

Mapping of *or*, a gene conferring orange color on the inner leaf of the Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*)

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Abstract The orange inner leaf of the Chinese cabbage is controlled by a single recessive gene (*or*), which causes abnormal accumulation of carotene. In the present study, an F₂ population consisting of 600 individuals was used for mapping *or* and developing new markers closely linked to this gene. Bulked segregant analysis was performed by screening 435 simple sequence repeat (SSR) markers well-distributed on 10 linkage groups and 16 SSR primers derived from nine bacterial artificial chromosome (BAC) clones. On the basis of linkage analysis, the *or* gene was mapped in a region covering a total interval of 4.6 centimorgans (cM) between two SSR markers derived from BAC clones AC172873 and AC189246 at the end of linkage group 9, which matches with chromosome 1 of A genome in Chinese cabbage. A genetic map of the *or* locus was constructed by using five SSR markers and two morphological markers. Three SSR markers were tightly linked to *or* and two of them, sau (C) 586 and syau19, were located on the same side at distances of 1.6 and 1.3 cM, respectively. The other marker, syau15, was located on the other side at a distance of 3.3 cM. The two morphological markers, orange flower and orange cotyledon (before cotyledon turns green during the germination period), were obtained by visual determination and screening of the

differences in the morphological traits between parents and the two segregated F₂ populations; the two markers were designated as *or-f* (orange flower) and *or-c* (orange cotyledon). It was suggested that these two markers co-segregate with orange inner leaf trait or that the three characters, namely orange inner leaf, orange flower, and orange cotyledon, are determined by the same gene. These markers could be very helpful for marker-assisted selection in Chinese cabbage hybrid breeding programs.

Keywords Chinese cabbage · Orange leaf · SSR marker · Morphological marker · Mapping · Marker-assisted selection

Introduction

Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) has been widely planted in China owing to its nutritional value and various ecological types. The inner leaf can be white, orange, or yellow in color. Colored head leaves have a brighter appearance as well as higher nutrition, and this trait has thus attracted more attention from researchers. Previous studies have revealed that the different colors of the inner leaves are mainly due to the various components of carotenoids. Carotenoids are essential components of the photosynthetic apparatus (Von Lintig et al. 1997; Pogson et al. 1998) and are responsible for the red, yellow, and

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orange colors in flowers, fruits, and other organs (Frank and Cogdell 1996). Chinese cabbage with orange leaves contains more carotenoid than that with the common white leaves. The total carotenoid content of the orange leaves is seven times higher than that of the white leaves, and, in particular, lycopene only exists in the orange leaves (Chen 2008). In addition, carotenoids are also important for human nutrition and health in such fields as conversion into vitamin A (Olson 1989), resistance to oxidation (Fraser and Bramley 2004), prevention of ultraviolet radiation injury (Mortensen and Skibsted 1997), and prevention of angiocardopathy and cancers (Yie et al. 2005; Molnar et al. 2004). An orange mutant was first found in cauliflower (*Brassica oleracea* var. *botrytis*) and the gene contributing to orange color in the edible curds of this plant was designated “*Or*,” which is a spontaneous single gene (Crisp et al. 1975) that causes abnormal accumulation of β -carotenoids (Crisp et al. 1975; Dickson et al. 1988; Li et al. 2001). Li et al. (2003) and Li and Garvin (2003) have conducted fine mapping and constructed a high-resolution genetic and physical map of *Or* in cauliflower. Furthermore, Lu et al. (2006) have reported the positional cloning and functional characterization of the *Or* gene and the authors suggested that the gene can serve as a new genetic tool for improving nutritional value in important food crops. The *or* gene has been studied in Chinese cabbage, and it was widely believed that *or* was a single recessive gene that also resulted in abnormal accumulation of β -carotenoids, which leads to the orange color in the inner leaves (Matsumoto et al. 1998).

The orange inner leaf in Chinese cabbage is usually accompanied by many other agricultural features that can be used as morphological markers. A morphological marker, as a kind of a genetic marker, can be obtained by examining the difference between morphological characters of plants without any laboratory procedure and is very suitable for application to a practical breeding program owing to its lower cost. In particular, almost all of these markers are co-segregated with the character of interest. Thus, the screening of some agricultural traits that co-segregate with the orange inner leaf is relevant.

To date, a few molecular markers linked to *or* have been developed, most of which are located quite far away from *or*, and it appears that all of them are dominant (Liu et al. 2003; Wang et al. 2007; Zhang

et al. 2008). Markers tightly linked to valuable traits should be an effective tool for accelerating the process in a breeding program. However, the dominant markers often fail to distinguish between homozygous and heterozygous genotypes and their use may be partly restricted in practice. It is thus essential to develop co-dominant markers for conducting marker-assisted selection (MAS). In addition, mapping of *or* is still in the initial stage, and the gene was mapped to linkage group 1 at a distance of nearly 19 centimorgans (cM) from a restriction fragment length polymorphism (RFLP) marker (Matsumoto et al. 1998). In a contrasting result, Zhang et al. (2008) reported that this gene is located on linkage group 9 by using two anchor simple sequence repeat (SSR) markers, and identified a nearest sequence characterized amplified region (SCAR) marker at a distance of 5 cM. Thus, mapping the *or* gene more precisely is important for fine mapping and for developing a map-based cloning strategy in the future. In this study, two morphological markers that co-segregated with *or* and five molecular SSR markers that were tightly linked to this gene were identified. Furthermore, *or* was mapped to a region covering a small interval between two BAC clones at the end of A9.

Materials and methods

Plant materials

Two F2 populations (07A163 \times Chiifu and PG-2 \times Chiifu) were established for the present studies. Chiifu (provided by Chungnam National University, Korea), used as the male parent, is the model material in the *B. rapa* Genome Sequencing Program (BrGSP), with white inner leaf. The female parents, 07A163 and PG-2, are inbred lines of orange inner leaf with the homozygous *or* allele. All the materials were planted in a greenhouse and a field in Shenyang, China, and the inner leaf color was determined by visual observation at maturation.

An F2 population consisting of 600 plants that was derived from the 07A163 \times Chiifu cross was used for the genetic analysis and SSR mapping of the *or* gene. The chi-square test was used to determine the fit of the segregation ratio of the F2 generation to the expected 3:1 ratio.

The population mentioned above (600 plants) was also used as the material for developing morphological markers of the *or* gene. As the female parents, the inbred lines 07A163 and PG-2 were bred from different resources. In order to verify the results obtained from the 07A163 × Chiifu cross, we also established another population of 158 F₂ plants derived from the PG-2 × Chiifu cross.

DNA isolation and pooling of samples for bulked segregant analysis (BSA)

Genomic DNA was isolated from fresh leaves of the parents and F₂ individuals following the procedure described by Murray and Thompson (1980) with minor modifications. The concentration of DNA was adjusted to 50 ng/μL for PCR amplification. Three bulk samples were prepared by using equal quantities of DNA of orange inner leaf individuals from the F₂ population. In order not to miss the markers that are located further away, each bulk sample was prepared by pooling only from three individuals. BSA (Michelmore et al. 1991) was performed with two parents and the three bulk samples (*oror*).

Morphological marker analysis

Two cross combinations (07A163 × Chiifu and PG-2 × Chiifu) were prepared for identifying morphological markers in this study. A morphological marker is a kind of genetic marker that can be identified by differences in the exterior appearances of some organ of the plant. In this study, we investigated four morphological traits of the Chinese cabbage, namely flower color, hairy leaf, wax leaf, and cotyledon color, to identify the markers that probably segregate with the orange inner leaf trait.

During the growth periods of the plants, differences in the morphological characters between the parents (07A163/Chiifu and PG-2/Chiifu) were screened. Then, the segregation of different morphological characters in F₁ and F₂ generations was observed to develop the markers that might be co-segregated with orange inner leaf. The cotyledon color was observed at the germination stage, hairy leaf and wax leaf were observed at the seedling stage, and inner leaf color was observed at the maturation stage. (When conducting the breeding program, we always make the seed undergo vernalization

treatment at the germination stage in low temperatures—often 0–2°C in a refrigerator for more than 20 days—for bolting and pollinating. During the vernalization stage, the seed coat comes off and the cotyledon color can be visually assessed. See Fig. 1). Two F₂ generations were preserved until the subsequent year in a greenhouse for observation of the flower color. To check the consistency of the data assessed by different people, three people were chosen to make visual observation of the morphological characters.

SSR marker analysis and mapping *or*

Five hundred SSR markers were designed according to the information from BrGSP. To screen for polymorphism between 2 parents, we selected 435 markers that were well distributed on 10 linkage groups of A genome to identify the markers linked to the *or* gene. PCR amplification was carried out in a total volume of 10 μL containing 25 ng template DNA, 0.8 μL of 2.5 mM dNTP, 1.0 μL of 10 × buffer (containing Mg²⁺), 1 μL of 0.5 μM primer, and 1 U *Taq* polymerase. The PCR amplification was performed on a Bio-Rad iCycler thermocycler as follows: PCR was initiated with 95°C for 1 min; followed by 30 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s; and ended with extension at 72°C for 3 min. The PCR products were separated on a 6% denaturing polyacrylamide gel, and were stained with AgNO₃.

After the identification of SSR markers linked to *or*, it was possible for us to confirm the linkage group in which the gene was located. In addition, we selected several BAC clones in the region suggested to contain *or* (www.brassica-rapa.org) in order to develop some markers flanking *or* and to map this gene to a certain small region. The primers were designed using the Primer Premier 5.0 software.

Linkage analysis

The identified SSR markers were used to survey 154 individuals homozygous for the *or* allele from 600 F₂ plants to calculate the recombination frequency. Linkage analysis was carried out by using JoinMap 3.0 (Van Ooijen and Voorrips 2001) and the genetic map was drawn by using MapChart (Voorrips 2002). The map distances in cM were calculated by the Kosambi function (Kosambi 1944).

Results

Segregation of the *or* gene in the F2 population

The genotype of individuals was visually determined by the color of the inner leaf at the maturation period. In the F2 population from the 07A163 × Chiifu cross, 446 and 154 individuals had white and orange inner leaves, respectively, which represents a segregation ratio of 2.9:1 ($\chi^2 = 0.142 < \chi_{0.05}^2 = 3.841$). In the population from PG-2 × Chiifu, 116 and 42 individuals had white and orange inner leaves respectively, which represents a segregation ratio of 2.76:1 ($\chi^2 = 0.211 < \chi_{0.05}^2 = 3.841$). The ratios of the two segregating populations accurately fit the expected segregation ratio of 3:1 of a single recessive gene.

Identification of morphological markers linked to the *or* gene

Of the four morphological traits surveyed in the present study, two morphological markers, orange flower and orange cotyledon (before the cotyledon turns green during the germination period), were visually determined by different people.

The orange leaf materials, 07A163 and PG-2, exhibited orange flowers and cotyledons. On the contrary, Chiifu showed normal yellow flowers and cotyledons (Fig. 1). The progenies of the F1 generation from the two crosses all had yellow flowers and cotyledons. The segregation ratio of the color of flower and cotyledon (yellow/orange) in the F2 population was identical to that of the inner leaf (2.9:1 and 2.76:1), which indicates that the morphological markers were controlled by a recessive gene as well.

On surveying the two F2 populations, no recombinant was found. Therefore, we concluded that the two morphological markers were co-segregated with the orange leaf trait (Table 1). Thus, the orange inner leaf plant was accompanied by orange flowers and cotyledons in contrast to the common yellow ones of the white inner leaf plant. We designated the two morphological markers (orange flower and orange cotyledon) as *or-f* and *or-c* respectively.

SSR marker analysis

For identifying the markers linked to *or*, 435 SSR primers were used to observe polymorphism. Of

these, 233 primers showed polymorphism at a ratio of 53% between the parents. BSA was conducted between the two parents and three bulk samples. Finally, three markers, namely ENA21, sau (N) 033, and sau (C) 586, were found at distances of 21.8 cM, 20.8 cM, and 1.6 cM, respectively, on the same side of the *or* gene (Table 2). These markers were positioned on A9. Of these, sau (C) 586, the nearer marker, yielded a band of the same size in 07A163 and the three bulk samples, which was significantly different from the size of the band obtained from Chiifu (Fig. 2).

Development of SSR markers flanking *or*

In order to map *or* and obtain tightly linked markers on both sides of the gene, we selected nine BAC clones on A9 (accession nos: KBrB063A04, KBrH050C15, KBrB017P15, KBrB043F18, KBrB087P03, KBrH010P01, KBrB089B13, KBrH010M08, KBrH138P004) at a certain distance on the other side of markers ENA21, sau (N) 033, and sau (C) 586 (www.brassica-rapa.org). Of the 16 SSR markers designed using the Primer Premier 5.0 software, two co-dominant markers, syau19 and syau15, were identified as closely linked to *or* and were derived from AC172873 and AC189246, respectively. The recombination frequencies of syau19 and syau15 were 1.3 and 3.3%, respectively, in the F2 population. The images of the two markers in two parents and three bulks homozygous for the *or* allele are shown in Fig. 2.

Mapping of the *or* locus

Finally, a genetic map of the *or* gene was constructed with five SSR markers and two morphological markers. Four co-dominant markers, namely ENA21, sau (N) 033, sau (C) 586, and syau19, were located on the same side of the gene. The co-dominant markers syau15 and syau19 tightly flanked the *or* gene at 3.3 and 1.3 cM, respectively, and were positioned at the end of linkage group 9 covering a total interval of 4.6 cM (Fig. 3). The two morphological markers, *or-f* and *or-c*, were co-segregated with *or* and were located at the same position.

The linkage group that we constructed covered a total interval of 25.1 cM. A gap of 19.2 cM exists between markers sau (N) 033 and sau (C) 586, which is mainly caused by insufficiency in the SSR primers

Fig. 1 Color of the cotyledons (a), flowers (b), and inner head leaf (c) of a normal white head leaf parent (Chiifu, *left*) and an orange head leaf parent (07A163, *right*). The flowers and cotyledons of the orange head leaf parent are also orange in contrast to the yellow ones of the normal white head leaf parent

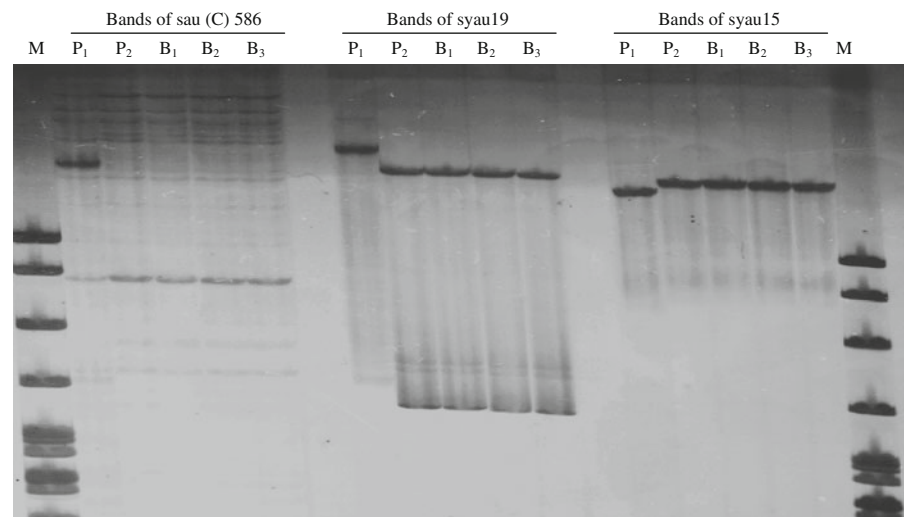


Table 1 Segregation of morphological markers (*or-f* and *or-c*) in the F₂ populations of two crosses

Morphological markers	Color of inner leaf	07A163 × Chiifu		PG-2 × Chiifu	
		No. of plants	No. of plants with marker	No. of plants	No. of plants with marker
Orange flower (<i>or-f</i>)	White	446	0	116	0
	Orange	154	154	42	42
Orange cotyledon (<i>or-c</i>)	White	446	0	116	0
	Orange	154	154	42	42
Segregation ratio	–	2.9:1	–	2.76:1	–

Table 2 Sequences of SSR markers and map distances from *or* gene on A9

Markers	Forward primer sequences (5′–3′)	Reverse primer sequences (5′–3′)	Map distance (cM)
ENA21	GAGTGTGGAGCAGATGA	GGAGACTTTGCCTTTGTGT	21.8
sau (N) 033	GCCAGATACGTTGCAGGTTT	TTGACATACCTGCATACTCAAAGA	20.8
sau (C) 586	AGGCTGCTGTCTCTCTCTCG	TGGGGTGATTAAGTGGTCAA	1.6
syau19	CCGAAGCCCAGATTAGGAG	GCCCATGCCTACGAATGATAT	1.3
syau15	CGTGATTAACGCCTTCCAATAT	TGTGACAGCGGCAGAAAATAG	3.3

Fig. 2 Amplifications of three SSR markers closely linking to *or* gene in two parents and three bulks (*oror*). *M*: Marker; *P*₁ (*OrOr*): Chiifu; *P*₂ (*oror*): 07A163; *B*₁, *B*₂, *B*₃: gene bulk with homozygous *or* allele

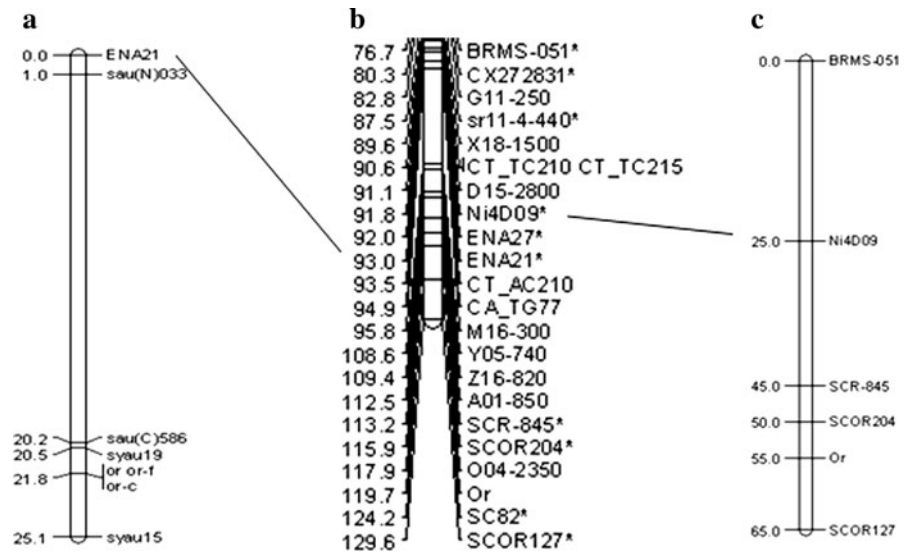
screened at the end of A9. The development of more markers in this region is essential for solving this problem.

Discussion

As a type of new breed, Chinese cabbage with orange inner leaves is becoming increasingly popular in people's lifestyle owing to its good nutritional value. *Brassica* crops with orange organs have been studied

for a long time. Crisp et al. (1975) discovered a mutant gene causing the edible curd to turn orange in cauliflower and designated the gene as "*Or*." Matsumoto et al. (1998) conducted RFLP studies on clubroot resistance and orange-yellow (*Oy*) pigmentation in Chinese cabbage and mapped "*Oy*" on linkage group 1. Genetic analysis indicated that the *Oy* gene is a recessive gene controlled by a single locus. Of the 600 individuals, 154 individuals showed orange inner leaf with a good segregation ratio of 2.9:1 ($\chi^2 = 0.142 < \chi_{0.05}^2 = 3.841$) in this study.

Fig. 3 **a** Partial genetic linkage map of the *or* locus on A9 constructed using five SSR markers and two morphological markers in this study. **b** Partial linkage group 9 of the published genetic map of Chinese cabbage (Yu et al. 2009). **c** A partial linkage map of the *or* region on A9 of Chinese cabbage published by Zhang et al. (2008). The genetic distances in cM shown on the left were calculated using the Kosambi function



This again confirmed that the orange inner leaf is controlled by a single recessive gene. To unify the name, we designated the orange inner leaf gene as “*or*,” in accordance with some subsequent reports. Although many genes in *Brassica* crops show homology, it has not yet been decided whether the genes leading to the orange mutant in cauliflower and Chinese cabbage are the same.

Lu et al. (2006) have conducted positional cloning of the *Or* gene and the accession number of this gene is reported. Based on this, we obtained the full-length sequences of this gene and BLASTed it in NCBI (<http://www.ncbi.nlm.nih.gov>). We found that the length of the *Or* gene locus is 9,240 bp, the *Or* mutant gene is from 1,711 to 8,162 bp and the coding sequence of the *Or* gene is from 2,555 to 6,274 bp. When performing nucleotide BLAST, we obtained a BAC clone (KBrB073I16) of Chinese cabbage that contained a DNA sequence (from 25,732 to 28,308 bp) showing an identity of 80% to parts of the coding domain of the *Or* gene (from 4,401 to 6,958 bp). When performing TBLASTN, we found that a DNA sequence (from 24,066 to 27,608 bp) of KBrB073I16 encodes a protein which shows an identity of 78% to the protein coded by the *Or* gene. In order to confirm the location of BAC clone KBrB073I16, we examined it on the BrGSP website (www.brassica-rapa.org) and with BAC clone accession numbers on A9 afforded by Korea group. Unfortunately, we did not find information on KBrB073I16 and were not able to decide whether this

clone is located in the region between KBrH010M08 and KBrB017P15 where the *or* gene was mapped in this study. Even so, we believe that the pigment gene should be homologous between Chinese cabbage and cauliflower and it will be interesting to carry out further studies on this issue.

Genetic markers in plants are mainly categorized into morphological markers, biochemical markers, cytological markers, and molecular markers (Staub et al. 1996). Morphological markers, which are based on the external characters of plants, can be obtained without laboratory procedures and are convenient and fast for visually distinguishing the materials of interest (Rieseberg and Ellstrand 1993). Thus far, these markers have been applied to the construction of genetic linkage maps in many plants (Dijkhuizen et al. 1994; Kaga et al. 1996; Yan et al. 2005). In this study, two morphological markers (*or-f* and *or-c*) were identified and they are strongly believed to co-segregate with the orange inner leaf trait. On the other hand, the three characters, namely orange inner leaf, orange flower, and orange cotyledon, may be determined by the same gene. Of the two morphological markers, orange cotyledon (*or-c*) can only be used to screen orange inner leaf plant before the cotyledon turns green on exposure to sunlight for long periods. Vernalization treatment of seed is often carried out for bolting and pollinating in the breeding procedure of Chinese cabbage, and visual observation of the cotyledon color during the vernalization stage is sufficient. Materials with orange inner leaf (*oror*)

can be observed at maturation, but require more time at the late growth stage. Though the two morphological markers are dominant, they may be used in the early stages such as the germination and flowering stages to distinguish *oror* from *Oror* and *OrOr* because this is less expensive, which would facilitate improvement in breeding efficiency.

Molecular markers have been studied for the major characters of Chinese cabbage, including clubroot resistance, male sterility, etc. (Piao et al. 2004; Saito et al. 2006; Feng et al. 2009; Wei et al. 2009). However, studies on the head color of the inner leaf have rarely been reported. In this study, an F2 population was used as the material for mapping the orange inner leaf gene (*or*). Three genotypes (*OrOr*, *Oror*, and *oror*) existed in the population and we cannot distinguish *OrOr* from *Oror* by the phenotype; thus, only plants homozygous for the *or* allele (*oror*) were used for pooling the DNA for the bulk samples used in BSA analysis. BSA was performed on two parents and three bulk samples pooled randomly using *oror* individuals. A polymorphic ratio of 53% was calculated by screening the primers between the two parents. All primers were designed from BrGSP, which has developed a number of genetic resources including two BAC libraries, 22 cDNA libraries (Trick et al. 2007), two genetic maps, a physical map (Choi et al. 2007; Mun et al. 2008), and a huge number of SSR markers. Chiifu, the model material of BrGSP, was also used in our studies and significant differences existed between the two parents. Thus, the polymorphism was maintained at a relatively high level.

As a co-dominant marker, SSR is sufficient to distinguish between homozygous and heterozygous genotypes in a segregation population (Zietkiewicz et al. 1994). In the present study, we identified five co-dominant SSR markers, three of which, namely sau (C) 586, syau19, and syau15, were closely linked to *or*. It is very convenient for us to be able to distinguish between the *Oror* and *oror* genotypes in the breeding process when transferring the *or* gene to white inner leaf materials. Furthermore, since syau19 and syau15 flank *or* on the two sides, these markers used in combination would enable us to screen orange inner leaf materials more precisely in the course of MAS. In contrast, the error rate is more likely to increase by using a marker on one side only (Zhang et al. 2007; Zhao et al. 2008).

It was reported that *or* was mapped on A9 by two anchor markers (Ni4D09 and BRMS-051) relatively far apart (Zhang et al. 2008), and we tested these markers for mapping *or* as well. However, no polymorphism existed between the parents. In the present study, we mapped the gene on the end of A9 covering an interval of 4.6 cM between the two markers syau19 and syau15. Furthermore, ENA21 has been used for the construction of genetic linkage groups in previous studies (Choi et al. 2007; Yu et al. 2009). Comparison between the present linkage group and previously reported linkage groups revealed that the markers ENA21 and Ni4D09 are positioned on the same side of *or* approximately 1–2 cM apart (Fig. 3). Considering that the map distances of ENA21 and Ni4D09 are 21.8 and 30 cM, respectively, we surmise that the gene is most likely to be the same one as that reported by Zhang et al. (2008). Matsumoto et al. (1998) mapped *or* on linkage group 1 with a different marker and mapping population. This may mean that the gene for the orange inner leaf is not identical to our gene or that the gene has been translocated in the course of evolution of Chinese cabbage.

The two markers (syau19 and syau15) flanking the *or* gene were developed from two BAC clones at the end of A9. In BrGSP, the studies of genetic map and physical map on A3 and A9 have nearly been completed (www.brassica-rapa.org) and we are able to estimate the physical map distance between two BAC clones. The real distance between KBrB017P15 (from 619.753 to 755.980 kb) and KBrH010M08 (from 1,427.251 to 1,555.321 kb) is 671.271 kb by examining the pseudochromosome of A9 constructed with BAC contig. Though the SSR primers that we screened in this study were distributed on 10 linkage groups of the Chinese cabbage, the density of primers at the end of A9 was still insufficient to fill in the gap between markers sau (N) 033 and sau (C) 586. High-resolution genetic linkage and physical maps are available for us to carry out a further study of *or*. On the basis of the existing information, we are preparing for fine mapping of this gene after the construction of a large-scale segregating population in the future.

Fine mapping of an interesting gene is essential for performing map-based cloning studies (Tanksley et al. 1995) and many genes of carotenoid mutants have been successfully isolated from some plants by map-based cloning (Ronen et al. 2000; Isaacson et al.

2002). In Chinese cabbage, we mapped *or* to a particular region and developed markers flanking this gene; on the basis of this finding, the isolation of *or* is also expected to be completed shortly after the fine mapping studies.

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References

- Chen PF (2008) The study of identify and accumulation mechanisms and regulation control of carotenoid on Chinese cabbage [D]: College of Horticulture and Protection. Yangzhou University, China
- Choi SR, Teakle GR, Plaha P, Kim JH, Allender CJ, Beynon E, Piao ZY, Soengas P, Han TH, King GJ, Barker GC, Hand P, Lydiate DJ, Batley J, Edwards D, Koo DH, Bang JW, Park BS, Lim YP (2007) The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. *Theor Appl Genet* 115:777–792
- Crisp P, Walkey DGA, Bellman E, Roberts E (1975) A mutation affecting curd colour in cauliflower (*Brassica oleracea* L. var. *botrytis* DC). *Euphytica* 24:173–176
- Dickson MH, Lee CY, Blamble AE (1988) Orange-curd high carotene cauliflower inbreds, NY 156, NY 163, and NY 165. *Hort Sci* 23:778–779
- Dijkhuizen A, Meglic V, Staub JE, Havey MJ (1994) Linkages among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. *Theor Appl Genet* 89:42–48
- Feng H, Wei P, Piao ZY, Liu ZY, Li CY, Wang YG, Ji RQ, Ji SJ, Zou T, Choi SR, Lim YP (2009) SSR and SCAR mapping of a multiple-allele male-sterile gene in Chinese cabbage (*Brassica rapa* L.). *Theor Appl Genet* 119:333–339
- Frank HA, Cogdell RJ (1996) Carotenoids in photosynthesis. *Photochem Photobiol* 63:257–264
- Fraser PF, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 43:228–265
- Isaacson T, Ronen G, Zamir D, Hirschberg J (2002) Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of β -carotene and xanthophylls in plants. *Plant Cell* 14:333–342
- Kaga A, Ohnishi M, Ishii T, Kamijima O (1996) A genetic linkage map of azuki bean constructed with molecular and morphological markers using an interspecific population (*Vigna angularis* x *V. nakashimae*). *Theor Appl Genet* 93:658–663
- Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugen* 12:172–175
- Li L, Garvin DF (2003) Molecular mapping of *Or*, a gene inducing β -carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *Genome* 47:588–594
- Li L, Paolillo DJ, Parthasarathy MV, DiMuzio EM, Garvin DF (2001) A novel gene mutation that confers abnormal patterns of β -carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *Plant J* 26:59–67
- Li L, Lu S, Halloran D, Garvin DF (2003) High-resolution genetic and physical mapping of the cauliflower high- β -carotene gene *Or* (Orange). *Mol Genet Genomics* 270:132–138
- Liu XC, Zhang FL, Zhang DS, Yang JP, Wang M, Xu JB (2003) Identification of a RAPD marker linked to orange-red internal head gene in Chinese cabbage. *Acta Agric Boreali-Sinica* 18:51–54
- Lu S, Joyce VE, Zhou XJ, Alex BL, Diana MO, Kelly MC, Brian JC, Dominick JP, David FG, Julia V, Leon VK, Hendrik K, Elizabeth DE, Cao J, Li L (2006) The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β -carotene accumulation. *Plant Cell* 18:3594–3605
- Matsumoto E, Yasui C, Met Ohi, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Euphytica* 104:79–86
- Michelmore RW, Paran I, Kesseli RV (1991) Identification of marker linked to disease-resistance gene by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA* 88:9828–9832
- Molnar J, Gyemant N, Mucsi I, Molnar A, Szabo M, Kortvelyesi T, Varge A, Molnar P, Toth G (2004) Modulation of multidrug resistance and apoptosis of cancer cells by selected carotenoids. *In Vivo* 18:237–244
- Mortensen A, Skibsted LH (1997) Importance of carotenoid structure in radical-scavenging reactions. *J Agric Food Chem* 45:2970–2977
- Mun JH, Kwon SJ, Yang TJ, Kim HS, Choi BS, Baek SH, Kim JS, Jin M, Kim JA, Lim MH, Lee SI, Kim HI, Kim HT, Lim YP, Park BS (2008) The first generation of a BAC-based physical map of *Brassica rapa*. *BMC Genomics* 9:280
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8:4321–4326
- Olson JA (1989) Provitamin A function of carotenoids: the conversion of β -carotene into vitamin A. *J Nutr* 119:105
- Piao ZY, Deng YQ, Choi SR, Park YJ, Lim YP (2004) SCAR and CAPS mapping of *CRb*, a gene conferring resistance to *Plasmodiophora brassicae* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Theor Appl Genet* 108:1458–1465
- Pogson BJ, Niyogi KK, Bjorkman O, Dellapenna D (1998) Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. *Proc Natl Acad Sci USA* 95:13324–13329
- Rieseberg LH, Ellstrand NC (1993) What can molecular and morphological markers tell us about plant hybridization. *Crit Rev Plant Sci* 12:213–241
- Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to β -carotene formation in plant chloroplasts discovered by map-based cloning of *Beta* and *old-gold* color mutations in tomato. *Proc Natl Acad Sci USA* 97:11102–11107

- Saito M, Kubo N, Matsumoto S, Suwabe K, Tsukada M, Hirai M (2006) Fine mapping of the clubroot resistance gene, *Crr3*, in *Brassica rapa*. *Theor Appl Genet* 114:81–91
- Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. *Hort Sci* 31:729–741
- Tanksley SD, Ganai MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends Genet* 11:63–68
- Trick M, Bancroft I, Lim YP (2007) The *Brassica rapa* genome sequencing initiative. *Genes Genomes Genomics* 1:35–39
- Van Ooijen JW, Voorrips RE (2001) JoinMap[®] 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands
- Von Lintig J, Welsch R, Bonk M, Giuliano G, Batschauer A, Kleinig H (1997) Light-dependent regulation of carotenoid biosynthesis occurs at the level of phytoene synthase expression and is mediated by phytochrome in *Sinapis alba* and *Arabidopsis thaliana* seedlings. *Plant J* 12: 625–634
- Voorrips RE (2002) MapChart, software for the graphical presentation of linkage maps and QTLs. *J Hered* 93:77–78
- Wang GC, Zhang FL, Yu YJ, Zhang DS, Zhao XY (2007) Identification of SCAR markers linked to orange head leaf gene in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Acta Hort Sin* 34:217–220
- Wei P, Feng H, Piao ZY, Li CY, Liu ZY, Wang YG, Ji RQ, Zou T, Ji SJ (2009) Identification of AFLP markers linked to *Ms*, a genic multiple allele inherited male-sterile gene in Chinese cabbage. *Breed Science* 59:333–339
- Yan Z, Denneboom C, Hattendorf A, Dolstra O, Debener T, Stam P, Visser PB (2005) Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. *Theor Appl Genet* 110:766–777
- Yie HP, Zhu ZL, Sun MM (2005) Synergistic formulation for preventing and/or treating diabetes. US Patent App 2008/0268066
- Yu SC, Zhang FL, Yu RB, Zou YM, Qi JN, Zhao XY, Yu YJ, Zhang DS, Li L (2009) Genetic mapping and localization of a major QTL for seedling resistance to downy mildew in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Mol Breed* 23:573–590
- Zhang SB, Zhu Z, Zhao L, Zhang YD, Chen T, Lin J, Wang CL (2007) Identification of SSR markers closely linked to *eui* gene in rice. *Hereditas (Beijing)* 29:365–370
- Zhang FL, Wang GC, Wang M, Liu XC, Zhao XY, Yu YJ, Zhang DS, Yu SC (2008) Identification of SCAR markers linked to *or*, a gene inducing beta-carotene accumulation in Chinese cabbage. *Euphytica* 164:463–471
- Zhao MF, Cai CM, Che RH, Xu XZ, Zheng JH, Fang SR, Wu CZ, Shi BH (2008) Molecular mapping of photoperiod-therom sensitive male sterile gene in rice 45S. *Mol Plant Breed* 16:1045–1049
- Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176–183