Association mapping of seed oil content in *Brassica napus* and comparison with quantitative trait loci identified from linkage mapping[1]

Jun Zou, Congcong Jiang, Zhengying Cao, Ruiyuan Li, Yan Long, Sheng Chen, and Jinling Meng

Abstract: Association mapping has been used increasingly in natural populations with rich genetic diversity to detect DNA-based markers that are associated with important agronomic traits. *Brassica napus* is an important oil crop with limited genetic diversity. "New-type" *B. napus* that is introgressed with subgenomic components from related species has been developed to broaden the genetic basis of "traditional" *B. napus*. In this study, new-type *B. napus* lines and a collection of traditional *B. napus* varieties from different countries were used as two different populations to evaluate seed oil content and to determine the efficacy of association mapping by comparison with previous study of linkage mapping. Relatively rich genetic diversity, but a higher level of linkage disequilibrium was observed in the new-type *B. napus* as compared with the traditional *B. napus*. Similarly, a larger variation in oil content and a greater number of associated markers were detected in the population of new-type *B. napus*. Meanwhile, more than half of the genetic loci, to which the associated markers corresponded, were located within the quantitative trait loci intervals identified previously in linkage mapping experiments, which demonstrated the power of association mapping in *B. napus*.

Key words: Brassica napus, genetic diversity, seed oil content, quantitative trait loci (QTL), association mapping.

Résumé : L'analyse d'association est de plus en plus utilisée au sein de populations naturelles riches en diversité génétique pour détecter des marqueurs génétiques qui sont associés à des caractères agronomiques. Le *Brassica napus* est une culture oléagineuse importante qui présente une diversité génétique restreinte. Des *B. napus* de « type nouveau », obtenus par introgression de composantes sub-génomiques provenant d'espèces apparentées, ont été développés pour élargir l'assise génétique des *B. napus* « traditionnels ». Dans ce travail, des lignées du *B. napus* de « type nouveau » ainsi qu'une collection de cultivars du *B. napus* de type « traditionnel » provenant de divers pays ont été employées pour former deux populations au sein desquelles la teneur en huile des graines a été mesurée et l'efficacité des analyses d'association a été comparée aux études antérieures fondées sur des cartes de liaison génétique. Une diversité génétique relativement élevée et un plus grand déséquilibre de liaison ont été observés au sein des *B. napus* de type « nouveau » par rapport au type « traditionnel ». Pareillement, une plus grande variation pour la teneur en huile et un plus grand nombre de marqueurs associés ont été détectés chez les *B. napus* de type « nouveau ». Plus de la moitié des locus génétiques auxquels correspondaient les marqueurs associés étaient situés au sein d'intervalles QTL identifiés lors d'expériences antérieures, ce qui démontre la puissance des analyses d'association chez le *B. napus*.

Mots-clés : Brassica napus, diversité génétique, teneur en huile dans les graines, locus de caractère quantitatif (QTL), analyse d'association.

[Traduit par la Rédaction]

Introduction

Mapping of quantitative trait loci (QTL) has proven to be a reliable way to dissect complex traits that are controlled by multiple genes (Salvi and Tuberosa 2005). Thousands of QTL that control different traits have been identified in various species through linkage mapping approaches (we refer to these as map-based QTL or M-QTL for simplicity) and

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Table 1. Summary of *Brassica napus* accessions used in this study.

Population new-type *B. napus*	No. of original parents			Accession
	B. carinata	*B. rapa*	*B. napus*	
Introgressed with A^r genomic components from *B. rapa* only	-	1	1	A1– A42, A47
	-	2	1	A43–A46
Introgressed with A^r from *B. rapa* and C^c from *B. carinata* simultaneously	1	1	1	B48–B88
	1	2	1	B89–B99
	1	3	1	B100–B103
Population traditional *B. napus*	Geographical origin			
Chinese varieties	Central China			C104–C121
	Southeast China			C122–C133
	Southwest China			C134–C141
	Northwest China			C142–C145
Varieties from other countries	Australia			D146–D149
	North America			D150–D153
	Europe			D154–D172

dozens of important genes have been cloned by fine mapping of QTL in crop species (Bernardo 2008). The oil content of seeds is one of the most important traits in oil crops and it behaves as a quantitative trait. Many QTL have been shown to control oil content in rapeseed (*Brassica napus*), as determined by linkage mapping of various populations (Zhao et al. 2005; Delourme et al. 2006; Qiu et al. 2006). However, the number of parents that have been used in previous genetic linkage mapping of QTL represent only a very small proportion of the germplasm of rapeseed, and it is not known how often the QTL can be detected repeatedly in practical breeding (Mackay et al. 2009).

In an alternative approach, QTL can be identified in a manner independent from linkage mapping populations by association analysis or association mapping (we refer to these QTL as association-analysis-based QTL or A-QTL for simplicity). The successful examples of association mapping in humans, animals, and forest trees, from which it is hard to produce and (or) maintain experimental linkage mapping populations (Weir 2008; Gailing et al. 2009), highlighted the advantages of the technique to crop geneticists, who were unsatisfied with the limited number of QTL that they could identify through traditional linkage mapping approach. The most significant improvement provided by association mapping, as compared with linkage mapping, is the higher probability of QTL detection, which results from the higher level of allelic diversity among the mapping population utilized, as compared with the limited diversity between two specific parental lines.

With other advantages, such as low cost, high efficiency with respect to time, and utilization of the unrestricted resources in which any breeding parents can be included, the association mapping has been widely used to identify QTL for complex traits in animals and crops (Myles et al. 2009) and more and more plant breeders have come to prefer A-QTL over M-QTL. Then, what is the relationship between A-QTL and M-QTL? How substantial is the overlap between the two types of QTL for a certain trait?

To answer these questions, we investigated QTL that control oil content in the seeds of *B. napus* by association mapping using a set of common molecular markers, which were mapped on the genetic map of a reference population, the TN (Tapidor × Ningyou 7) doubled haploid (DH) population that derived from a cross of the cultivars Tapidor and Ningyou 7 (Qiu et al. 2006), to compare A-QTL with previously mapped M-QTL. In particular, two types of population of *B. napus* were used for the association mapping in this study. One is the conventional cultivars of *B. napus* from different countries, and the other is the lines of new-type *B. napus* which had been developed through the introgression of A^r/C^c subgenomic components from *B. rapa* (A^rA^r) and *B. carinata* ($B^cB^cC^cC^c$) by interspecific crosses to increase the diversity of rapeseed (Li et al. 2004; Qian et al. 2005, 2006; Zou et al. 2010). Genetic diversity and linkage disequilibrium (LD) were evaluated, and association mapping were processed in the two populations, respectively. The accordant locations of the A-QTL with M-QTL would demonstrate the power of association mapping in *B. napus*.

Materials and methods

Plant material

A total of 172 accessions composed of two populations were used in this study (Table 1). The first population, population of conventional cultivars of *B. napus*, comprised 69 cultivars including 42 Chinese cultivars from 13 provinces spread throughout the whole country and 27 cultivars collected from the countries other than China (Table S1).[4] The second population, population of new-type *B. napus*, was made up with 103 lines including 47 lines with different A^r subgenomic components that had been introduced from *B. rapa* (Qian et al. 2006), and 56 accessions with different proportions of A^r components from *B. rapa* and C^c components from *B. carinata* (Li et al. 2004).

Phenotyping

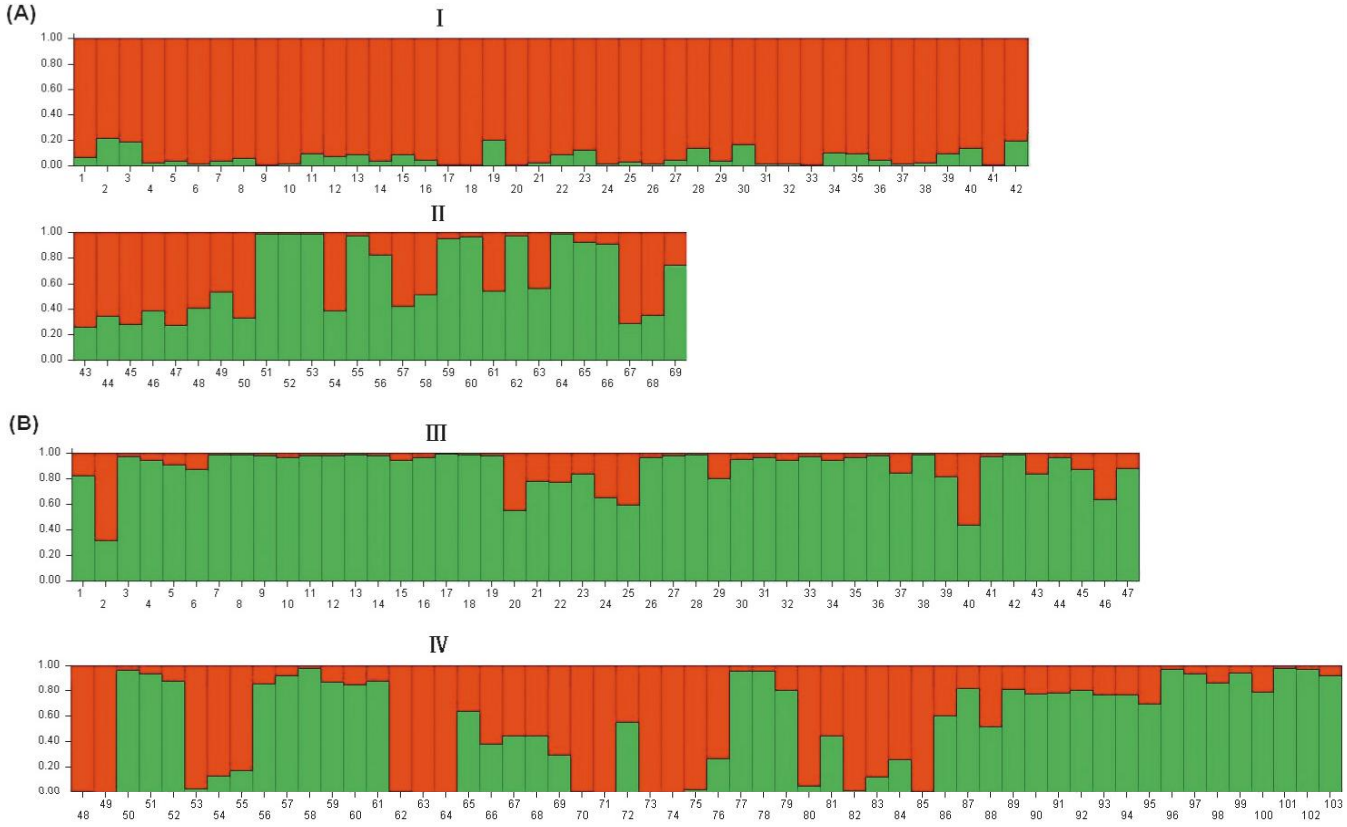
The 172 tested accessions were planted in Huazhong Agricultural University, Wuhan, China in 2005–2006 and 2006–2007 as two experiments. The field experiments were arranged as three rows per plot (10 plants per row) with

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

Table 2. Summary of genetic polymorphism in traditional and new-type *B. napus*.

Population		Traditional *B. napus*	New-type *B. napus*	Total
Polymorphic markers		340	360	378
Locus-specific markers to 96 loci on TN genetic map	Total alleles	281	293	310
	Average alleles per locus	2.9	3.1	3.2
	Population-unique alleles	7	12	19
Unassigned markers	Total number	59	67	68
	Population-unique markers	12	17	29

Fig. 1. Population structure in the accessions of the traditional *B. napus* (A) and new-type *B. napus* lines (B). The *x*-axis shows the individual accessions, and the *y*-axis shows the *Q* value of the population structure when $K = 2$. According to Table 1, the 69 accessions of traditional *B. napus* varieties (A) included groups I and II; the 103 accessions of new-type *B. napus* lines (B) included groups III and IV.



three replications for each accession following a randomized block design. The oil content of the seeds was measured by near-infrared reflectance spectroscopy, and was expressed as a percentage of oil in total seed dry mass (%). The mathematical model for oil content measurement was first established with the results by residue method on analysis for the seed populations from 1288 accessions of rapeseed (the oil content varied from 31.02% to 53.59%), and then to calibrate the measurement of near-infrared reflectance spectroscopy (Gan et al. 2003). For each accession in every experiment, the average value of seed oil content from three repetitions was used for association analysis.

Genotyping

Genomic DNA was extracted from the leaves of young seedlings. In total, 115 pairs of simple sequence repeat (SSR) primers were used in this study. Generated SSR markers enabled the identification of 116 loci on the genetic map of the TN DH population of *B. napus* (Long et al. 2007; Fig. S1[4]). Each particular amplification product (band) was taken as a distinct marker and genotyped dominantly as "0" or "1" in individual accessions along with Tapidor and Ningyou 7, the two parents of the TN DH population. For each accession: if the marker was identical with either Tapidor or Ningyou 7, it was assigned to the corresponding mapped locus; if only one marker was generated from a pair of primer and this marker was not identical with either Tapidor or Ningyou 7, this marker will be regarded as a new allele at the corresponding locus; if one or more markers were generated from a certain pair of primer besides of the locus-specific marker identified, the additional marker(s) were named as unassigned-marker(s).

Fig. 2. Linkage disequilibrium (LD) scatter plot based on all pairwise comparisons between adjacent loci in the traditional *B. napus* population (A) and new-type *B. napus* population (B). Plots of LD are represented by R^2 against genetic distance (in cM).

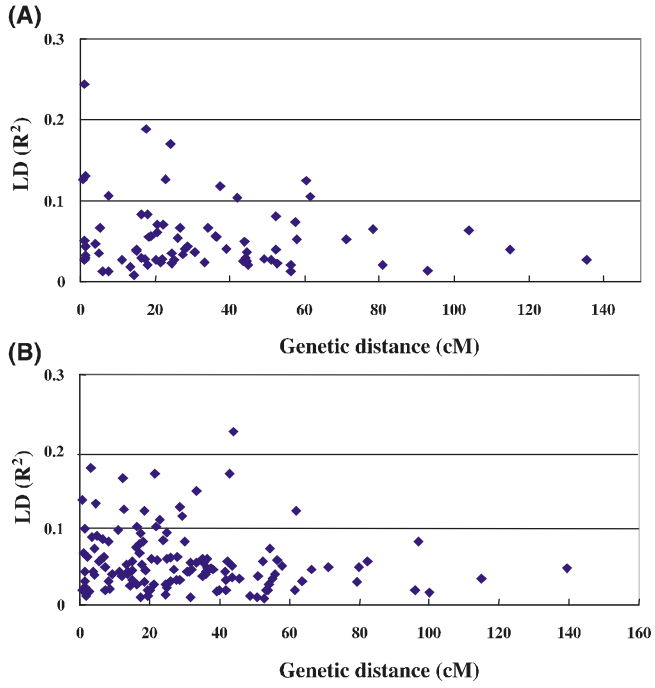
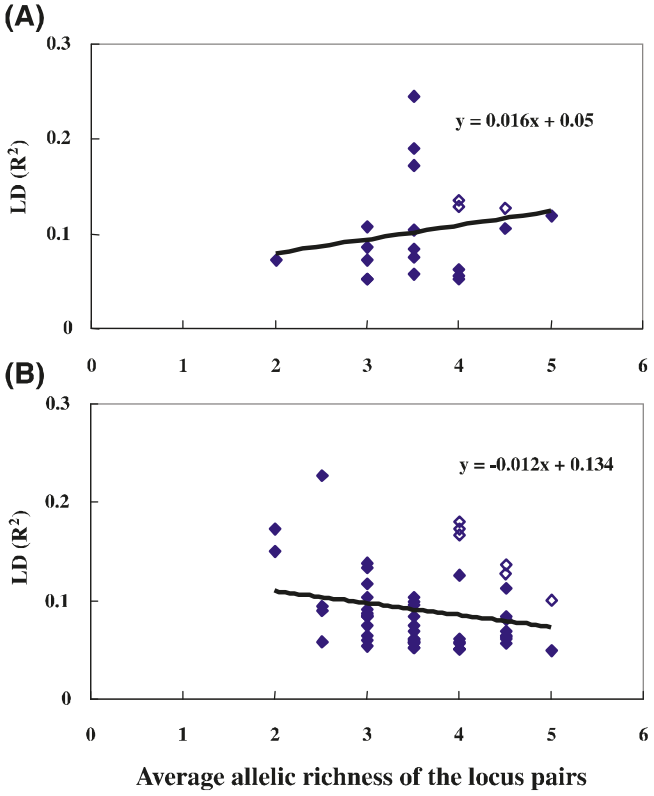


Fig. 3. Dependence of linkage disequilibrium (LD) on allelic diversity and unique alleles in the traditional *B. napus* (A) and the new-type *B. napus* (B). Plots of intrachromosomal LD are represented by R^2 against the average level of allelic richness of the locus pairs, which is represented by the average number of the alleles detected in the two loci involved in one LD pair. The hollow and solid diamonds represent the LD pairs between two loci with and without unique alleles, respectively. Only significant LD pairs (P <0.001 and $R^2 > 0.05$) were calculated.



Polymorphism analysis

Molecular diversity within all 172 accessions and between the two populations was calculated separately with the locus-specific markers and unassigned markers. The χ^2 test was used to test for deviation of each polymorphic marker distribution between the two populations from the null hypothesis H_o: $P_{\text{new-type } B.\ napus(i)} = P_{\text{traditional } B.\ napus(i)}$ (Li et al. 2002). Markers were considered to be unique if they predominated in only one of the populations at a significant level, as determined with the χ^2 test, and rare if they occurred in less than 0.5% of the accessions investigated. Polymorphic markers that occurred in more than 0.5% of the investigated accessions were referred to as widespread or common markers. These statistical analyses were conducted with Microsoft Office Excel and the software POPGENE 1.3 (Yeh et al. 1997).

Population structure evaluation

The software STRUCTURE 2.2 (Pritchard et al. 2000) (http://pritch.bsd.uchicago.edu/software.html) was used to evaluate the genetic structure of the two populations, namely, population from the new-type *B. napus* and the population for the traditional *B. napus*. The calculations were carried out under an *admixture ancestry* model and *correlated allele frequency* model, and the lengths of *burn-in* period and Markov chain Monte Carlo steps were both set to be 100 000 for the assumed K value (number of existing subpopulations), which was varied from 1 to 10. Five independent iterations were run for each K value, and the stability of the value ln $Pr(X/K)$ and the obtained Q value (proportion of genomic comprising that originated from every subpopulation for each cultivar) among different iterations were both considered to identify the correct K value. The final **Q** matrix of the identified K value was the average result of five independent iterations.

Linkage disequilibrium analysis

The LD was evaluated using the software Powermarker (Liu and Muse 2005). Given that SSR are multiallelic markers, LD was estimated using squared allele-frequency correlations (R^2) according to Malysheva-Otto et al. (2006). The rapid permutation test (1000) was performed to detect the significance of pairwise LD. The loci were considered to be in significant LD if $P < 0.01$. Rare alleles (occurred in less than 0.5% of all the accessions) were excluded because the allele frequency affected the evaluation. The plots of LD for pairs of intrachromosomal loci versus genetic distance in centimorgan (cM) between loci in pairs were drawn from R^2 values.

Association analysis

The general linear model (trait variation = marker effect + population structure + residual error) in TASSEL 2.1 Standalone software (available at http://www.maizegenetics.net), which takes population structure as the covariate, was used

to test the marker–trait association in each population and each experiment. Markers that were associated with variation in seed oil content at a significance level of $P \leq 0.1$ ("*P*_Marker") were admitted. "R^2_Model" and "R^2_Marker" indicated the associated contribution to the variation in seed oil content of the model and the marker itself, respectively.

Results

Genetic polymorphism and population structure in the accessions of traditional *B. napus* and new-type *B. napus*

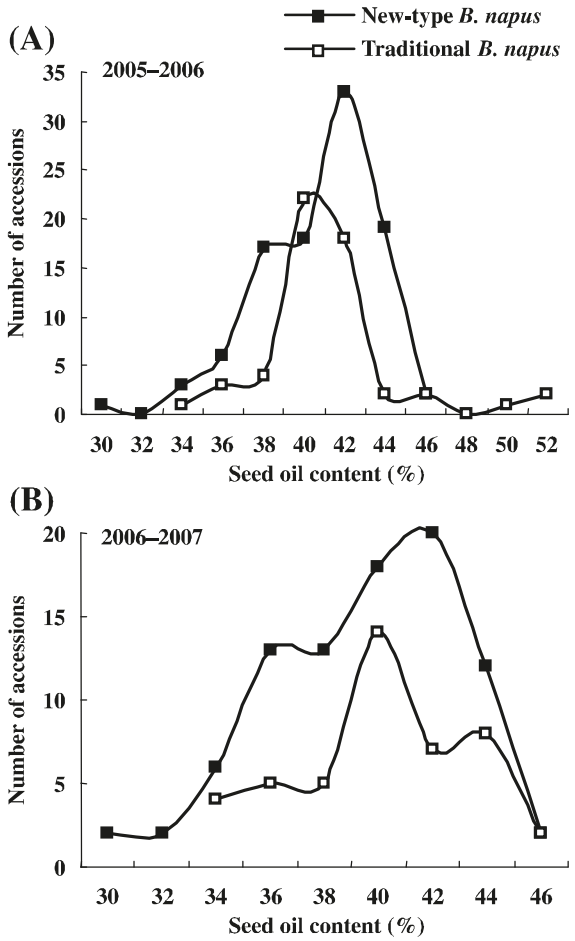
To evaluate the genetic polymorphism of the accessions in the population of traditional *B. napus* and new-type *B. napus*, 378 SSR markers were used in which 310 were locus-specific markers representing 96 genetic loci distributed in all of the 19 linkage groups of TN genetic map (Table 2, Fig. S1), and 68 were unassigned. Eighty-four percent of the markers were shared between the two populations, and the population of new-type *B. napus* contained more unique markers and alleles than that of the traditional *B. napus*. Although there were just six original parents involved in the resynthesis of the new-type *B. napus* (one parental *B. napus*, three parental *B. rapa*, and two parental *B. carinata*), the new-type *B. napus* was clearly more diverse than the traditional *B. napus* varieties, which were collected worldwide.

The genetic structures for the population of traditional *B. napus* and the population of new-type *B. napus* were evaluated separately with 273 SSR markers from 85 of 96 loci, to satisfy the requirement of minimum genetic distance (larger than 5 cM) (Fig. S1). The value of α (degree of admixture) in each population varied greatly in particular runs and among different iterations under the assumption $K = 1$, which suggested that subpopulations existed within the populations. When K was set from 2 to 10, variation in the ln $Pr(X/K)$ value and the obtained Q value (for each accession) among different iterations was much smaller under the assumptions of $K = 2$, 3, and 5 as compared with other assumed K values. According to the concept of using the smallest value of proper K that captures the major structure in the population, we eventually identified the **Q** matrix under the assumption of $K = 2$ for each population (Fig. 1). This indicated that the 69 accessions of traditional *B. napus* and the 103 accessions of the new-type *B. napus* could each be divided into two apparent subpopulations. Interestingly, the two subpopulations of traditional *B. napus*, subpopulation I and II, were composed of all of accessions of Chinese cultivars (42) and 27 cultivars bred from countries other than China, respectively. The subpopulation III and IV were composed of new-type *B. napus* with different A^r subgenomic components, and with different proportions of A^r and C^c components, respectively.

Linkage disequilibrium analysis in the accessions of new-type *B. napus* and the traditional *B. napus*

The LD in the accessions was also evaluated with the 96 loci resulting in 4560 possible intrachromosomal and interchromosomal locus pairs. A significance level of $P < 0.001$ was set for the occurrence of LD for all locus pairs. Of all locus pairs, 25.1% and 12.2% were in LD for the new-type

Fig. 4. Phenotypic variation among the populations of the traditional *B. napus* and the new-type *B. napus* in the experiment carried out in 2005–2006 (A) and in 2006–2007 (B), respectively.



B. napus and the traditional *B. napus* populations, respectively. Of all possible intrachromosomal locus pairs (332), 31.6% and 14.5% were in significant LD for the new-type *B. napus* and the traditional *B. napus*, respectively, whereas 24.5% and 11.9% of all interchromosomal locus pairs (4228) were in significant LD for the new-type *B. napus* and the traditional *B. napus*, respectively. LD was more prevalent between loci on the same chromosome than for loci on independent chromosomes. The overall level of LD in the traditional varieties was low, as indicated by the small proportion of marker pairs in significant LD and the low mean R^2 of these pairs.

Figure 2 shows the LD decay scatter plots for significant, adjacent pairwise comparisons for the populations of new-type *B. napus* and the traditional *B. napus*. Though no significant LD decay was presented in two populations, as the interlocus distance increased, the R^2 value decreased. Intrachromosomal LD extended to distances of up to 80 cM with $R^2 > 0.05$ or up to 30 cM with $R^2 > 0.2$ in the new-type *B. napus*. The distance over which LD decayed was quite similar for both populations, with LD extending over a marginally larger distance for the traditional *B. napus* than the new-type *B. napus*. Values of R^2 were increased over distances of up to 120 cM ($R^2 > 0.05$) in the traditional *B. napus*.

Table 3. Number of markers associated with seed oil content in traditional and new-type *B. napus* and coincidence with oil-content-related QTL identified in the TN DH population.

			Contribution to oil content variation		Comparison with linkage mapping	
Population	Year of experiment	No. of associated markers	Marker genotype	Population structure and residual error	No. of identified loci on TN map	No. of loci in QTL intervals
Traditional *B. napus*	2006	23	5% ~ 13%	2%	22	12
	2007	27	6% ~ 15%	6%		
New-type *B. napus*	2006	93	3% ~ 30%	1%	46	26
	2007	85	3% ~ 15%	10%		
Total		150			54	32

In Fig. 3, the plot of R^2 versus the average allelic richness of the two loci involved in one significant intrachromosome LD pair ($P < 0.001$ and $R^2 > 0.05$) exemplifies the dependence of LD on allelic diversity. The allelic diversity of the locus pairs was represented by the mean value of the number of alleles detected in the two loci involved in one LD pair. Although there was no significant coefficient relativity was detected for the two linear regressions curves, but the curves presented the current of the correlation. Different patterns of dependence were observed between the new-type *B. napus* lines and the traditional *B. napus*. A decreased R^2 value for the locus pairs which represented richer allelic diversity was observed in the new-type *B. napus*, whereas an increased R^2 value was observed in the traditional *B. napus*. Interestingly, in both of the populations, the R^2 value for locus pairs with unique alleles tended to be higher than that for the locus pairs without unique alleles at the same level of allelic diversity (Fig. 3).

Marker–trait association analysis for seed oil content variation and its comparison with linkage mapping results

Phenotypic data were obtained for 99 and 88 accessions of new-type *B. napus* and for 55 and 45 accessions of traditional *B. napus* from the experiments carried out in 2005–2006 and 2006–2007, respectively. The variation of seed oil content exhibited a continuous distribution in both populations, and the variation extent was 15% on average (Fig. 4). It is notable that some accessions of the new-type *B. napus* had extremely low oil content (below 30%) and some accessions of the traditional *B. napus* varieties had extremely high oil content (over 50%).

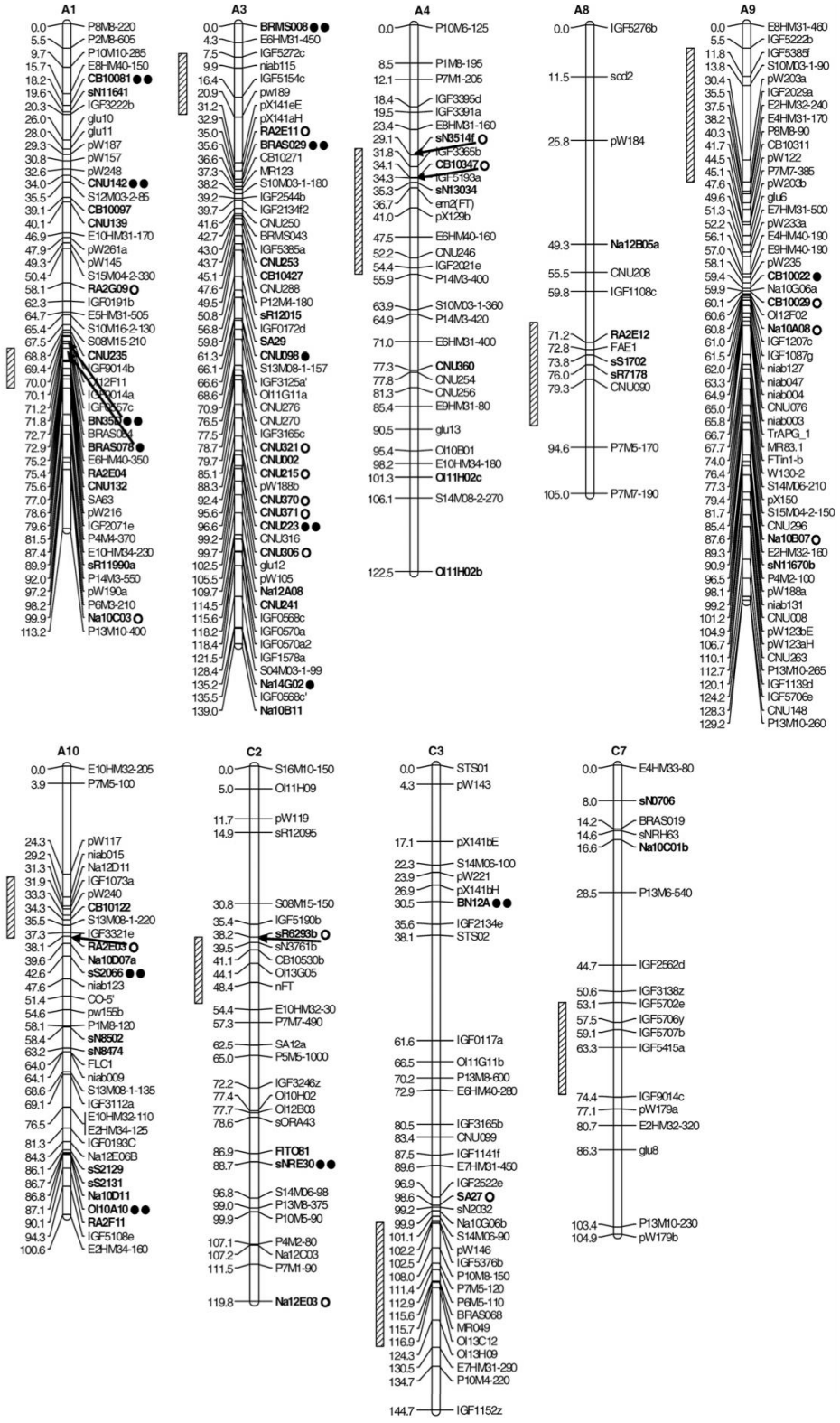
Through general linear model analysis, which considered the effect of population structure on variation in seed oil content as well as the effect of markers, a total of 150 distinct markers showed association with seed oil content variation in either of the two different populations, among which 27 markers were found to show an association in both populations (Table 3 and Table S2[4]). For the traditional *B. napus* population, the minimum effect of particular markers was higher than that among the new-type *B. napus* population, but the maximum effect did not exceed that among the new-type *B. napus* population. The population structure and residual error for phenotypic variation were similar in the two populations. However, the number of associated markers that were obtained specifically from the population of new-type *B. napus* was five times greater than that obtained from the population of traditional *B. napus* varieties. Particular markers were found to be associated with at least 3% of the seed oil content variation ("R^2_Marker") among the accessions of new-type *B. napus* in both experiments, and at most 30% in the first experiment. In contrast, the effect of population structure, including residual error, accounted for only 1% of phenotypic variation in the first experiment but up to 10% in the second, in which residual error was increased owing to missing data.

Given that a number of seed oil-content-related M-QTL have been obtained via linkage mapping of the TN DH population from multiple environments (Qiu et al. 2006; C. Jiang et al. unpublished data), we were very interested to determine whether, or how large a proportion of, these M-QTL could function in a random and (or) ordinary genetic background. By checking the corresponding locations of associated markers on the TN genetic map to identify the A-QTL, we compared the overlap between the M-QTL and A-QTL. Among the 150 distinct associated markers that we identified from the two *B. napus* populations, 96 locus-specific markers corresponded to 54 distinct genetic loci on the TN genetic map, which identified 54 A-QTL associated with oil content variation of *B. napus*. There were nine distinct M-QTL intervals related to seed oil content variation previously identified by Qiu et al. (2006) based on the TN DH population. When comparing the location of these M-QTL and A-QTL, we found that 6 of the 54 A-QTL fell in the intervals of four of the nine M-QTL that were located on linkage group A1, A4, A10, and C2 (Fig. 5). It indicated that nearly half of the M-QTL for oil content in seeds would be retrieved by association mapping.

Discussion

In the present study, we analysed the genetic polymorphism, population structure, and LD in two populations of *B. napus*, namely, the traditional *B. napus* and new-type *B. napus*. The accessions of new-type *B. napus* showed richer SSR diversity and more specific markers than that of the traditional varieties. The higher level of LD and the different pattern of dependence on allelic diversity in the genome of the new-type *B. napus*, as compared with the traditional *B. napus*, indicated the short history of recombination of the former and the frequent genetic linkage that resulted from introgression, as well as the significance of these factors in association mapping. Marker–trait association analysis showed that a total of 150 distinct markers and 54 A-QTL identified through the two populations were associated with variation in seed oil content, and the number of associated markers that were obtained specifically from the new-type *B. napus* population was five times greater than that obtained

Fig. 5. The comparison between linkage QTL and associated loci. It exhibits the nine linkage groups on which QTL related to seed oil content variation in TN genetic population were identified by Qiu et al. (2006). The genetic linkage map was published by Long et al. (2007), and the corresponding loci of assessed SSR primers in this study are shown in bold type. The scratched rectangle at the left of each linkage group indicates the QTL interval that was identified by Qiu et al. (2006) via linkage mapping. The circles at the right of each linkage group indicates associated loci of the seed oil content variation among the population of new-type *B. napus* (empty circle), traditional cultivars (single solid circle), or both (double solid circles). The corresponding loci of assessed primers (bold) without circles indicates nonassociation was obtained among the populations we used in this study.



from the traditional *B. napus* population. More than half of the loci, to which these associated markers corresponded on the *B. napus* TN genetic map, especially those detected in the new-type *B. napus*, were located in the QTL intervals that were identified during previous linkage mapping, which demonstrated the power of association mapping in *B. napus*.

The choice of populations and the correct evaluation of the population structures and LD decay are considered to be crucial for association mapping (Sorkheh et al. 2008). Most previous studies mainly considered populations with distant origins where they had been cultivated, to increase the level of allelic diversity (Zhao et al. 2007; Hasan et al. 2008; Yan et al. 2009). In this study, in addition to the traditional *B. napus* varieties, we utilized a number of accessions of new-type *B. napus*, which might carry more distinct alleles that are derived from genomic rearrangement during interspecific hybridization (Zou et al. 2010). Both populations exhibited a simple population structure, which well agreed with the pedigree and origin of the lines, for example, the difference between those cultivars from China and those from other countries. This demonstrated that breeding origin had a substantial impact on population structure. A previous study also reported a distinctive Chinese *B. napus* population that usually experienced interspecific hybridization such as with *B. rapa*, though some other Chinese cultivars showed relatively higher genetic similarity with cultivars from Australia, Canada, and Europe (Chen et al. 2008).

Recently, it was reported that the LD beyond about 2 cM decayed rapidly in canola quality winter rapeseed (*B. napus*) revealed with AFLP markers (Ecke et al. 2010). However, a more precise result about the structure and extent of LD in the whole genome in a core collection of various *B. napus* is still expected. Since physical map information is still limited in *B. napus*, it is difficult to develop high-density single-nucleotide polymorphism maps for high-throughput locus-specific assay, which could help to provide accurate knowledge about the extent of LD in *B. napus* (Hasan et al. 2008). Owing to the limited number of markers and the large genetic distances between most of the markers identified in TN genetic map used in this study, we might not obtain sufficiently accurate information on LD decay in the two populations. However, the low level of LD in the traditional *B. napus* suggested that large numbers of markers would be required to maintain power in association analysis in *B. napus*. In contrast, the high level of LD in the new-type *B. napus* resulted possibly from the short history of resynthesis and genetic linkage because of introgression of large exotic genomic fragments, and should enable A-QTL to be identified with fewer markers. On the other hand, exotic introgression, frequent recombination, and substantial genomic alterations in the genome of the new-type *B. napus* could provide many novel favourable alleles, which should promote the efficiency of association mapping. Although the population size of the new-type *B. napus* was only twice that of the traditional *B. napus*, the number of associated markers detected specifically in the new-type *B. napus* population was five times greater than the number detected in the traditional *B. napus* population. Given that the estimated population structures and their effects were similar in both populations, the higher probability of detection of associated markers in the new-type *B. napus* than in the traditional *B. napus* should be related to the higher genetic diversity of the novel genetic resource represented by the former population (Table 2).

Four of nine M-QTL (44%) identified previously (Qiu et al. 2006) were validated by six overlapped A-QTL from association mapping approach in this study. On the other hand, it seems that only a small amount of A-QTL (6 in 54, or 11%) could be covered by M-QTL from linkage mapping approach. However, this low coverage does not mean a low efficiency of M-QTL identification. As the improvement on the power of QTL identification (Long et al. 2007), and the implement of multiple-environmental experiments, the number of M-QTL for seed oil content identified from the same TN DH population could be as much as that of A-QTL identified in this experiment (C. Jiang et al. unpublished data), and a significant higher portion of A-QTL (32 in 54, or 60%) would be retrieved by M-QTL. This suggests that association mapping and traditional linkage mapping will complement each other. An important reason for the growing enthusiasm of crop geneticists for association mapping is that it is much more likely that the detected associated loci will function in a common genetic background rather than just a specific background, such as an experimental genetic population. After decades of M-QTL mapping studies, a few of the identified M-QTL realized their function in breeding programs, but their expected effects usually "disappeared" after introgression into another background (Salvi and Tuberosa 2005). For association mapping, we suggest the use of populations of modern varieties rather than those with extreme phenotypic expression that are eliminated from breeding resources, because we may obtain QTL from those extreme materials but not elite alleles suitable for breeding purposes. In this study, the traditional *B. napus* varieties that we used were commonly planted during the past 20 years, but were considered previously to be elite varieties. Using this population, we aimed to identify additional QTL as well as elite alleles that were not detected through previous linkage mapping. The new-type *B. napus* used in this study were also used as intermediate materials in our breeding program, because of the introgression of favourable alleles and the appearance of novel alleles that resulted from genomic alterations induced by interspecific crosses (Zou et al. 2010) as well as their canola quality, which will lead to the immediate utilization in canola breeding without any impairment to the oil quality in the canola seed (Chen et al. 2010). Therefore, the addition of novel germplasm for association analysis would significantly enhance the power of association mapping and greatly benefit the practical breeding programs.

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