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Submission date: 28-Jun-2019 07:16PM (UTC+0700)

Submission ID: 1147698864

File name: SABRAO-J-Breed-Genet-48-2-189-199-Nurhasanah.pdf (363.67K)

Word count: 8864

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CONSTRUCTION OF A HIGH FIDELITY GENETIC LINKAGE MAP USING AFLP AND SSR MARKERS IN RAPESEED (*Brassica napus* L.)

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SUMMARY

High-density and high-quality genetic linkage maps are valuable tools for genome-wide analyses. In this study, a high fidelity genetic linkage map has been developed in an F1DH population generated from a cross between the winter and spring type rapeseed. The map contained 568 genetic loci covering 2003 cM of the rapeseed genome, with an average markers density of 2.77 cM. The linkage groups represented A1 to A10 and C1 to C9 of the rapeseed genome with the exception of C6, which could not be identified. The failure to map the C6 chromosome might be due to the presence of a reciprocal translocation between homeologous segments of A7 and C6 chromosomes, since the genetic mapping population was developed from the cross between spring and winter type rapeseed. About 17% of SSR primer pairs amplified more than one polymorphic locus. This should be considered as a minimum level of homology analysis in the rapeseed genome. From 40 duplicated loci, 38 duplications were between the A and C genome showing a high level of similarity between the homeologous A and C genomes. An obvious evidence for homoeology was observed between A1-C1, A3-C3, A9-C9 and between A10-C9 of the rapeseed genome.

Key words: AFLP, genetic linkage map, molecular markers, SSR/microsatellite

Key findings: The principle of a high fidelity (HF) map produced a high accuracy of linkage map and diminished the impact of errors on map length resulting in high-quality genetic linkage map which is very useful for genome wide analyses and quantitative trait loci (QTL) mapping.

Manuscript received: October 2, 2015; Decision on manuscript: March 25, 2016; Manuscript accepted: April 3, 2016.

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Communicating Editor: Jung-Hyun Shim

INTRODUCTION

Rapeseed (*Brassica napus*) is the main oilseed crop in most of Europe, Canada and parts of China (Raymer, 2002). It is utilized to produce vegetable oil for human consumption, biodiesel and for industrial purposes. The meal is also used as animal feed. Its significant economic value and broad utilization makes rapeseed one of the most important crops in the world. Therefore, many research projects are aimed to improve the understanding of the

inheritance of desired characters in rapeseed and to identify the genes contributing significantly to the variation of the respective traits.

Molecular markers have been developed to investigate the inheritance of traits in the species and facilitate the construction of genetic linkage maps. High-density and high-quality genetic linkage maps are valuable tools for genome analyses, quantitative trait loci (QTL) mapping and marker-assisted selection in breeding

programs. They are also a pre-requisite for further map-based cloning to elucidate the genes controlling important agronomical traits.

Various types of molecular marker system have been applied in rapeseed genetics and breeding research (Mailer *et al.*, 1997; Lombard *et al.*, 2000; Lowe *et al.*, 2004; Hasan *et al.*, 2006). Some of the molecular marker systems, such as RAPD (random-amplified polymorphic DNA) and RFLP (restriction fragment length polymorphism) are assayed on agarose or polyacrylamide gel systems. They result in low-throughput profiling and usually lead to low-density and unsaturated genetic linkage maps. In addition, RAPD marker system also deals with problems of reproducibility, while RFLP is very laborious and time consuming.

SSR (simple sequence repeat/microsatellite) and AFLP (amplified fragment length polymorphism) markers are considered to be more effective in this regard, since they can be assayed on highly parallel genotyping platforms such as capillary electrophoresis systems and the analysis can be multiplexed in different ways (Ecke *et al.*, 2010). The use of these multiplexed marker systems allows a fast and efficient genetic analysis.

Comparisons between RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis showed that SSR markers have the highest expected heterozygosity and AFLP markers the highest effective multiplex ratio compared to the other marker types (Powell *et al.*, 1996; Yue *et al.*, 2002). In addition, SSR markers are more desirable because of their high information content, codominant inheritance pattern, even distribution along chromosomes, reproducibility, and locus specificity (Kashi *et al.*, 1997). On the other hand, the AFLP marker system is considered the most efficient method for generating large numbers of DNA markers for genetic linkage and diversity studies due to having the highest multiplex ratio of all PCR-based marker systems (Myburg *et al.*, 2001). Therefore, the use of the AFLP and SSR markers systems will enable the construction of well saturated genetic linkage maps and high-resolution mapping of genomic regions.

In this study, we constructed a well saturated genetic linkage map in rapeseed using AFLP and SSR markers which is very

useful for further use in genome wide analyses and quantitative trait loci (QTL) mapping.

MATERIALS AND METHODS

Mapping population

The population used in this study consisted of 93 doubled-haploid (DH) lines developed from an F1 plant (F1DH) generated from a cross between an inbred line, 'Express 617', of the winter rapeseed variety 'Express' and the resynthesized rapeseed line 'RS239'. The resynthesized line 'RS239' is a spring type rapeseed derived from an artificial interspecific hybridization of *B. rapa* (yellow sarson) and *B. oleracea* (cauliflower).

DNA extraction and marker analysis

DNA was extracted from young leaves of 2-3 weeks old plants using Nucleon PhytoPure plant extraction kits (GE Healthcare, Illustra™) according to the manufacturer's instructions and quantified using Bio-Rad Fluorescent DNA Quantification Kit (Bio-Rad Laboratories CA, USA). SSR marker analysis was carried out by KWS breeding company, following the general procedure of SSR marker analysis. For AFLP analysis, DNA was digested with EcoRI and MseI. Restriction, ligation, pre-amplification and selective amplification for AFLP analysis were performed following the multiplex protocol published by Ecke *et al.* (2010).

The amplification products were separated on an ABI PRISM 3100 genetic analyzer (Applied Biosystems) with GeneScan-500 LIZ size standard (Applied Biosystems). The raw data were collected using Genotyper software version 3.7 NT (Applied Biosystems). The markers were scored using GeneMapper software version 3.7 (Applied Biosystems).

High fidelity (HF) genetic linkage map construction

1 Linkage analyses were performed using MAPMAKER/EXP 3.0 (Lincoln *et al.*, 1993). Graphical representations of the linkage groups were produced using MapChart (Voorrips, 2002). The genetic linkage maps were constructed using a Perl script that

controls MAPMAKER and automates the mapping process according to the principle of a high fidelity (HF) map. The HF map is a subset of markers, in which all marker orders are supported by a minimal log-likelihood difference of 3.0 with a maximal distance between markers of 30 cM. In addition, during the construction of the HF map double crossovers (DC) were used as a proxy for marker quality since most scoring errors would occur as DC in the map. When the number of DC shown by a marker exceeded the expected number of DC by more than 3 the marker was excluded from the HF map. The expected number of DC was roughly estimated by multiplying the product of the recombination frequencies between the marker and its flanking markers with the number of informative genotypes.

Markers were assigned to linkage groups using MAPMAKER's 'group' command. Starting with a random set of 5 highly informative markers from a linkage group whose map fit the above mentioned criteria for a HF map, the Perl script used repeated cycles of the MAPMAKER commands 'try', 'insert', and 'ripple' to test whether markers fit into the HF map of a linkage group. Markers that fit were used to expand the HF map. All other markers assigned to the respective linkage group were then tested again against the expanded HF map. This cycle was repeated until no additional fitting markers were found. After processing all linkage groups a full map was constructed by the script by placing all remaining markers individually relative to the markers of the HF map at their most likely position without changing the distances between the HF markers.

The fit of marker segregation ratios to the 1:1 segregation ratio expected in a doubled haploid population was tested for each marker locus by a χ^2 test ($P = 0.05$). Markers that significantly deviated from the 1:1 segregation ratio were marked as skewed markers. Markers with strongly skewed segregations (not significantly different from a 3:1 segregation ratio or beyond) were initially excluded from the HF map construction. After the basic HF map was constructed, the strongly skewed markers were then tested and mapped into the HF or full map if the following criteria were fulfilled: the marker

was unambiguously linked to a specific linkage group and the distances to the neighboring markers was not larger than 20 cM. The marker was permanently discarded from map construction if it was significantly linked to markers on more than one linkage group.

To optimize the map, each linkage group was constructed 120 times starting with a random subset of 5 highly informative markers to calculate possible variants of the HF map. The optimal variant was chosen with the following criteria: the HF map should comprise as many markers as possible, the markers in the HF map should be as evenly distributed as possible, contain as few double crossover as possible and the first marker of the full map should preferably be a marker on the HF map.

RESULTS

Linkage map construction

A genetic linkage map was developed using 484 AFLP and 240 SSR markers. As many as 568 genetic loci were defined on the map. The map covers 2003 cM of the rapeseed genome, organized in 23 linkage groups (Figure 1). In addition to 19 major linkage groups there were three triplets and one marker pair.

The map was aligned to an AFLP and SSR map developed in a cross between a resynthesized line 'R53' and 'Express 617' based on shared markers (Ecke *et al.* 2010). In the marker analysis the line 'R53' had been included. Therefore a direct comparison of the AFLP markers showing a polymorphism in the cross between 'RS239' and 'Express 617' and between 'R53' and 'Express 617' was possible. In addition, the SSR markers were aligned to established SSR linkage maps (Lowe *et al.* 2004; Piquemal *et al.* 2005; Radoev *et al.* 2008; Sharpe and Lydiate, unpublished data; Ecke, unpublished data). Based on the alignments, the linkage groups of the map could be assigned names according to the A and C nomenclature of the *Brassica napus* linkage groups (Parkin *et al.*, 1995; Lagercrantz and Lydiate 1996; Brassica.info: <http://www.brassica.info/resource/maps/Ag-assignments.php>, 22.1.2015).



Figure 1. Genetic linkage map of *B. napus* from the cross of “Express” and “RS239”. Marker positions are presented in absolute positions from the first marker on the HF map of the respective linkage group in cM. The markers in bold are markers in the high fidelity (HF) map. Markers in italic are markers placed individually relative to the markers of the HF map at their most likely position without changing the distances between the HF markers. The markers with skewed segregations are designated with ‘+’ if skewed towards “Express” alleles, and ‘-’ if the “RS239” allele is the more frequent one.

The linkage groups represented A1 to A10 and C1 to C9 of the rapeseed genome with the exception of C6 which could not be identified (Figure 1). Linkage group C8 was split into two unlinked parts, C8a and C8b, indicating that this linkage group was not entirely covered by the markers. Four additional linkage groups, namely LG20, LG21, LG22 and LG23 could not be aligned to the linkage groups of the reference maps. These four small linkage groups together covered 53.6 cM of the genome.

Most of the linkage groups had markers on the HF map as the first marker on the full map. Only on linkage groups A6, A9, C3, C7 and C8b placed markers of the full map preceded the first marker on the HF map,

resulting in negative positions of these markers since the marker positions on the linkage groups were calculated from the first marker on the respective HF map.

Marker density and size of linkage groups

A marker density of one marker per 2.77 cM was calculated from the present map. The 19 major linkage groups had marker density ranging from 1.49 to 4.54 cM, in which the most saturated linkage group was A8 and the least saturated one was C7. The sizes of the linkage groups, numbers of markers and marker densities of the groups are presented in Table 1.

Table 1. Linkage group size, number of markers and marker density per linkage group of rapeseed developed from a cross of “Express” and “RS239”.

Linkage group	Size (cM)	Number of markers	Marker density (cM)
A1	203.6	54	3.77
A2	107.0	42	2.55
A3	173.5	72	2.41
A4	84.2	22	3.83
A5	104.9	31	3.38
A6	101.7	40	2.54
A7	96.3	34	2.83
A8	29.7	20	1.49
A9	102.9	48	2.14
A10	64.3	27	2.38
C1	94.1	24	3.92
C2	97.0	46	2.11
C3	104.4	46	2.27
C4	179.4	48	3.74
C5	135.9	50	2.72
C7	113.4	25	4.54
C8a	24.6	14	1.76
C8b	39.5	22	1.80
C9	93.0	48	1.94
LG20	24.7	2	24.7
LG21	16.4	3	5.47
LG22	7.0	3	2.33
LG23	5.5	3	1.83
Total	2003.0	724	2.77

The linkage map developed in this study displayed a high correlation of $r = 0.88$ ($P = 10^{-6}$) between the length and number of markers per linkage group. It indicates that the markers are evenly distributed across the genome. However, a non-uniform marker distribution was observed in some linkage groups, for instance, gaps of more than 20 cM were found on linkage groups A3, C4, LG20

and two of these gaps on C7. On the other hand, some marker clusters were observed on linkage groups A2, A6, A9, C2, C5 and C9 (Figure 1).

Skewed segregation markers

About 38% of the mapped markers showed a significant ($P = 0.05$) deviation from the

expected 1:1 segregation ratio. The markers with skewed segregations were observed for both types of markers and the percentage of skewed AFLP markers (38.2%) was not significantly different from the percentage of SSR markers (40%). Of the markers with skewed segregations, 93 showed an excess of 'RS239' alleles (33.1%) while an excess of 'Express' alleles was observed in 188 markers (66.9%). The majority of the markers with distorted segregations were clustered on linkage groups A2, A5, A7, A8, C1, C5, C7, C8b, and C9. In addition, 93% of the markers that could not be mapped (193 markers), consisting of 157 AFLP and 36 SSR markers, showed strongly skewed segregations.

Detection of duplicated regions in the linkage map

Using SSR primer pairs, which can amplify more than one homoeologous locus, duplicated regions can be detected. Forty out of 186 SSR

primer pairs used, amplified more than one polymorphic locus. These duplications showed a high level of similarity between the homoeologous A and C genomes.

From 40 duplicated loci, 38 duplications were between the A and C genome, indicating possible homoeologous regions between the two genomes. Homoeologous regions with two shared markers were detected between, A2-C2, A4-C4, A5-C5, A6-C5, and A8-C3 (figures not shown). More testimony for homoeology was observed between A1 and C1, A3 and C3, A9 and C9 and between A10 and C9 (Figure 2). A1 and C1 shared 5 duplicated loci, which covered the largest duplicated regions of about 99 and 73 cM, respectively. The highest number of duplicated loci was observed between A3 and C3 with seven duplicated loci. C9 shared four markers on the lower part of the linkage group with A9 and 5 markers in the central part with A10.

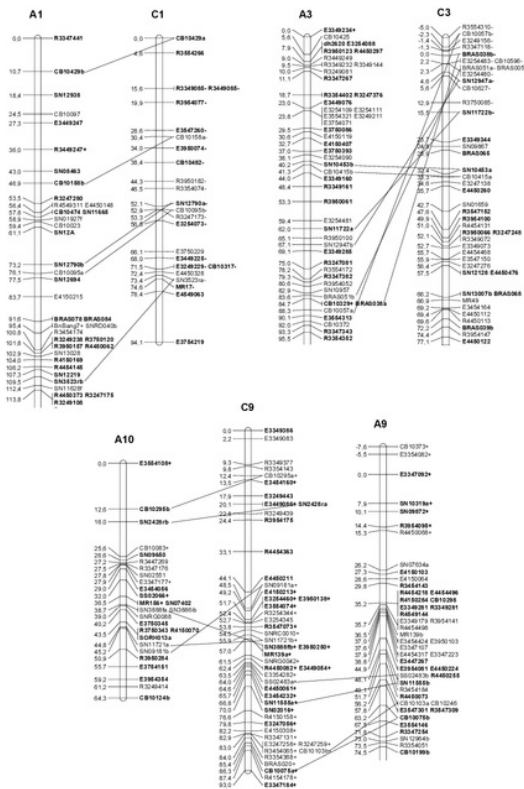


Figure 2. The duplicated regions observed in the genome of *Brassica napus*. Duplicated loci are connected by lines.

DISCUSSION

Genetic linkage map

High-quality genetic linkage maps are very important for genome wide genetic analyses. Several factors can affect the accuracy of linkage group construction, such as the frequency of double crossing overs, errors in scoring and data input, marker type, size and type of the mapping population, and the mapping approach.

In this study, a genetic linkage map covering 2003 cM of the rapeseed genome was constructed using 724 AFLP and SSR markers. Lombard and Delourme (2001) estimated the range of the rapeseed genome from 2.127 to 2.480 cM in a study on consensus linkage map construction. Based on this estimation, the map developed in this study covered about 81 to 94% of the rapeseed genome. A larger map of 2,619 cM was developed by Piquemal *et al.* (2005). In relation to this map our genetic linkage map covers 76.5% of the largest rapeseed map developed so far. Nevertheless the size of the map is not easily comparable, since the type of marker, mapping population, and mapping approach applied were different.

Genotyping or scoring errors can inflate map length because in the mapping they would appear as double crossovers, introducing faux recombination events into the calculation of marker distances. Especially with high density maps comprised of hundreds of markers this effect has the potential to markedly increase map length. In contrast to others genetic linkage maps previously developed, which have large map sizes, the map developed in this study was based on the principle of a high fidelity (HF) map. In this HF map only a subset of markers whose order was supported by a minimal log-likelihood difference of 3.0 with a maximal distance of 30 cM between markers was actually mapped. The remaining markers were only placed individually relative to the markers of the HF map without changing the distances between the HF markers. In addition, during the construction of the HF map the number of double crossovers per marker was used as a quality control criterium and markers showing an unexpectedly high number of double crossovers were excluded from the HF map, further diminishing the impact of errors on

map length. A similar approach was applied by Qiu *et al.* (2006) in the development of a high stringency linkage map for the QTL analysis of seed oil and erucic acid content in rapeseed.

In this research, the map generated has an average marker density of one marker per 2.77 cM which is a high marker density compared to others map generated in rapeseed. Lombard and Delourme (2001) constructed three individual maps. The maps cover about 2023, 1574 and 1911 cM with 590, 344 and 340 mapped markers on each of the maps, respectively. The map developed from a cross between winter and spring type rapeseed showed the highest density with an average marker distance of 3.6 cM per marker, compared to 5.0 and 6.2 cM per marker for the other two maps, which were developed in crosses between two winter and two spring types, respectively.

Resynthesized lines of *B. napus* are generated from its diploid ancestors, *B. rapa* and *B. oleracea*. These genotypes show a high degree of polymorphism to conventional rapeseed phenotypically and in marker analyses (Becker *et al.*, 1995), indicating a high degree of allelic differences. High genomic divergence between the cross in this study is also exhibited from different type of rapeseed used as the parents, a winter ('Express') and spring ('RS239') rapeseed cultivars. By crossing a resynthesized rapeseed line ('RS239') with an elite cultivar ('Express') a highly polymorphic mapping population could be developed, easing the construction of a saturated genetic linkage map which is very important for further genome analyses in breeding programs in rapeseed.

Generally, the markers mapped in the current study were mostly evenly distributed across the genome as shown by a high correlation of $r = 0.88$ ($P = 10^{-6}$) between the length and number of markers per linkage group. Some non-uniform marker distributions, such as gaps and marker clusters observed on several linkage groups might be due to differences in the relation of physical distances and recombination frequencies in different parts of the genome (Tanksley *et al.*, 1992; Lichten and Goldman, 1995).

Segregation distortion

Marker segregations deviating from expected Mendelian segregation ratios are described as

distorted, disturbed or skewed segregations. A high number of distorted segregations were observed among the mapped markers (38%) in this research. Additionally, there were about 193 unmapped markers, in which 93% of them displayed strongly skewed segregations. Segregation distortion has been documented in a number of species, including rapeseed (Foisset and Delourme, 1996; Foisset *et al.*, 1997). According to Foisset *et al.* 1996, there are two main hypotheses that could explain their existence, i.e. gametic selection or selection specific to in vitro microspore culture. Based on the latter hypothesis it was also proposed that disturbed segregations were a common feature in microspore-derived DH populations (Piquemal *et al.*, 2005), due to specific parental alleles favorable for in vitro androgenesis or subsequent plant regeneration.

Contrary to the hypotheses above, Ferreira *et al.*, (1994), comparing maps constructed from a doubled haploid and an F2 population generated from the same F1 plant derived from a cross between an annual canola cultivar (Stellar) and a biennial rapeseed (Major), observed similar rates of distorted segregations of 30% and 24% in the doubled haploid and the F2 population, respectively, although the sets of markers showing skewed segregations were different in the two populations. In addition, Xu *et al.*, (1997) found that there were no significant differences in the rates of distorted markers between F2, BC1 and DH populations in rice.

Lombard and Delourme (2001) noticed a higher frequency of markers that did not segregate according to the expected Mendelian ratio in a cross between winter and spring type rapeseeds (24.6%) than in crosses of two winters (14%) or two spring type (8.8%) parents. In this study a cross between a spring type resynthesized rapeseed, 'RS239', and the winter type inbred line 'Express 617' resulted in more than one third of the mapped markers showing significant deviations from the expected 1:1 segregation ratio. These results indicate that the rate of disturbed segregations may also depend on the genetic distance between the parents of a cross, resulting in high percentages of distorted marker segregations in wide crosses. Kianian and Quiros (1992) proposed that a relatively large number of disturbed segregations might be due to genomic divergences between the parents of a cross and that the rate of disturbed

segregations will increase with the level of divergence. In rice, it has been also reported that wide crosses between sub-species can lead to a higher frequency of markers with distorted segregation (Lin *et al.*, 1992, Lin and Ikehashi, 1993).

A comprehensive study explained that segregation distortion is an indication of linkage between molecular markers and distorting factors such as recessive lethal genes and incompatible alleles (Lyttle, 1991). It assumes that a high frequency of segregation distortion in wide crosses is due to the segregation of many incompatible alleles or recessive lethal genes in the population.

Undetected genome regions

An indication of an incomplete coverage of the entire genome was observed in this study as linkage group C8 was split into two unlinked parts, C8a and C8b (Table 1; Figure 1.), and linkage group C6 was missing altogether. On the other hand, four additional small linkage groups were found that could not be aligned to the reference maps. Some of these may constitute the missing parts of C8 or C6. It may also be that some of the unmapped markers actually represent loci from the missing part or the undetected linkage groups.

A large number of markers (724 markers) were used to develop the genetic linkage map in this research; nevertheless linkage group C6 still could not be mapped. The same was found by Butruille *et al.* (1999) who also could not map linkage group C6 in a composite map developed from inbred and DH populations generated from the cross of winter and spring-type cultivars of *B. napus*, the same type of cross which was used in the construction of the present mapping population. An abnormal segregation of loci on C6 due to the presence of a reciprocal translocation between homeologous segments of A7 and C6 was proposed as a reason by Butruille *et al.*

Evidence for a reciprocal translocation between linkage group A7 and C6 was identified in several genetic mapping populations developed from crosses between spring and winter or annual and biennial rapeseed genotypes (Parkin *et al.*, 1995; Sharpe *et al.*, 1995; Butruille *et al.*, 1999; Udall *et al.*, 2005). Frequently, the annual rapeseed type parent is proposed to be the

origin of the translocation (Osborn *et al.*, 2003; Howell *et al.* 2008). According to Osborn *et al.* (2003), the homoeologous segments of chromosomes A7 and C6 underwent homoeologous recombination at meiosis resulting in gametes with rearranged chromosomes. The lines derived from those gametes could have i) normal or translocated genotypes resulting from alternate segregations, containing either non-translocated A7 and C6 chromosomes or reciprocally translocated chromosomes with a segments of A7 on C6 and vice versa; ii), duplication of a segment of A7 on C6 or of C6 on A7 resulting from adjacent-1 segregation, or iii) the most rare event of adjacent-2 segregation, contain either two A7 homologs or two C6 homologs without exchanged portions of chromosomes, that is a duplication and corresponding deletion of whole chromosomes. The first configuration resulted in normal and viable gametes. Whereas the second and the third could produce non-viable gametes because it has either duplication and deficiency for different regions or whole chromosomes for adjacent-1 and adjacent-2 segregations, respectively (Griffiths *et al.*, 2000).

Even though in allotetraploid species like rapeseed, the deficiency of chromosome A7 or C6 or parts of them will not cause a lethal effect on pollen due to the presence of other homoeologous regions on other chromosomes (Osborn *et al.*, 2003), it might lead to unbalanced marker segregations and further prevent the mapping of the affected linkage group (adjacent-1 segregation case), or result in an aneuploidy of the linkage group (adjacent-2 segregation case). Both situation could be a reason for the failure to map chromosome C6 in our study.

Detection of duplicated regions

Rapeseed (*Brassica napus* L.) is derived from an interspecific hybridization of *Brassica rapa* (A genome) and *Brassica oleracea* (C genome). Therefore, the chromosomes A1–A10 of *B. napus* correspond to the A genome of *B. rapa*, and the chromosomes C1–C9 to the C genome of *B. oleracea*. Cytogenetic investigations indicated that the basic Brassica genomes are related (Attia and Röbbelen, 1986). The genomes are partially homologous and apparently derived from a

common ancestral genome (Mizushima, 1950; Lagercrantz and Lydiate, 1996).

A high degree of duplications within the diploid genomes of *B. rapa* and *B. oleracea* have also been described in previous mapping studies (Kianian and Quiros, 1992; Parkin *et al.*, 2003). A high level of similarities between the homoeologous A and C genomes explaining the duplication of *Brassica napus* loci were reported in some investigations as well (Ferreira *et al.*, 1994; Parkin *et al.*, 1995; Mayerhofer *et al.*, 2005; Udall *et al.*, 2005). Analysis of synteny between the genomes of the Brassica species identified the homoeologous linkage groups of the three genomes. It shows that the shape and ancestral blocks in all of the three diploid Brassica genomes remained unaltered during evolution and only minimal macro-level changes occurred after polyploidization (Panjabi *et al.*, 2008).

In this study, thirty-eight SSR primer pairs amplified two genetic loci in the linkage map showing the duplication of loci between the A and C genomes, allowing the detection of regions where homeology is obvious. An unambiguous homeology involving 5 primer pairs between A1 and C1 covering 99 and 73 cM, respectively, with the same order of markers (Figure 2) shows a large syntenious region between these two linkage groups. Piquemal *et al.* (2005) observed homeology between linkage groups A1 and C1 covering regions of 100 and 80 cM, respectively, which is in line with results of Parkin *et al.* (2003) showing that those two linkage groups are completely collinear. Some homeology between A and C genome were observed between A3-C3, A8-C3, A2-C2, A6-C5, A10-C5, A10-C9 (some figures are not shown).

The number of SSR markers is only 33.15% of the total markers mapped in the linkage map. Therefore, the results found in this study are not optimal for homeology analysis. Thus, the detection of homoeologous regions in this study has to be considered as a lower limit of the extent of duplications in the rapeseed genome.

ACKNOWLEDGEMENTS

This project was supported and funded by the breeding companies Deutsche Saatveredelung AG, KWS SAAT AG, Norddeutsche Pflanzenzucht Hans-Georg Lembke

KG (NPZ) and SW seed GmbH. The doubled-haploid lines of the mapping population were developed by NPZ. The SSR marker analyses were carried out by KWS.

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