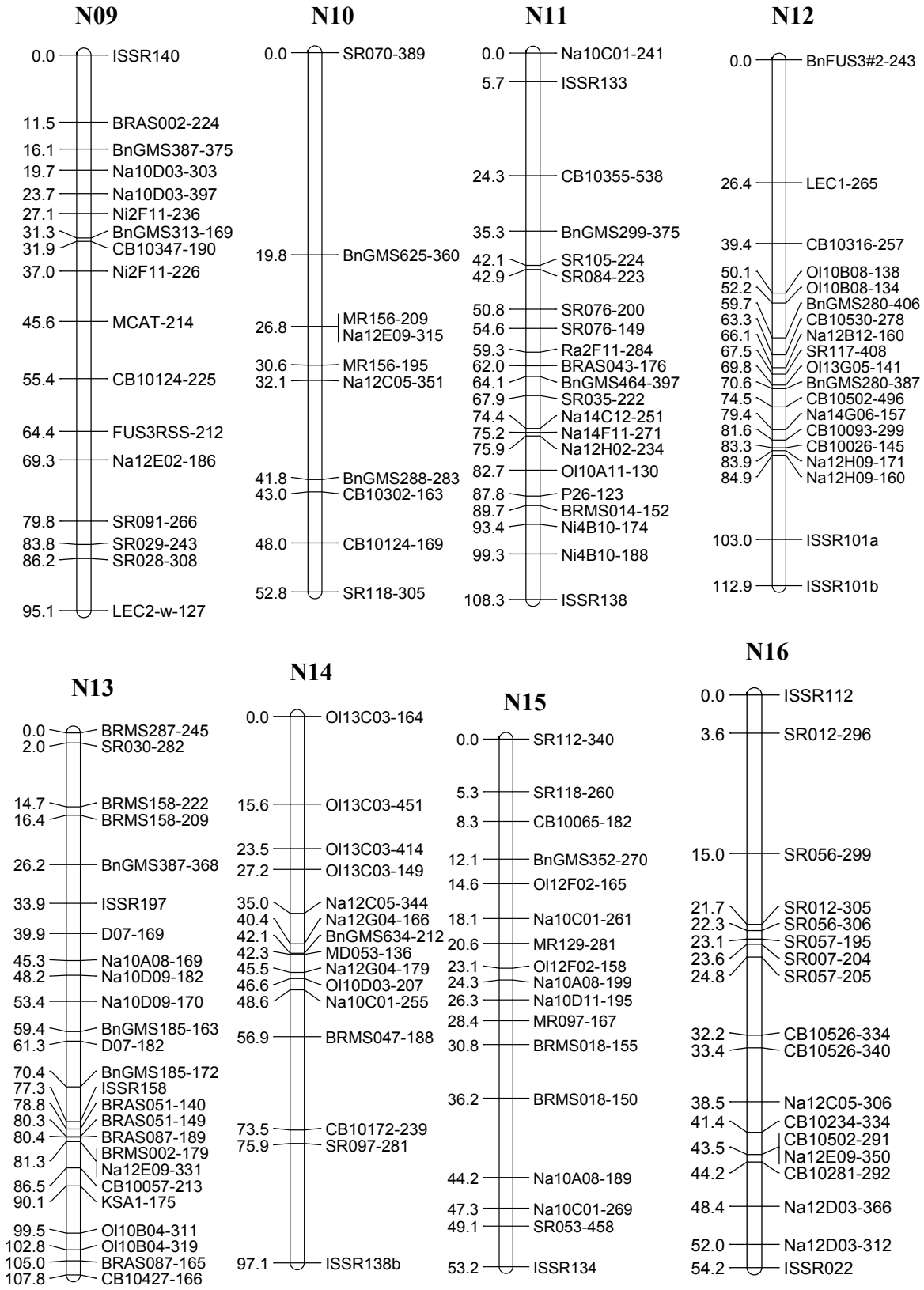

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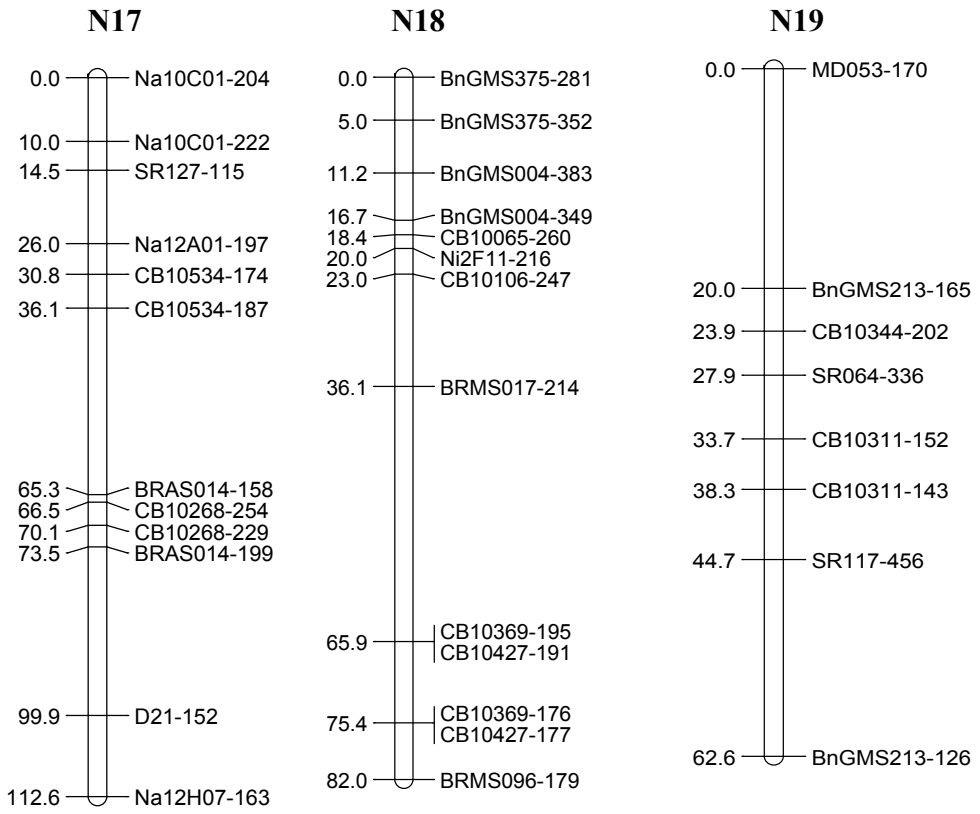
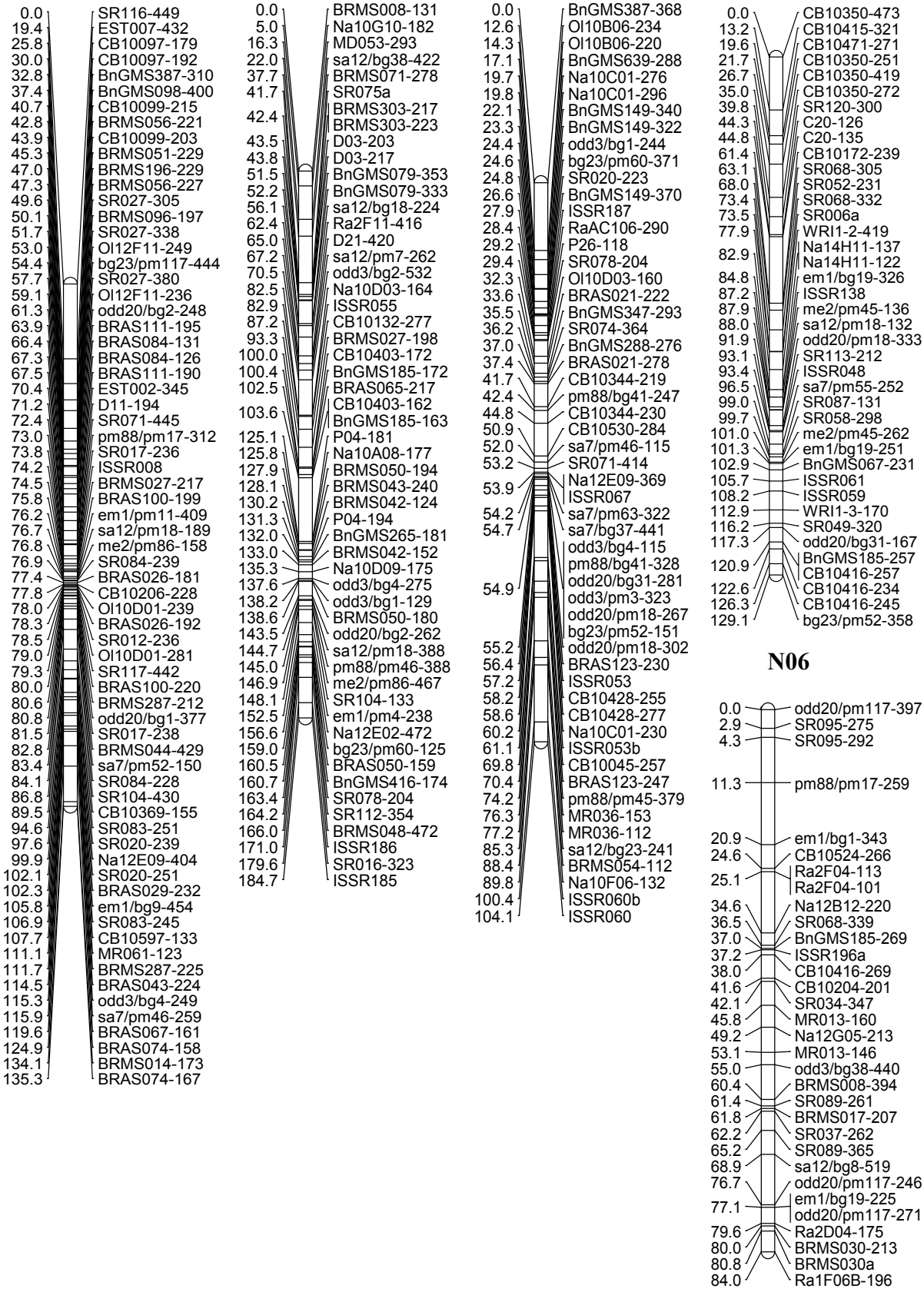


Figure 2: A RI-based genetic map for *Brassica napus*.

LGs	Total markers	Common markers*	SSR	ISSR	SRAP	SCAR	Length (cM)	Marker density (cM/marker)
N01	73	23	58	1	14	-	137.0	1.9
N02	40	4	25	4	9	2	129.1	3.2
N03	54	11	39	3	12	-	184.7	3.4
N04	55	18	34	6	15	-	104.1	1.9
N05	54	13	32	11	11	-	153.9	2.9
N06	32	5	23	1	8	-	84.0	2.6
N07	46	10	32	1	13	-	134.6	2.9
N08	45	7	36	2	7	-	130.6	2.9
N09	38	8	21	2	12	3	101.9	2.7
N10	38	7	23	1	13	1	117.4	3.1
N11	40	8	32	2	6	-	146.0	3.7
N12	35	10	20	2	11	2	94.0	2.7
N13	64	12	34	3	26	1	209.4	3.3
N14	18	9	16	1	1	-	83.7	4.7
N15	32	14	22	1	7	1	104.6	3.3
N16	37	10	24	2	11	-	108.9	2.9
N17	35	7	23	1	11	-	163.3	4.7
N18	25	5	19	-	5	1	121.9	4.9
N19	35	2	26	-	1	8	155.8	4.5
Total	796	183	539	44	193	19	2464.9	3.1
Average	41.9	18.1	54.1	4.6	19.3	4.1	129.7	3.1

*Common markers represent the markers which are common to DH and RIL maps

Table 3: Main characteristics of integrated genetic map for *Brassica napus*.



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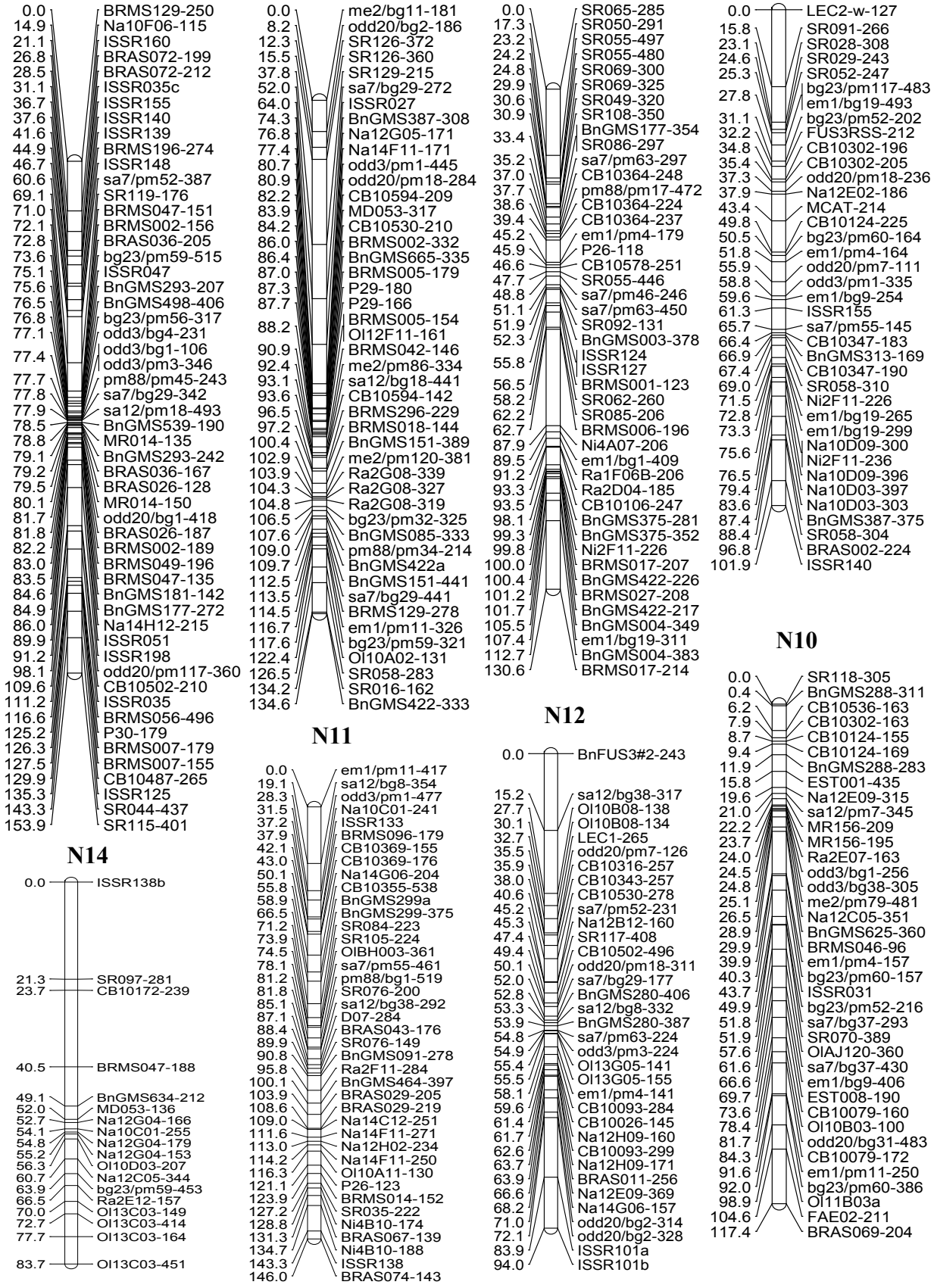

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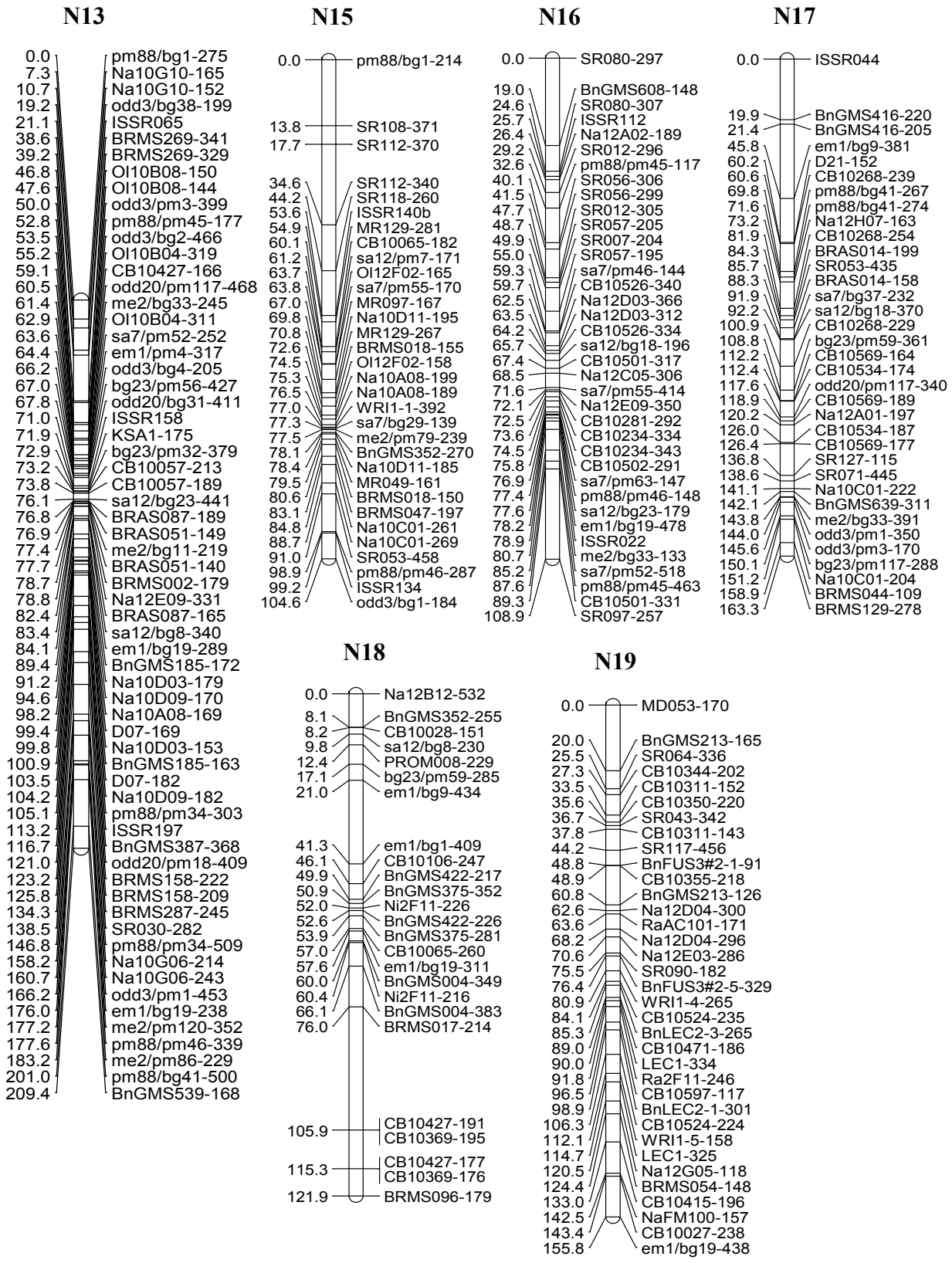
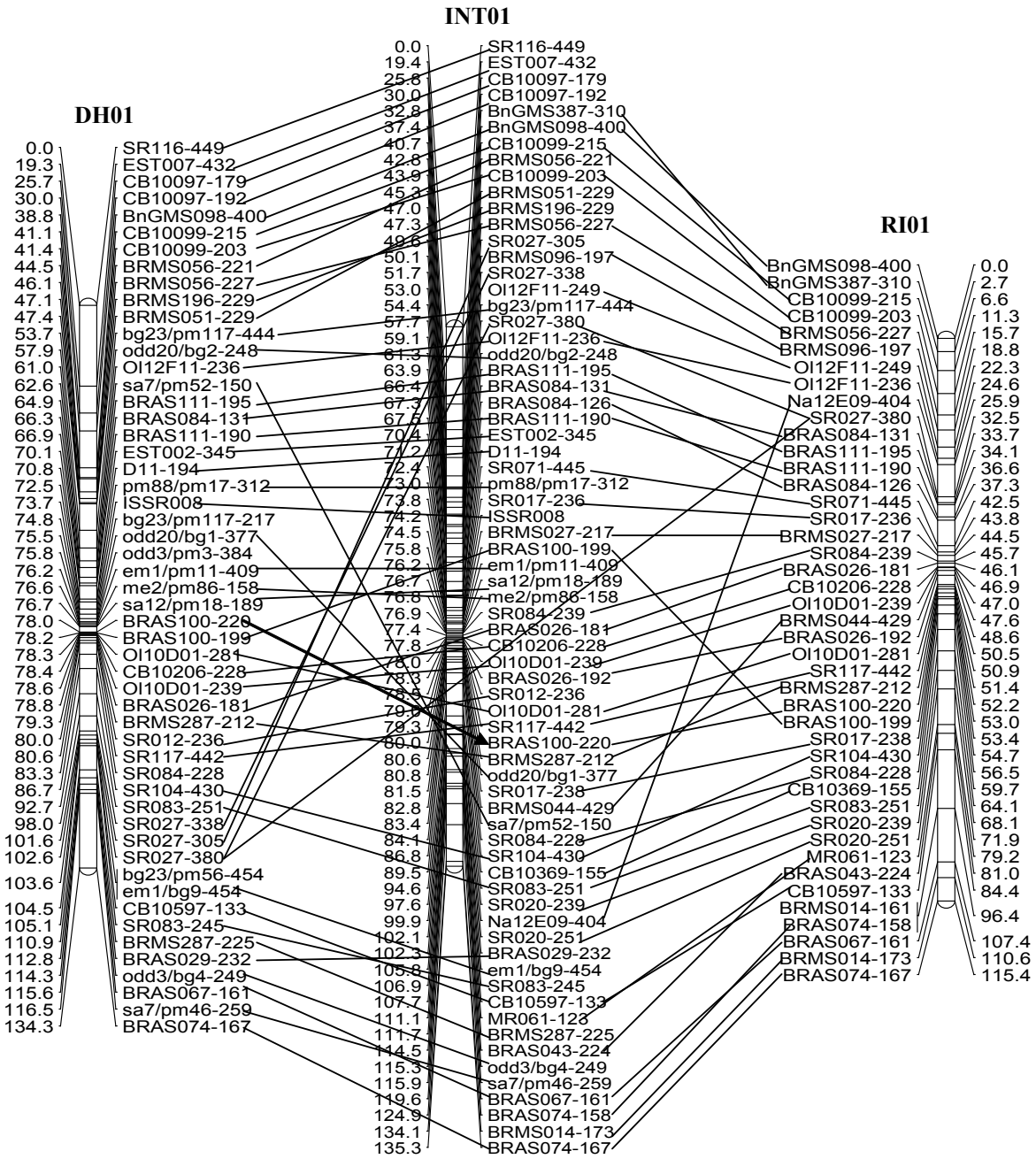


Figure 3: An integrated genetic map for *Brassica napus*.

for N14 and N01, respectively (Table 3).

Comparison of individual maps and integrated genetic map

Common markers among homologous LGs allowed the comparison of marker order between these individual maps and the integrated map. One hundred and ninety-four molecular markers were found in common between two populations; however, just 183 (94.3%) were arranged onto the resulting 19 integrated LGs. Of these mapped common markers, 170 (92.9%) markers were assembled onto the same LGs of the DH and RIL maps, nine located onto N01 (A genome) or N11 (C genome), N03 (A genome) or N13 (C genome), N04 (A genome) or N 14 (C genome), N07 (A genome) or N17 (C genome), N08 (A genome) or N18 (C genome) and N09 (A genome) or N19 (C genome) in the DH and RI maps, which suggests a synteny between the A and C genomes, while the remaining four markers distributed on different LGs whithout any relationship. The integrated map is generally in agreement with the two individual maps, and the two individual maps complement each other on the integrated map with small translocations. Regarding the length of LGs, five LGs (N02, N03, N07, N18, N19) in the integrated map are 10 cM longer than in the individual maps, whereas eleven LGs (N01, N04, N05, N06, N08, N09, N10, N11, N13, N15, N17) in the integrated map are similar to the longer ones of the individual maps, while the remaining 3 LGs (N12, N14 and N16) were over 10 cM shorter than the longer ones of



Note: DH01 represents LG01 in the DH-based map, INT01 represents LG01 in the integrated map, RI01 represents LG01 in the RIL-based map

Figure 4: Comparison of LG01 between DH-based map, RIL-based map and integrated map.

the individual maps. This result revealed that most of LGs (84.2%) in the integrated map were longer or similar to the related longer LG in individual maps, which resulted in a significantly longer coverage for the integrated map.

Regarding marker order, for example on LG 01 (Figure 4), most of the markers on individual maps shared the similar order; however, there were five translocatios (sa7/pm52-150, odd20/bg1-377, SR027-238, SR027-305 and SR027-338) detected between the DH and integrated maps. Similarly, between the RI and integrated maps, three markers (BRAS100-199, BRMS044-429 and Na12E09-404) translocated, however, only one translocation (SR027-380) was found between the DH and RI polulations.

Discussion

Generating sharable and lab-to-lab reproducibile results is becomming the most important and final purpose of genetic map construction, inspite of using different materials and experimental systems in different labs. In this study, two individual genetic maps were constructed with different types of markers, such as SSR, SRAP, ISSR and SCAR. Further these individual maps were combined into an integrated genetic map using DH and RI populations in *Brassica napus*. With the public SSR markers (16; 34) found between individual maps, all the LGs were anchored to the corresponding chromosomes of *Brassica napus*. A total of 796 loci could be mapped onto the integrated map, whereas, only 620 and 349 markers were assembled on the DH and RI map, respectively. The integrated map covered a total genetic distance of 2464.9 cM, which is in the mean confidence-interval estimates of genome length estimated as 2,127–2,480 cM (30), and thus, seemed to indicate near-complete genome coverage. The differences of map lengths in different studies are usually attribuable to scoring errors, type of markers, population size, recombination frenquency, LOD values, and the software employed [43]. Previously, Qi et al.[44], reported that the length and observed genome coverage in barley was greater with MAPMAKER than with JOINMAP. Pradhan et al. [45], also observed reduction in the total genetic length although they mapped more markers in comparision to other maps in *Brassica juncea*. In our study, similar evidence was observed that the length of the DH-based map was longer than the RI-based map due to one additional type of marker (SRAP) used, which filled large existing gaps on LGs, and eventually improved the genome coverage, assuming different markers are developed from different principles and amplifiy different genome regions, for example, SSR, ISSR and SRAP are based on SSR regions, inter-SSR regions and ORF regions, respectively.

Marker segregation distortion is a common phenomenon in crops [27,46,47], especially in maps derived from DH population regardless of marker types [16,23,24,29]. This distortion probably results from gametic or zygotic selection, or from a specific selection derived from the production of plants in vitro microspore culture. In the study by Lombard and Delourme [30], the segregation bias was towards certain parents, with a corresponding region for microspore-culture responsiveness being identified in these parents. This finding suggests that distortion segregation is related to the genes controlling microspore responsiveness during haploid production. In our study, the result of goodness fit test (χ^2 test) revealed that 362 (50.6%) and 174 (32.6%) molecular markers violated the expected Mendelian segregation ratio of 1:1 for the DH and RI populations, repectively, the results suggested that the RI population is more nomal than the DH population. However, when a χ^2 test was used on distorted markers for the DH population, 48.3% markers biased to *Polo*, and 51.7% markers biased to *Topas*, which suggests that this DH population is not distorted but rather a standard normal population. This conclusion is further supported by the similar response to microspore culture observed in both parents (data not published). Therefore, double χ^2 tests are recommended for fitting Mendelian segregation ratio of 1:1. However, among the distorted markers of the RI population, 64.9% markers biased to *Polo*, and 35.1% markers biased to *Topas*, the result of second χ^2 test showed a significant violation to the Mendelian segregation ratio of 1:1, this could be due to the smaller population size.

Genetic maps based on multiple populations and multiple types of molecular markers offer many advantages over a map based on a single population and one type of molecular marker. Likewise, in this study, a DH and a RI population were developed from the same F_1 cross, which provided an opportunity to compare these populations as well as to contruct an integrated map from them. A higher percentage of markers (85.6%) were assembled onto the main 19 LGs in the DH map than in the RI map (65.5%). It could be argured that additional type of marker (SRAP) used in the DH population and the DH population size was larger than RI population. Most common markers (94.3%) were mapped onto the same LGs and found at similar positions in individual maps, which suggests that DH and RI populations are both ideal for map constructions and complement each other.

Synteny is the preserved order of genes on chromosomes of related species which results from descent from a common ancestor. A chromosomal region of one species is said to be syntenic with a chromosomal region in another species if the regions carry two or more homologous genes. During evolution, chromosome rearrangements result in disruptions of synteny [30]. In our study, seventeen molecular markers showed synteny between the A genome and the C genome in DH, eight markers in RI and 26 markers in the integrated map.

Compared with other crops such as rice and soybean [48], there are relatively few public SSR markers available in *Brassica*, although several research groups have presented a number of SSR markers with different technology [16,34,35,37,49]. SSR development has been improved since the early expensive technology of prob hybridization to an easy online source-based method. In our study, a large number of SSR sequences (including (AT)n, (CT)n,and (GA)n) were downloaded from a website, and a number of SSR primers were developed, tested and adopted to construct the genetic map. For the newly developed and linked SSR markers, 92 markers were assigned onto the A genome and 45 markes were mapped onto the C genome, nine distributed both on the A and C genome LGs. We believe that this method is accessible and efficient for SSR development and map construction. As SSR based markers, ISSRs are semi-arbitrary markers and are easy and quick to develop and use. Two kinds of ISSR primer, with or without an anchoring end were used in this study. Seventy-seven sucessfully amplified, 36 primers detected polymorphism between the two parents, and 29 ISSR markers were integrated onto the DH map, distributed on 13 LGs. All the mapped ISSR markers filled gaps, 3 of the markers, ISSR185, ISSR060 and ISSR44, were mapped onto the ends of N03, N04 and N17 respectively, which consquently improved map length by up to 43.4 cM, directly. In the RI population, 7 ISSR markers were mapped onto the ends of N04, N05, N11, N14, N15 and N16, respectively, which improved the map length by up to 81.5 cM, directly. This result indicates that ISSR is desirable and suitable for map construction in combination with

SSR markers. Sequence-related amplified polymorphisms (SRAP) have proved to be a simple approach and an efficient system for the framework contruction of genetic maps [9,10]. We selected 64 primer pairs for map construction, and 199 polymorphic bands were detected from these primer pairs, with 191 of them assigned onto the DH map, distributed on 19 LGs, which saturated the map greatly. This result supported that SRAP could be valuable to saturate the genetic map.

Nineteen SCAR markers related to fatty acid synthsis pathway genes were integrated onto the present maps. This result offers detailed references for related agronomic quantitative trait loci (QTL) mapping and marker-assisted selection (MAS). The current maps and previously developed genetic maps could play an important role in QTL mapping and map-based gene coloning in *Brassica napus*.

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