A Genetic Linkage Map of Nonheading Chinese Cabbage

Jian-Feng Geng^{1,2}, Cheng-Song Zhu¹, Xiao-Wei Zhang², Yan Cheng, Yuan-Ming Zhang³, and Xi-Lin Hou³

State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China

ADDITIONAL INDEX WORDS. *Brassica rapa* ssp. *chinensis*, doubled haploid population, SSR, RAPD, SRAP, ISSR, segregation distortion

ABSTRACT. *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt, known as nonheading chinese cabbage in China, is an important vegetable in eastern Asia and its genetic improvement requires a genetic linkage map. The first genetic linkage map of nonheading chinese cabbage using 112 doubled haploid lines derived from a released F₁ hybrid cultivar Shulü between two lines SW-3 and Su-124 was constructed in this paper. One hundred thirty-eight molecular markers were mapped into 14 linkage groups. Among these markers, there were 77 sequence-related amplified polymorphism markers, 27 simple sequence repeat markers, 21 random amplification polymorphic DNA markers, and 13 intersimple sequence repeat markers. Chi-square tests showed that 54 markers are distorted from Mendelian segregation ratios, and the direction of the distortion is mainly toward the maternal parent SW-3. The distortion affects not only the estimation of genetic distance, but also the order of distorted markers on a same linkage group. Given a specific marker order, the authors proposed a multipoint approach to correct the linkage map in an unbiased manner in an F₂ population while considering distorted, dominant, and missing markers. A new method was used to correct the linkage map in the doubled haploid population mentioned earlier considering new, distorted, and missing markers. The total length of the corrected linkage map was 1923.75 cM, with an average marker spacing of 15.52 cM. The map will facilitate selective breeding and mapping of quantitative trait loci.

The genus *Brassica* includes many important vegetables: chinese cabbage [*B. rapa* ssp. *pekinensis* (Lour.) Hanelt], nonheading chinese cabbage (*B. rapa* ssp. *chinensis*), cabbage (*B. oleracea* L. var. *capitata* L.), broccoli (*B. oleracea* var. *italica* Plenck), cauliflower (*B. oleracea* var. *botrytis* L.), kale (*B. oleracea* var. *acephala* DC.), and rape [*B. napus* L. ssp. *oleifera* (Delile) Sinskaya] (Nozaki et al., 1997). Nonheading chinese cabbage, which originated from China, is one of the most important vegetables in eastern Asia. However, there are fewer detailed selective breeding programs worldwide. Recently, we have established a selective breeding program focusing on the improvement of important agronomic traits (e.g., disease resistance and quality). Such a breeding program requires the development of a large number of DNA markers for the detection of quantitative trait loci (QTL), and marker-assisted selection to speed up the breeding process and to increase the selection efficiency.

Genetic linkage maps that are essential to the detection of QTL and other applications have been constructed for nearly all

economically important plants. Although there are a number of genetic maps reported in *B. rapa* (Ajisaka et al., 1995; Nozaki et al., 1997; Song et al., 1991; Zhang et al., 2006), a linkage map for nonheading chinese cabbage is unavailable.

Sequence-related amplified polymorphism (SRAP) recently developed by Li and Quiros (2001) is aimed at amplifying the open reading frames with particular primer pairs. The primers consist of core sequences and three selective nucleotides, and the core sequences contain “filler” sequences (nonspecific constitution) and specific sequences. The forward primer is 17 bp (10-bp filler + CCGG + three selective nucleotides) whereas the reverse primer is 18 bp (11-bp filler + AATT + three selective nucleotides). They can pair with exons and promoters (or introns) respectively. The polymorphisms revealed by these primer pairs originate from the variations of the lengths of the involved introns, promoters, and spacers among individuals and species. Sequence-related amplified polymorphisms are easily amplified in crops (Li and Quiros, 2001), and may combine simplicity, reliability, moderate throughput ratio, and facile sequencing of selected bands, so it has been widely used in the comparative analysis of biotype (Budak et al., 2004a), in the evaluation of genetic diversity (Budak et al., 2004b; Ferriol et al., 2003), and in the construction of genetic maps (Li and Quiros, 2001; Sun et al., 2007). In theory, moreover, SRAP can detect any kind of sequence differences, including base changes and insertions and deletions, so there are as many of these markers as single nucleotide polymorphisms (SNPs) in a genome. In contrast to SNPs, however, a primer combination in the SRAP protocol can detect multiple loci in a genome without known sequence information. Intersimple sequence repeat (ISSR) is derived from simple sequence repeat (SSR), which amplifies specifically the region

Received for publication 20 Feb. 2007. Accepted for publication 11 July 2007. This work was supported in part by the 863 program (no. 2006AA10Z1C9) to X.-L. Hou; the 973 program (no. 2006CB101708), the National Natural Science Foundation of China (no. 30671333), Jiangsu Natural Science Foundation (BK2005087), and NCET (NCET-05-0489) to Y.-M. Zhang; and the Program for Changjiang Scholars and Innovative Research Team in University, the Ministry of Education (IRT0432).

We thank Genyi Li for providing the sequences of sequence-related amplified polymorphism primers and for improving the presentation of the paper.

¹Contributed equally to this paper.

²Current address: Institute of Horticulture, Henan Academy of Agricultural Science, Zhengzhou, 450002, China

³Corresponding authors. E-mail: hxl@njau.edu.cn or soyzhang@njau.edu.cn.

Table 1. Sequences of sequence-related amplified polymorphism primers used in the development of genetic linkage maps for nonheading chinese cabbage.

Name of primer	Sequences of primer (5' → 3')	Name of primer	Sequences of primer (5' → 3')
me1	TGA GTC CAAACC GGA TA	pm15	TAGGGTATATGTGCGGGA
me2	TGA GTC CAAACC GGA GC	pm19	CTTCAAGCGGTGCATTCC
me3	TGA GTC CAAACC GGA TG	pm34	GGGACGAACAGACAACGA
me4	TGA GTC CAAACC GGA CA	pm36	GCCCATCACAGTGTCAAAC
me5	TGA GTC CAAACC GGG AT	fc1	TCAAGGGCAGGTAAGAACAA
me6	TGA GTC CAAACC GGG CT	fc8	GTGCTTTACTGTTTGCTCC
me7	TGA GTC CAAACC GGT AA	fc9	AATCTTCCTCAGGTTTCA
me8	TGA GTC CAAACC GGT GC	odd38	TACAGAACTTAGAACCGAAA
em1	GAC TGC GTA CGAATT ATT	odd40	CAGCAACACCAACAATCTC
em2	GAC TGC GTA CGAATT TGC	odd44	AATGTCTTTGTTTCAGCCAC
em3	GAC TGC GTA CGAATT GAC	odd53	CCATCGTGCAATCTTTCTTC
em4	GAC TGC GTA CGAATT TGA	sa12	TTCTAGGTAATCCAACAACA
em5	GAC TGC GTA CGAATT AAC	sa17	ATAAGAATCAGCAGACGCAT
em6	GAC TGC GTA CGAATT GCA	sa20	TAGAGGAAGATGCAATGTTG
em7	GAC TGC GTA CGAATT ATG	ga3	TCATCTCAAACCATCTACAC
em8	GAC TGC GTA CGAATT CTG	ga18	GGCTTGAACGAGTGACTGA
em9	AGG CGG TTG TCAATT GAC	ga24	TTTTGGGACTGATAGCATTC
em11	TGT GGT CCG CAAATT TAG	ga32	TTGCGATGTATTGTTATTAG
em14	GAAGTCGTCGAGATGGTC	ga43	TCATGGATCTACGAACCTCA

between two microsatellite motifs (Zietkiewicz et al., 1994). Compared with random amplified polymorphic DNA (RAPD), ISSR produces more reliable and reproducible result. Inter-simple sequence repeat has been broadly applied to investigate

genetic diversity, phylogenetic relationships and germplasm identification, and construction of genetic linkage maps (Blair et al., 1999; Kantety et al., 1995; Kojima et al., 1998; Moreno et al., 1998). Therefore, it is feasible to make use of SRAP,

Table 2. Sequences of simple sequence repeat primers used in the construction of genetic linkage maps for nonheading chinese cabbage.

Name of primer	Sequences of forward primer (5' → 3')	Sequences of reverse primer (5' → 3')
BRMS036	GGTCCATTCTTTTTCATCTG	CATGGCAAGGGGTAACAAACAT
BRMS040	TCGGATTTGCATGTTCTGACT	CCGATACACAACCAGCCAACTC
BRMS042	GGATCAGTTATCTGCACCACAA	TCGGAATTGGATAAGAATTCAA
BRMS043	GCGATGTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTC
BRMS088	TATCGGTACTGATTCGCTCTTCAAC	ATCGGTTGTTATTTGAGAGCAGATT
Na12D04	ACGGAGTGATGATGGGTCTC	CCTCAATGAAACTGAAATATGTGTG
Na14G02	TTCCCTTTATTGAGCAAGCTG	TCCCGGTGCGTAAGATATTG
FITO007.2	GGTCGGCACGAGAGCTTTAAG	CCTAAAATTTAATCGCACGCCG
FITO033	GTAACCACGAAGATGCTGA	TCTAACCCTCCATACACTCC
FITO045	ATGGCTGTAGAAACACATTGA	CTGACAACACGAGCATCTTAC
FITO047	TTTATGTGTGAGGCAGATGA	AGTAGGTTGATTGTATTGACCAG
FITO105	GGTGAGCAAAGGATACAAAG	AAACCGAATAAACCGAAA
FITO152	AGAGGCAGGTGGATTCT	AGTGTCTTGGTAGTCAGAGCC
FITO172	TTTAGAGAGTTCCAGTCCTC	CAAGGTCAGTTTGGCATT
BrH80A08-FLC1	TTCCCAAGCTTGCTGGTACT	GAGATTTCCCTCGCTTGATG
BrH04D11-FLC2	GCGCCAATTATAAATTTGATTTTC	TCCTCCTGAACCTGGTCTTG
BrH80C09-FLC3	CAGTGAAGTTCAACCGCAGTA	CATGAGTGAACATAAAACAGTGAAA
O111H02	TCTTCAGGGTTTCCAACGAC	AGGCTCCTTCATTTGATCCC
BN9A	GAGCCATCCCTAGCAAACAAG	CGTGGAAGCAAGTGAGATGAT
O110F09	AGAGAGCGAGATTGATTGGC	AAACGACCACGAGTGATTCC
C20	GACAAGGAGGCAGCATAAAAA	GGAAGATGGATTGCTCTACGG
D3	GGAGCCAGGAGAGAAGAAGG	CCCAAACTTCCAAGAAAAGC
D4	TGTCAGTGTGTCCACTTCGC	AAGAGAAAACCAATAAAGTAGAACC
D7	TGAAACTAGGGTTTCCAGCC	CTTCACCATGTTTGTGCCC
D11	GCCAACTTAATTGATGGGGTC	CCTCAATGTTCTCTCTCTCTCTC
D21	ATCATCAATCCTGACGAGGC	CGCGCACACACACACAC
P4	AACTTTGCTTCCACTGATTTT	TTGCTTAACGCTAAATCCATAT
P26	GGTGGCTCTAATTCCTCTGA	ATCTTTCTCTACCAACCCC
P29	ACCTCCTGCAGATTCGTGTC	GCTGACCTTTCTTACCGCTC
P30	AAATTGTTTCTCTCCCAT	GTGTTAGGGAGCTGGAGAAT

Table 3. Molecular markers analysis for distorted, linked, and unlinked markers in nonheading chinese cabbage.

Marker	Primers (n)	Markers (n)	Distorted markers (n)	Distorted markers (%)	Linked markers (n)	Unlinked markers (n)	Unlinked markers (%)
SRAP	90	154	67	43.5	77	77	50.0
SSR	40	43	23	53.5	27	16	37.2
RAPD	22	32	16	50.0	21	11	34.4
ISSR	8	16	7	43.8	13	3	18.8
Total	160	245	113	46.1	138	107	43.7

ISSR, intersimple sequence repeat; RAPD, random amplified polymorphic DNA; SRAP, sequence-related amplified polymorphism; SSR, simple sequence repeat.

Table 4. Test for goodness-of-fit for Mendelian segregation ratios and genetic distances (GD) with and without considering marker segregation distortion for the eighth linkage group in nonheading chinese cabbage.

Name of marker	Genotype (n) ^z		χ^2_c value ^y	GD (cM)	GD considering segregation distortion (cM)
	<i>MM</i>	<i>mm</i>			
<i>RC20</i>	47.00	65.00	2.58	0	0
<i>pm4em2-1</i>	60.25	51.75	0.50	8.68	8.68
<i>em9me8-1</i>	63.00	49.00	1.51	12.20	12.20
<i>issr42</i>	75.98	36.02	13.55	12.30	12.30
<i>BrH80A08_FLC1</i>	82.57	29.43	24.27	10.25	13.77
<i>em2me3-2</i>	74.00	38.00	10.94	6.93	9.33
<i>em2me3-3</i>	74.00	38.00	10.94	0.0	0.0
<i>em4me7-1</i>	55.00	57.00	0.01	16.17	16.17

^zThe number of genotypes includes the theoretical number of missing marker genotypes.

$$^y\chi^2_c = \frac{(|n_{MM} - 56| - 0.5)^2}{56} + \frac{(|n_{mm} - 56| - 0.5)^2}{56}.$$

GD, genetic distances.

Table 5. Main characteristics of markers and genetic distances (GD) per linkage group in nonheading chinese cabbage.

Linkage group	Total markers (n)	Distorted markers (n)	Missing genotypes (n)	GD (cM)		Corrected GD (cM)	
				Total	Avg spacing	Total	Avg spacing
LG1	27	8	41	530.66	20.41	542.98	20.89
LG2	13	7	14	211.94	17.67	220.71	18.40
LG3	22	10	49	412.55	19.65	429.29	20.47
LG4	11	1	14	90.74	9.05	91.04	9.11
LG5	5	1	4	33.47	8.37	33.47	8.37
LG6	13	1	4	129.71	10.81	129.71	10.81
LG7	13	4	35	144.43	12.04	146.69	12.23
LG8	8	4	12	66.53	9.51	72.05	10.35
LG9	5	5	9	20.09	5.03	25.48	6.37
LG10	5	0	12	66.31	16.58	66.31	16.58
LG11	4	4	12	21.76	7.26	21.57	7.19
LG12	4	4	1	23.42	7.81	28.91	9.64
LG13	4	3	3	66.32	22.11	71.16	23.72
LG14	4	2	3	43.96	14.66	44.38	14.80
Total	138	54	213	1861.89	15.02	1923.75	15.52

Avg, average; GD, genetic distances.

ISSR, and other markers in the construction of a moderately saturated genetic map.

Microspore culture and anther culture are commonly used to produce doubled haploid (DH) lines. The DH lines have been widely used to explore the genetic architecture of complex traits (Kuginuki et al., 1997; Voorrips et al., 1997), and to develop further elite parental lines for hybrid seed

production (Chen and Beversdorf, 1990), because genetically homozygous DH lines are usually obtained in a single generation (Burr et al., 1988). However, high distortion segregation frequencies are frequently reported in the DH populations of *B. rapa* (Ajisaka et al., 1999; Zhang et al., 2006), *B. oleracea* (Voorrips et al., 1997), and *B. napus* (Cloutier et al., 1995).

Segregation distortion is usually observed for some or all markers in both experimental and natural populations (Lytte, 1991). The distortion affects linkage tests (Garcia-Dorado and Gallego, 1992) and the estimation of recombination

fractions between distorted markers (Lorieux et al., 1995a, b). Currently, most statistical methods used for map construction ignore the fact that some molecular markers are distorted (Jiang and Zeng, 1997; Lander and Green, 1987). Recently,

Zhu et al. (2007) proposed a multi-point approach to correct unbiasedly a linkage map given a specified marker order in an F_2 population, which is especially useful to deal with distorted, dominant, and missing markers.

In this paper, therefore, we establish the first genetic linkage map of nonheading chinese cabbage containing new, distorted, and missing markers using the method of Zhu et al. (2007). The map will facilitate selective breeding and mapping of QTL.

Materials and Methods

PLANT MATERIALS. One hundred twelve DH lines were derived from a newly released F_1 hybrid cultivar Shulü of nonheading chinese cabbage between other two lines SW-3 and Su-124. The maternal parent SW-3, a self-incompatible line, was developed from cultivar Aijiaohuang by molecular-assisted selection; the paternal parent Su-124, an inbred line, was derived from cultivar Suzhouqing. There are a number of different traits between these two parents, including leaf color, peduncle color, disease resistance, hot tolerance, cold hardiness, and quality.

DNA EXTRACTION. About 0.3 g fresh bud of flower was used to extract genomic DNA using the cetyl-trimethyl-ammonium bromide (CTAB) method. DNA was quantified by comparing with λ DNA through running agarose gels.

SEQUENCE-RELATED AMPLIFIED POLYMORPHISM ANALYSIS. The sequences of SRAP primers were provided by G. Li at the University of Manitoba, Canada, and are shown in Table 1. Reactions were performed in a 10- μ L volume containing 30 ng DNA, 0.5 μ mol·L⁻¹ forward and reverse primers, 0.2 mmol·L⁻¹ deoxyribonucleotide triphosphate (dNTPs), 1.5 mmol·L⁻¹ MgCl₂, 1 $\times$ polymerase chain reaction (PCR) buffer (Tri-HC; pH, 8.3; 100 mM; KCl, 500 mM), and

Fig. 1. A genetic linkage map of nonheading chinese cabbage. LG1a through LG14a represent linkage groups without considering segregation distortion; LG1b through LG14b represent linkage groups considering segregation distortion. The markers with stars are distorted. *,**A significance level of 0.05 and 0.01 respectively.

0.75 U *Taq* DNA polymerase. The protocol of PCR reaction was as follows: 94 °C for 3 min, then 94 °C for 30 s, 37 °C for 30 s, 72 °C for 90 s for five cycles; 94 °C for 30 s, 48 °C for 30 s, 72 °C for 90 s for 33 cycles; and finally 72 °C for 7 min. The products were separated on 5% vertical polyacrylamide gel. The gel was run at 70 W constant power

for 1.5 to 2 h until xylene cyanol reached toward the bottom of the gel. After electrophoresis, the gel was stained by AgNO_3 solution.

SIMPLE SEQUENCE REPEAT ANALYSIS. The sequences of SSR primers were shown in Table 2. The SSR reactions were performed in a 10- μL volume containing 20 ng DNA, 0.5 $\mu\text{mol}\cdot\text{L}^{-1}$ forward and reverse primer, 0.2 $\text{mmol}\cdot\text{L}^{-1}$ dNTPs, 1.0 $\text{mmol}\cdot\text{L}^{-1}$ MgCl_2 , 1 $\times$ PCR buffer, and 0.5 U *Taq* DNA polymerase. The PCR procedure was the following: 94 °C for 5 min, then 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s for 35 cycles, then 72 °C for 7 min. The products were separated on 5% vertical polyacrylamide gel. The gel was run at 60 W constant power for 1 to 1.5 h until xylene cyanol reached toward the bottom of the gel. After electrophoresis, the gel was stained by AgNO_3 solution.

Fig. 1. (continued).

RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS. The sequences of RAPD primers were obtained from Nanjing Sunshine Biotechnology Co., Ltd. (Nanjing, China). The RAPD reaction was performed in a total volume of 20 μL , with 1 $\times$ PCR buffer, 2.0 $\text{mmol}\cdot\text{L}^{-1}$ MgCl_2 , 0.25 $\text{mmol}\cdot\text{L}^{-1}$ dNTPs, 0.2 $\mu\text{mol}\cdot\text{L}^{-1}$ primer, and 0.5 U *Taq* DNA polymerase, and 20 ng template DNA. The procedure of PCR amplification was as follows: 94 °C for 3 min, then 94 °C for 30 s, 37 °C for 45 s, 72 °C for 90 s for 42 cycles, then 72 °C for 7 min. The PCR products were electrophoresed on 1% horizontal agarose gel in 1 $\times$ Tris-Acetate-EDTA (TAE) buffer with a voltage of 5 $\text{V}\cdot\text{cm}^{-1}$ and visualized under ultraviolet light after staining in 1 $\mu\text{g}\cdot\text{mL}^{-1}$ ethidium bromide.

INTERSIMPLE SEQUENCE REPEAT ANALYSIS. The sequences of RAPD primers were obtained from Nanjing Sunshine Biotechnology Co., Ltd. The ISSR reaction was performed in a total volume of 20 μL , with 1 $\times$ PCR buffer, 2.0 $\text{mmol}\cdot\text{L}^{-1}$ MgCl_2 , 0.2 $\mu\text{mol}\cdot\text{L}^{-1}$ primer, 0.25 $\text{mmol}\cdot\text{L}^{-1}$ dNTPs, and 0.5 U *Taq* DNA polymerase, and 20 ng template DNA. The PCR amplification procedure was as follows: 94 °C for 4 min, then 95 °C for 45 s, 52 °C for 45 s, 72 °C for 45 s for 40 cycles, then 72 °C for 7 min. The PCR products were electrophoresed on 1.5% horizontal agarose gel in 1 $\times$ TAE buffer with a voltage of 5 $\text{V}\cdot\text{cm}^{-1}$ and

Fig. 1. (concluded).

visualized under ultraviolet light after staining in $1 \mu\text{g}\cdot\text{mL}^{-1}$ ethidium bromide.

RECONSTRUCTION OF GENETIC LINKAGE MAP. Let the order of m markers on the same chromosome be $M_1, M_2, \dots, M_m$ in a DH population, r_k be the recombination fraction between the k th and $(k+1)$ th markers, s_k ($0 \leq s_k < \infty$; $k = 1, 2, \dots, m$) be the viability coefficients of $M_k M_k$ relative to $m_k m_k$ at the k th marker, x be a dummy variable defined as $x_k = 1$ and -1 for a homozygote of P_1 and P_2 respectively, and z be an indicator of the phenotype of the k th marker M_k . If the distortion is incited by viability selection, the markers linked to the viability locus show distorted Mendelian segregation ratios. Sometimes the locus is known as segregation distortion locus (or SDL). In the construction of a linkage map, therefore, it is necessary to estimate $2m - 1$ coefficients, including s_k ($k = 1, 2, \dots, m$) and r_k ($k = 1, 2, \dots, m - 1$). The case where $s_k = 1$ ($k = 1, 2, \dots, m$) represents typical Mendelian segregation.

If we incorporate the previous viability model into the methods of both Lander and Green (1987) and Jiang and Zeng (1997), the logarithm likelihood is defined by

$$\ln L = \sum_{i=1}^n \log [q_{z_{i1}}^T \mathbf{H}_{z_{i2}}(r_1) \mathbf{H}_{z_{i3}}(r_2) \cdots \mathbf{H}_{z_{im}}(r_{m-1}) c] \quad (1)$$

where n represents population size; $q_{z_{i1}}$ denotes the column vector of the prior probability $p(x_1)$, $q_{z_{i1}}^T = [\text{Pr}(x_1 = 1), \text{Pr}(x_1 = -1)]$, and $c^T = [1, 1]$, T denotes the transpose of a matrix or vector; and the transition probability matrix $\mathbf{H}(r_k)$ from marker M_k to M_{k+1} is

$$\begin{pmatrix} \frac{s_{k+1}(1-r_k)}{s_{k+1}(1-r_k) + r_k} & \frac{r_k}{s_{k+1}(1-r_k) + r_k} \\ \frac{s_{k+1}r_k}{s_{k+1}r_k + (1-r_k)} & \frac{1-r_k}{s_{k+1}r_k + (1-r_k)} \end{pmatrix}.$$

Maximum likelihood estimates for all parameters in Eq. (1) may be directly solved for if there are no missing genotypes; if this is not the case, an expectation and maximization algorithm (Dempster et al., 1977) should be adopted, including the following steps:

1. **Initialization:** The viability coefficient $s_k^{(0)}$ ($k = 1, \dots, m$) is initialized with 1, and the initial value of the recombinant fraction $r_k^{(0)}$ ($k = 1, \dots, m - 1$) along with the order of markers is obtained from Mapmaker 3.0 software (Lander et al., 1987).
2. **E-step:** Let $A_k = (a_{k,icd})$, denoted by $P(x_k x_{k+1} | z_1, \dots, z_m)$ in a 2×2 matrix form, where $a_{k,icd}$ is the c th row and d th column element of A_k for the i th individual. Using the multi-point method, the posterior probabilities $P(x_k x_{k+1} | z_1, \dots, z_m)$ for each individual can be calculated by

$$P(x_k x_{k+1} | z_1, \dots, z_m) = \frac{p(x_k x_{k+1}) p(z_1, \dots, z_m | x_k, x_{k+1})}{\sum_{x_k, x_{k+1}} p(x_k x_{k+1}) p(z_1, \dots, z_m | x_k, x_{k+1})} \quad (2)$$

3. **M-step:** The recombination fraction between the k th and $(k+1)$ th markers and the selection coefficients (s_k) can be updated as

$$r_k^{(1)} = \frac{\sqrt{\sum_{i=1}^n a_{k,i12} \sum_{i=1}^n a_{k,i21}}}{\sqrt{\sum_{i=1}^n a_{k,i12} \sum_{i=1}^n a_{k,i21}} + \sqrt{\sum_{i=1}^n a_{k,i11} \sum_{i=1}^n a_{k,i22}}} \quad (3)$$

$$s_k^{(1)} = \frac{X(k) \sqrt{\sum_{i=1}^n a_{k-1,i11} \sum_{i=1}^n a_{k-1,i12}} + W(k) \sqrt{\sum_{i=1}^n a_{k,i11} \sum_{i=1}^n a_{k,i21}}}{X(k) \sqrt{\sum_{i=1}^n a_{k-1,i21} \sum_{i=1}^n a_{k-1,i22}} + W(k) \sqrt{\sum_{i=1}^n a_{k,i21} \sum_{i=1}^n a_{k,i22}}} \quad (4)$$

4. where $X(k)$ and $W(k)$ are indicator variables, $X(k) = 1$ if $1 \leq k < m$ or takes a value of zero if this condition is not met; $W(k) = 1$ if $1 < k \leq m$ or takes a value of zero if this condition is not met. If $\sum_{i=1}^n a_{k,icd} = 0$, Eq. (3) and Eq. (4) are unavailable. In this case, the iterated algorithm [i.e., Newton-Raphson (Lorieu et al., 1995b)] works well. In addition, $s_k = 1$ if there are no significant differences for all chi-square tests. The E-step and M-step are iterated until convergence occurs.

Results

POLYMORPHISM SCREENING OF PRIMERS BETWEEN PARENTS. Before the construction of a linkage map, the two parents were screened for polymorphism with 160 SRAP primer combinations, 200 SSR primer combinations, 120 RAPD primers, and 58 ISSR primers (Table 3). In total, 160 of 538 primers (combinations) (29.7%) could produce polymorphic loci, including 90 SRAP primer combinations, 40 SSR primer combinations, 22 RAPD primers, and eight ISSR primers. With 90 polymorphic SRAP primer combinations, a total of 154

polymorphic loci were selected at an average of 1.7 loci per primer combination. Meanwhile, 43 SSR polymorphic loci, 32 RAPD polymorphic loci, and 16 ISSR polymorphic loci were obtained. All these polymorphic markers were performed in the DH line population.

CONSTRUCTION OF GENETIC LINKAGE MAP. One hundred twelve DH lines derived from a released F_1 hybrid cultivar Shulü were genotyped for 138 linked molecular markers, including 77 SRAP markers, 27 SSR markers, 21 RAPD markers, and 13 ISSR markers. The results of a chi-square test showed that 54 of 138 markers were distorted from Mendelian segregation ratios, and the direction of distortion was mainly toward the maternal parent SW-3. As an illustrative example, the results for both chi-square tests and the genetic distances between adjacent markers with and without considering segregation distortion for the eighth linkage group are listed in Table 4. Results from Table 4 show that the distortion affects the estimation of genetic distance between distorted markers. Therefore, it is necessary to correct the bias of genetic distances between distorted markers.

In the construction of a genetic linkage map using molecular markers, there are three steps. The first step is to cluster markers into linkage groups. One hundred thirty-eight molecular markers were assembled into 14 linkage groups with Mapmaker 3.0 software. The second step is to estimate pairwise genetic distances in each of the linkage groups. In this paper, the genetic distances between adjacent markers were calculated twice. The first time was using Mapmaker 3.0 without considering segregation distortion, and the second time was adopting the new multipoint method mentioned earlier while considering segregation distortion. The results in Table 5 show that the length of each linkage group ranges from 21.57 to 542.98 cM and all these molecular markers covered 1923.75 cM for the whole genome, with an average marker spacing of 15.52 cM. The genetic distances between distorted markers with the consideration of segregation distortion were usually longer than those without the consideration of segregation distortion, although there were one or two exceptions that were observed on linkage groups LG 1 to LG 3. Therefore, it is important and necessary to correct the bias of genetic distances between distorted markers. The third and last step is to optimize the orders of all mapped markers in all linkage groups. The optimized linkage groups were showed in Fig. 1.

Discussion

Genetic maps for *B. rapa* have been published (Ajisaka et al., 1999; Chyi et al., 1992; Foisset et al., 1996; Song et al., 1991; Voorrips et al., 1997). The total length of the current map in this study was comparable with those reported, although we mapped fewer markers. However, the number of linkage groups on the current map does not match the 10 expected chromosomes in the genome. To integrate the linkage groups with the 10 chromosomes, further research needs to be done through adding more individuals in the DH population, developing more molecular markers, and using different types of mapping populations such as F_2 , recombinant inbred lines, and backcrosses.

Marker segregation distortion is a common phenomenon in vegetable crops (Foisset et al., 1996; Kim et al., 1999; Voorrips et al., 1997). Fifty-four distorted markers, accounting for 39.1% of the total markers, were observed in this study. In DH

populations, a skewed segregation ratio has been often observed in many other plants (Foisset et al., 1996), and their existence probably results from gametic or zygotic selection, or from a specific selection derived from the production of plants by in vitro microspore culture. The distortion is known to bias the estimation of recombination fraction between consecutive markers and the order of distorted markers on the same linkage group (Lorieux et al., 1995a, b). Most quantitative geneticists who are interested in QTL mapping do not prefer to use distorted markers for QTL mapping, because the basic assumption of Mendelian segregation is violated. Too many distorted markers will cause tremendous information loss in QTL mapping if these markers are removed from the marker map (Luo et al., 2005). Hence, it is necessary to develop a new statistical method to reconstruct a molecular linkage map to adjust the bias of the estimates of the genetic distances between adjacent markers.

Murigneux et al. (1993) suggest that a more strict linkage test standard be used when segregation distortion existed (i.e., relatively higher logarithm of odds (LOD) value). Lorieux et al. (1995b) developed software called MapDisco to reconstruct a molecular linkage map considering distorted markers. The software by Manly and Olson (1999) also offers options to compute the recombination fractions in case of deviation from the Mendelian hypothesis. However, nearly all cited programs cannot determine whether the corrected values are close to the true ones. The reason is that the previously mentioned investigations are seldom addressed in theoretical simulation studies. More recently, we developed an alternative algorithm for reconstructing a molecular linkage map given a specified marker order while considering distorted, dominant, and missing markers using an F_2 population as an example (Zhu et al., 2007). Our analysis is similar to the method of Lander and Green (1987) in spirit, but we extend construction of a genetic linkage map to more general situations, considering distorted, dominant, and missing markers. In this paper we simplify our method from an F_2 population to a DH population. These results are fundamentals for QTL mapping in nonheading chinese cabbage.

Literature Cited

- Ajisaka, H., Y. Kuginuki, K. Hida, S. Enomoto, and M. Hirai. 1995. A linkage map of DNA markers in *Brassica campestris*. *Breed. Sci.* 45(suppl.): 195–197.
- Ajisaka, H., Y. Kuginuki, M. Shiratori, K. Ishiguro, S. Enomoto, and M. Hirai. 1999. Mapping of loci affecting the cultural efficiency of microspore culture of *Brassica rapa* L. syn. *campestris* L. using DNA polymorphism. *Breed. Sci.* 49:187–192.
- Blair, M.W., O. Panaud, and S.R. McCouch. 1999. Inter-simple sequence repeats (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 98:780–792.
- Budak, H., R.C. Shearman, I. Parmaksiz, and I. Dweikat. 2004a. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theor. Appl. Genet.* 109:280–288.
- Budak, H., R.C. Shearman, I. Parmaksiz, R.E. Gaussoin, T.P. Riordan, and I. Dweikat. 2004b. Molecular characterization of buffalograss germplasm using sequence-related amplified polymorphism markers. *Theor. Appl. Genet.* 108:328–334.
- Burr, B., F.A. Burr, K.H. Thompson, M.C. Albertson, and C.W. Stuber. 1988. Gene mapping with recombinant inbred in maize. *Genetics* 118:519–526.

- Chen, J.L. and W.D. Beversdorf. 1990. A comparison of traditional and haploid-derived breeding populations of oilseed rape (*Brassica rapus* L.) for fatty acid composition of the seed oil. *Euphytica* 51:59–65.
- Chyi, Y.S., M.E. Hoenecke, and J.L. Sernyk. 1992. A genetic linkage map of restriction fragment length polymorphism loci for *Brassica rapa* (syn. *campestris*). *Genome* 35:746–757.
- Cloutier, S., M. Cappadocia, and B.S. Landry. 1995. Study of microspore culture responsiveness in oil-seed rape (*Brassica napus* L.) by comparative mapping of a F₂ population and two microspore-derived populations. *Theor. Appl. Genet.* 91:841–847.
- Dempster, A.P., N.M. Laird, and D.B. Rubin. 1977. Maximum likelihood from incomplete data via EM algorithm. *J. R. Stat. Soc. B.* 39: 1–38.
- Ferriol, M., B. Picó, and F. Nuez. 2003. Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theor. Appl. Genet.* 107:271–282.
- Foisset, N., R. Delourme, P. Barret, N. Hubert, B.S. Landry, and M. Renard. 1996. Molecular-mapping analysis in *Brassica rapa* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny. *Theor. Appl. Genet.* 93:1017–1025.
- García-Dorado, A. and A. Gallego. 1992. On the use of the classical tests for detecting linkage. *J. Hered.* 83:143–146.
- Jiang, C.J. and Z.B. Zeng. 1997. Mapping quantitative trait loci with dominant and missing markers in various crosses from two inbred lines. *Genetica* 101:47–56.
- Kantety, R.V., X. Zeng, and J. Bennetzen. 1995. Assessment of genetic diversity in popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Mol. Breed.* 1:365–373.
- Kim, J.H., C.S. Jang, K.W. Cho, and Y.P. Lim. 1999. AFLP and RAPD mapping of chinese cabbage (*Brassica rapa* L. var *pekinensis*), p. 17–21. In: *Proceedings of the Plant and Animal Genome Conference VII*. San Diego, California, USA. 17–21 Jan. 1999.
- Kojima, T., T. Nagaoka, and K. Noda. 1998. Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theor. Appl. Genet.* 96:37–45.
- Kuginuki, Y., H. Ajisaka, M. Yui, H. Yoshikawa, K. Hida, and M. Hirai. 1997. RAPD markers linked to a clubroot-resistance in *Brassica rapa* L. *Euphytica* 98:149–154.
- Lander, E.S. and P. Green. 1987. Construction of multilocus genetic linkage map in humans. *Proc. Natl. Acad. Sci. USA* 84:2363–2367.
- Lander, E., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and nature populations. *Genomics* 1:174–181.
- Li, G. and C.F. Quiros. 2001. Sequence-related amplified polymorphism (SRAP): A new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.* 103:455–461.
- Lorieux, M., X. Perrier, B. Goffinet, C. Lanaud, and D. González de León. 1995a. Maximum likelihood models for mapping genetic markers showing segregation distortion. 2. F₂ population. *Theor. Appl. Genet.* 90:81–89.
- Lorieux, M.B., G.X. Perrier, D. González de León, and C. Lanaud. 1995b. Maximum likelihood models for mapping genetic markers showing segregation distortion. 1. Backcross population. *Theor. Appl. Genet.* 90:73–80.
- Luo, L., Y.M. Zhang, and S. Xu. 2005. A quantitative genetics model for viability selection. *Heredity* 94:347–355.
- Lyttle, T.W. 1991. Segregation distortion. *Annu. Rev. Genet.* 25: 511–557.
- Manly, K.F. and J.M. Olson. 1999. Overview of QTL mapping software and introduction to MapManager QTL. *Mamm. Genome* 10:327–334.
- Moreno, S., J.P. Martin, and M. Ortiz. 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica* 101:117–125.
- Murigneux, A., D. Barloy, P. Leroy, and M. Beckert. 1993. Molecular and morphological evaluation of doubled haploid lines in maize. 1. Homogeneity within DH lines. *Theor. Appl. Genet.* 86:837–842.
- Nozaki, T., A. Kumazaki, T. Koba, K. Ishikawa, and H. Ikehashi. 1997. Linkage analysis among loci for RAPDs, isozymes and some agronomic traits in *Brassica campestris* L. *Euphytica* 95:115–123.
- Song, K.M., J.Y. Suzuki, M.K. Solcum, P.H. Williams, and T.C. Osborn. 1991. A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. *Theor. Appl. Genet.* 82:296–304.
- Sun, Z., Z. Wang, J. Tu, J. Zhang, F. Yu, P.B. McVetty, and G. Li. 2007. An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers. *Theor. Appl. Genet.* 114:1305–1317.
- Voorrips, R.E., M.C. Jongerius, and H.J. Kanne. 1997. Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. *Theor. Appl. Genet.* 94:75–82.
- Zhang, X., J. Wu, J. Zhao, X. Song, Y. Li, Y. Zhang, D. Xu, R. Sun, Y. Yuan, C. Xie, and X. Wang. 2006. Identification of QTLs related to bolting in *Brassica rapa* ssp. *pekinensis* (syn. *Brassica campestris* ssp. *pekinensis*). *Agr. Sci. China* 5:265–271.
- Zhu, C.S., C.S. Wang, and Y.M. Zhang. 2007. Modeling segregation distortion for viability selection I. Reconstruction of genetic linkage maps with distorted markers. *Theor. Appl. Genet.* 114:295–305.
- Zietkiewicz, E., A. Patalski, and D. Labuda. 1994. Genomic fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20:176–183.