Pepper is an important horticultural crop worldwide. Among the five cultivated species of pepper, *Capsicum annuum* (2n = 24 (2x)) is most widely used as a vegetable, a spice, and a food colorant. Pepper fruits, especially of mature red pepper, are an excellent source of natural pigments. The carotenoid pigments of pepper include capsanthin, capsanthin-5,6-epoxide, capsorubin, zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene and cucurbitaxanthin A, and are synthesized and accumulated during fruit ripening. There are a great number of variations on the color and carotenoid content in pepper germplasm. And the most highly valued characteristic is high content of carotenoid. To breed and select carotenoid rich varieties, red-to-yellow isochromic fractions ratio and the capsanthin-to-zeaxanthin ratio are most useful and appropriate index together with the total carotenoid content (Hornero-Mendez et al. 2000, 2002, Wall et al. 2001). Because capsanthin is the main carotenoid, the relative content for capsanthin reaches 60% in carotenoid of red pepper fruits (Hornero-Mendez et al. 2002).

Capsanthin, the main carotenoid of red pepper fruits, is beneficial for human health. To breed pepper (*Capsicum annuum* L.) with high capsanthin content by marker-assisted selection, we constructed a linkage map of doubled-haploid (DH) lines derived from a cross of two pure lines of *C. annuum* (‘S3586’ × ‘Kyoto-Manganji No. 2’). The map, designated as the SM-DH map, consisted of 15 linkage groups and the total map distance was 1403.8 cM. Mapping of quantitative trait loci (QTLs) for capsanthin content detected one QTL on linkage group (LG) 13 at 90 days after flowering (DAF) and one on LG 15 at 45 DAF; they were designated Cst13.1 and Cst15.1, respectively. Cst13.1 explained 17.0% of phenotypic variance and Cst15.1 explained 16.1%. We grouped DH lines according to the genotypes of markers adjacent to Cst13.1 and Cst15.1 on both sides. The DH lines with the alleles of both QTLs derived from ‘S3586’ showed higher capsanthin content at 45 and 90 DAF than the other lines. This is the first identification of QTLs for capsanthin content in any plant species. The data obtained here will be useful in marker-assisted selection for pepper breeding for high capsanthin content.

**Key Words:** pepper (*Capsicum annuum* L.), capsanthin, QTL, linkage map.
and color variation of fruits (Hurtado-Hernandez and Smith 1985). Genes for carotenoid biosynthesis pathway, for instance, PSY for phytoene synthase, Lcyb for lycopene-β-cyclase, Crtz for β-carotene hydroxylase and CCS for capsanthin–capsorubin synthase were identified so far (Bouvier et al. 1994, 1998 Hgueney et al. 1995, Romer et al. 1993). These are key genes for control yellow, orange and red colors of fruits (Hu et al. 2001, Lefebvre et al. 1998). Especially, CCS controls the red color (Tian et al. 2015). Further, orange and yellow colors of fruits are due to deletion or silencing of CCS gene (Ha et al. 2007, Lang et al. 2004, Rodriguez-Uribe et al. 2012).

Although there are many variations in pigment content in pepper germplasm, few studies about genetic controls of quantitative variations in carotenoid content have been reported. In one study, 4 QTLs were identified for fruit color of red pepper fruits, by quantifying lightness, chroma and hue parameters (Ben Chaim et al. 2001). In another study, 2 QTLs, pc8.1 and pc10.1, were identified that control chlorophyll content. The QTL pc8.1 also affected carotenoid content in ripe fruit. However, in subsequent generations there was not consistent effect of this QTL on carotenoid content (Brand et al. 2012).

Therefore, to access the genetic mechanisms for controlling content of red color pigment, capsanthin, in pepper, we mapped QTLs for the content in DH population derived from a cross between genetic resource and local cultivar. The genetic resource ‘S3586’ has high capsanthin content and local cultivar ‘Kyoto-Manganji No. 2’ has low capsanthin content. In QTL mapping using population with fixed genotypes such as DH and recombinant inbred lines, utilization of all biological replication in one analysis is very important factor for reduction of nongenetic residual variance and increase in accuracy of the mapping (Broman and Sen 2009). Because our segregating population is DH, we can create multiple individuals with the same genotype for two experiments. Hence, we performed QT mapping mainly using phenotypes from two datasets (Experiments 1 and 2) at one time. Further, we discuss how to increase capsanthin content by marker-assisted selection in practical breeding of pepper using QTLs detected in this study.

**Materials and Methods**

**Plant materials**

The pepper genetic resource ‘S3586’ (C. annuum, Laboratory of Plant Genetics and Breeding, Shinshu University, Matsushima et al. 2009) was crossed with cultivar ‘Kyoto-Manganji No. 2’ (C. annuum, Biotechnology Research Department, Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Research Center, Seika, Kyoto, Japan, Minamiyama et al. 2012). A segregating doubled-haploid (SM-DH) population (n = 141) was developed by another culture of an F1 plant as described by Dumas de Vaulx et al. (1981).

SM-DH lines were grown in a greenhouse of the Bio-technology Research Department (Seika, Kyoto, Japan) during the summers of 2013 (Experiment 1) and 2016 (Experiment 2). One plant of each SM-DH line was used for analysis. Seeds were sown in trays filled with vermiculite on 11 March 2013 and 4 February 2016. After 4 weeks, seedlings were transplanted into rockwool cubes; the cubes were placed on rockwool slabs on 17 May 2013 and 22 April 2016. The temperature in the greenhouse was maintained above 16°C. Plants were grown in hydroponic solution (M nutrient prescription, M Hydroponic Research Co., Ltd., Aichi, Japan) with an electrical conductivity of 1.0 to 1.2 dS/m. Five fruits were harvested from each plant at each of the two ripening stages, 45 and 90 days after flowering (DAF). 45 DAF was turning color stage and 90 DAF was full maturity stage. Usually, we harvest ‘Kyoto-Manganji No. 2’ at green mature stage, about 30 to 35 DAF, but at this stage capsanthin mostly is not detected. The peduncles and the seeds were removed, and the fruits were cut into small pieces and kept at –30°C until analysis.

**Pigment extraction and saponification**

Because a large proportion of capsanthin in pepper fruits is esterified with many kinds of fatty acids, qualitative and quantitative analysis of all capsanthin esters by high-performance liquid chromatography (HPLC) is very difficult. Generally, prior to HPLC analysis, capsanthin esters are hydrolyzed by saponification and then the capsanthin monomer is quantified (Hornero-Mendez et al. 2000, Howard et al. 2000).

Frozen sample (1 g fresh weight) was powdered using a mortar and pestle with sea sand and extracted with ethanolic pyrogallol (3% w/v). Extraction was repeated until the complete loss of color. All extracts were pooled and made up to 50 mL with ethanolic pyrogallol in a volumetric flask.

Each aliquot (10 mL) of the extract was mixed with 1 mL of potassium hydroxide (60% w/v). The tubes were placed in a 70°C water bath for 30 min with shaking continuously during saponification. The tubes were then cooled in water to room temperature, and 22.5 mL of sodium chloride (10 g/L) was added into each tube. Then the suspension was extracted three times with 15 mL of n-hexane/ethyl acetate (9:1 v/v). The upper layer was collected, and evaporated to dryness, and the residue was dissolved in 5 mL of ethanolic pyrogallol (3% w/v). All samples were filtered through 0.45-μm nylon membrane filters (Minisart-RC, Sartorius, Göttingen, Germany).

**HPLC analysis**

Qualitative and quantitative HPLC analysis was performed according to the modified method of Goda et al. (1995) using a Shimadzu LC-10A quaternary pump equipped with a diode array detector (Shimadzu, Kyoto, Japan) and a Cosmosil SC18-AR II reverse-phase column (Nacalai Tesque, Kyoto, Japan) protected by a guard cartridge (Nacalai Tesque). The oven was operated at 40°C. The sample injection volume was 10 μL. Samples were
eluted with acetone in water as follows: 70% acetone for 5 min; 70%–90% linear gradient for 5 min; 90% acetone for 5 min; 90%–100% linear gradient for 20 min; and 100% acetone for 5 min. The flow rate was 1.0 mL/min. For quantification, a capsanthin standard was obtained from Extrasynthese S.A. (Lyon, France) and a β-carotene standard was obtained from Wako Pure Chemical Industries (Osaka, Japan). All samples were analyzed before and after saponification. Loss of capsanthin during saponification was calculated from the loss of β-carotene. All analysis was carried out in 3 to 5 replications.

Heritability of traits

We used one-way ANOVA tables for phenotypes of the SM-DH lines in each experiment, using line (genotype) as a factor, to estimate heritability ($h^2$) of capsanthin content at 45 DAF and 90 DAF. The phenotypic value of the $i$th line in the $j$th replicate, denoted as $y_{ij}$, is expressed as:

$$y_{ij} = \mu + g_i + e_{ij} \ (i = 1, 2, ..., m; j = 1, 2),$$

where $\mu$ is the intercept, $g_i$ is the effect of the $i$th line, $e_{ij}$ is the residual error with $\sim N(0, \sigma^2_e)$ and $e_{ij} \sim N(0, \sigma^2_e)$, and $m$ is the number of lines ($m = 98$ (45 DAF) or 94 (90 DAF)).

The expectations of sums of squares between and within lines, $S_B$ and $S_W$, are expressed as:

$$E(S_B) = 2(m-1)\sigma^2_g + (m-1)\sigma^2_e$$

and

$$E(S_W) = m\sigma^2_e$$

From these formulae, estimates of the genetic variance $\hat{\sigma}^2_g$ and residual variance $\hat{\sigma}^2_e$, $\hat{\sigma}^2_g$ and $\hat{\sigma}^2_e$, are obtained as:

$$\hat{\sigma}^2_g = S_B / m$$

and

$$\hat{\sigma}^2_e = \left\{S_B - (m-1)\hat{\sigma}^2_g \right\} / \left\{2(m-1)\right\}.$$

Heritability was estimated as:

$$h^2 = \hat{\sigma}^2_g / (\hat{\sigma}^2_g + \hat{\sigma}^2_e).$$

Isolation of genomic DNA and genotyping

Genomic DNA from the leaves of parental lines and the SM-DH population was isolated using a Nucleon PhytoPure DNA extraction kit (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, England). Simple sequence repeat (SSR), single nucleotide polymorphism (SNP), sequence characterized amplified repeat (SCAR) and cleaved amplified polymorphic sequence (CAPS) primer pairs used in this study were selected on the basis of the published marker locus data (Gulyas et al. 2006, Kim and Kim 2006, Lee et al. 2004a, 2004b, Mimura et al. 2010, 2012, Minamiyama et al. 2006, 2007, Nagy et al. 2007, Sugita et al. 2006, 2013, Yi et al. 2006). Some SSR markers designed by Minamiyama et al. (2006) were newly mapped in this study (Table 1). PCR with SSR primers (Sugita et al. 2006, 2013) was performed by a post-labeling method with a bar-coded split tag as described in Konishi et al. (2015). PCR products were sequenced on a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment length was determined by GeneMapper v3.7 software (Applied Biosystems). Labeling and analysis of SSR markers developed by Minamiyama et al. (2006) were performed as in that report. The 5’ ends of the forward primers were labeled with D2-, D3- or D4-fluorescent dye. PCR products were sequenced on a Beckman CEQ 200XL sequencer (Beckman Coulter, Fullerton, CA, USA). Fragment length was determined on a CEQ 8000 genetic analysis system (Beckman Coulter). SNPs were genotyped by the Tm-shift method as in Fukuoka et al. (2008). PCR using SCAR and CAPs markers was performed as in Lee et al. (2004b), Gulyas et al. (2006) and Kim and Kim (2006). PCR products were separated on 2% agarose gels and stained with ethidium bromide.

Construction of a linkage map and QTL analysis

AntMap software (Iwata and Ninomiya 2006) was used to construct LGs; the order of markers was determined using the Kosambi mapping function. The map was compared to the KL-DH map (Sugita et al. 2013; downloaded from VegMarks, http://vegmarks.nivot.affrc.go.jp/) and to the pepper genome (chromosomes) sequence data (CM334 ver. 1.55, Kim et al. 2014; available from the Pepper Genome website, http://peppergenome.snu.ac.kr). Composite interval mapping was performed in QTL Cartographer ver. 2.5 software (https://brcwebportal.cos.ncsu.edu/qtlcart/WQTLCart.htm, Wang et al. 2005). Forward and backward stepwise regression was performed with a threshold of $P < 0.05$. To

Table 1. SSR markers newly mapped in this study

| Marker name  | Forward primer (5’-3’) | Reverse primer (5’-3’) | Motif                  | Linkage group | Expected product size (bp) |
|--------------|------------------------|------------------------|------------------------|--------------|---------------------------|
| CAMS_061-2   | cgattcagtggggtgtcatc  | cgcactgaaaagggagttgg  | (gt)3...(tg)5...(tg)4...(ga)3 | 8            | 231                       |
| CAMS_206-2   | tggagcactggtaaacacac  | gtgcaacaacccccttgctgc  | (ac)22cc(ac)13         | 14           | 286                       |
| CAMS_308     | gtctggtcgatgtgtcattc  | tcggaagatgcgataaagtggtg | (ta)3(g3)(ta)7tgtag(11) | 14           | 215                       |
| CAMS_487-2   | ggtattgattgaacgattagcgt | ttgcagctgtggagaataaa  | (ag)13                 | 2            | 260                       |
| CAMS_604-2   | atgggctatgatacaagaca  | cttggctgtcaggttattttg  | (ta)n8                 | 14           | 158                       |
| CAMS_667-2   | cgactcgaggtgaatgtcctac  | ggccaccccacctttttagc  | (ag)12...(ga)4...(ga)3 | 9            | 222                       |
| CAMS_818     | gctgactcctctctctctc   | ccccatagttggttaggaatac | (ctt)5...(ca)5         | 14           | 268                       |
| CAMS_852     | gctgactcctctctctctc   | tggcgacggtccgtatcag   | (tct)9                 | 15           | 256                       |

All markers in this Table were designed by Minamiyama et al. (2006).

a Expected product size is indicated for ‘Kyoto-Manganji No. 2’.
establish empirical LOD thresholds at the 5% level, 1000 permutation tests were performed. Seven datasets were prepared (Supplemental Table 1) using phenotypes of two cultivations (Experiments 1 and 2) at two ripening stages (45 and 90 DAF). These datasets were used to perform QTL mapping. In dataset No. 7, all of the phenotypes at two ripening stages (45 DAF and 90 DAF) in two cultivations were entered as a single phenotype.

**Analysis of the effect of QTLs on capsanthin content**

The SM-DH lines were grouped according to the genotypes of the markers linked to the QTLs, and the phenotypes of the groups were compared to each other.

### Results

**Phenotypic characterization of capsanthin content in parents and SM-DH population**

Capsanthin content increased during ripening (from 45 DAF to 90 DAF) in both parental lines and was higher in ‘S3586’ than in ‘Kyoto-Manganji’ at both ripening stages (Figs. 1, 2). This result agreed with that of Konishi and Matsushima (2011).

The capsanthin content in the SM-DH population evaluated in experiments 1 (2013) and 2 (2016) showed a normal distribution (Fig. 3). Capsanthin content of parental lines and average capsanthin content of SM-DH was higher in experiment 1 than in experiment 2 at both ripening stages (45 and 90 DAF) and a histogram of capsanthin content in experiment 2 shifted lower than in experiment 1. In 45 DAF, the distribution pattern in experiment 1 was different from that in experiment 2 (Fig. 3). Heritability of capsanthin content was quite low at 45 DAF and it was 0.155 at 90 DAF (Table 2).

**Fig. 1.** Fruits of parental lines at two ripening stages. (A) ‘S3586’, 45 DAF (B) ‘S3586’, 90 DAF (C) ‘Kyoto-Manganji No. 2’, 45 DAF (D) ‘Kyoto-Manganji No. 2’, 90 DAF. Bars indicate 5 cm.

**Fig. 2.** Capsanthin content of parental lines at two ripening stages. Values are means of 7–9 measurements in two experiments. Error bars represent the standard deviation. Means sharing the same letter are not significantly different according to the Tukey–Kramer multiple-comparison test.

**Fig. 3.** Frequency distribution of capsanthin content in SM-DH lines. (A) 45 DAF. (B) 90 DAF. Arrowheads indicate the mean values for the parents and average of SM-DH.
Table 2. Heritability of capsanthin content

| Trait               | h^2  |
|---------------------|------|
| Content at 45 DAF   | -0.068 |
| Content at 90 DAF   | 0.155 |

**Linkage map construction**

To construct a genetic map (designated as the SM-DH map), 160 SSR, 24 SNP, 3 SCAR and 1 CAPS markers were used. The map consisted of 15 LGs covering a total distance of 1403.8 cM (Fig. 4). The average distance between markers was about 9 cM. In this study, 8 new SSR markers were mapped (Table 1). We were able to assign 14 of the 15 LGs of the SM-DH map to LGs of the KL-DH map (Sugita et al. 2013), which covers nearly the entire genome of C. annuum (Fig. 5). Comparison with the pepper genome (CM334 ver. 1.55) using the BLAST program showed that the SM-DH map covered 75% of the genome.

**QTL analysis**

We carried out QTL analysis using 4 datasets on capsanthin content at 45 DAF and 90 DAF obtained from two experiments (Experiments 1 and 2). Further, we also performed QTL analysis using the data on the content at 45 DAF and 90 DAF from each experiment (Dataset 5 and 6, Supplemental Table 1).

Analysis of capsanthin content at 45 DAF from two experiments detected a significant QTL on LG15 (LOD score, 4.95; Table 3), which was designated Cst15.1. The LOD score peak was positioned between the SSR markers GPMS001 and CAMS378. The additive effect of this QTL was 501.0, and the allele that increased capsanthin content was derived from 'S3586' (Table 3). An insignificant LOD score peak at 90 DAF was observed close to Cst15.1 (Fig. 6).

Analysis of capsanthin content at 90 DAF from two experiments detected a significant LOD peak on LG13 (LOD score, 4.02; Fig. 6, Table 3), which was designated Cst13.1. The LOD peak was positioned between the SSR markers EPMS376/HpmsE072 and ge075-422pmc0296C (Fig. 6). The additive effect of this QTL was 778.0 and its allele that increased capsanthin content was derived from 'S3586' (Table 3). In analysis of the content from each single experiment (Dataset1-4), Cst15.1 was detected at 45 DAF in experiment 1, and at 90 DAF in experiment 2. Cst13.1 was also detected at 90 DAF in experiment 1 (Table 3). A new QTL, Cst7.1 was detected only at 90DAF in experiment 2. At 45 DAF of experiment 2, we could not detect any significant QTL.

We also carried out QTL analysis using combined 45 DAF and 90 DAF data (Dataset 7), which we considered as variations of a single phenotype during ripening. In this analysis, we detected Cst15.1 but not Cst13.1 (Supplemental Fig. 1).

We grouped SM-DH lines according to the genotypes of markers adjacent to Cst15.1 and Cst13.1 on both sides and calculated the mean capsanthin content of each group at each ripening stage. Lines with the homozygous genotypes of Cst15.1 or Cst13.1 derived from 'S3586' had higher capsanthin content than the other lines at both 45 DAF and 90 DAF (Table 4). At 45 DAF, the ‘S3586’ allele of Cst15.1 seemed to be more effective in increasing capsanthin content than that of Cst13.1.

**Discussion**

In this study, to access the mechanisms for the genetic control in variation of capsanthin content of pepper (C. annuum), QTL mapping using SM-DH lines derived from a cross of high content genetic resource line, 'S3586' and cultivar ‘Kyoto-Manganji No. 2’ was performed. Capsanthin content of SM-DH lines at two ripening stages (45 DAF and 90 DAF) differed between the two experiments, and its difference ascribes to the variation of the environmental (cultivation) conditions (Fig. 3). It is known that carotenoid accumulation is regulated by light signaling (Nisar et al. 2015). In Capsicum fruit, light irradiation at immaturity stage of fruit increases the expression of Psy gene for phytoene synthase (Nagata et al. 2015). Phytoene synthase is an enzyme upstream in capsanthin biosynthesis (Supplemental Fig. 3) and expression level of Psy and content of total carotenoid positively correlate (Rodriguez-Uribe et al. 2012). In this study, total global solar radiation at the nearest observation point from fruit setting (DAF 0) to immaturity stage (DAF 40) was 785 and 633 MJ/m^2 in experiments 1 and 2, respectively (Supplemental Fig. 2). This difference of light condition may account for the difference of capsanthin content in two experiments. In order to verify this hypothesis, it is necessary to investigate into gene expression for carotenoid biosynthesis in the fields under different light condition.

To improve accuracy of QTL detection, we took into account variation among year in the analysis according to Broman and Sen (2009) and found Cst15.1 at 45 DAF and Cst13.1 at 90 DAF (Table 3). Cst 15.1 was detected at 45 DAF of experiment 1 and 90 DAF of experiment 2, but Cst 13.1 was detected at only experiment 1. Hence, it is possible that Cst 15.1 has more stable and large effect than Cst 13.1. We could select SM-DH lines with higher capsanthin content at both ripening stages in both experiments by using markers adjacent to the two QTLs (Table 4), suggesting that the QTLs have stable effects on capsanthin content under environmental conditions tested here.

It is important to breed high-capsanthin-content peppers to be used as health beneficial vegetables. Because the pepper fruits to be used as vegetables are usually harvested before they are fully matured (ex. 90 DAF), it is necessary to accumulate QTLs that increase capsanthin content at early stage (ex. 45 DAF). In particular, Cst15.1 seems to be efficient for this purpose (Tables 3, 4). Capsanthin is accumulated during the development of pepper fruit (Fig. 2), and Cst15.1 was the only QTL detected when capsanthin content at both 45 DAF and 90 DAF was considered as a
Fig. 4. Linkage map of the SM-DH population ('S3586' × 'Kyoto-Manganji No. 2'). SSR markers “PM”, “ge” and “es” were reported by Sugita et al. (2006, 2013), “CAMS” and “CaEMS” by Minamiyama et al. (2006) and Mimura et al. (2010, 2012), “Hmps” by Lee et al. (2004a), “HmpsE” by Yi et al. (2006), and “GPMS” and “EPSMS” by Nagy et al. (2007). Newly mapped SSR markers are indicated with asterisks (*). SNP markers “TC”, “CA”, “CO” and “CK” were reported by Sugita et al. (2013). SCAR markers BF7/BR9 and BF6/BR8 were reported by Lee et al. (2004b) and CRF3SCAR by Gulyas et al. (2006). The CAPS marker AFRF3CAPS was reported by Kim and Kim (2006).
Detection of QTLs for capsanthin content in pepper

Fig. 5. Comparison between KL-DH and SM-DH maps. KL01–KL12 are 12 linkage groups of the KL-DH map corresponding to the 12 pepper chromosomes (Sugita et al. 2013). SM01–SM15 are linkage groups of the SM-DH map constructed in this study. Identical markers on both maps are connected by lines.

Fig. 6. Positions of two QTLs for capsanthin content on the SM-DH linkage map. Positions of QTLs with 1-LOD support intervals are shown by black boxes. Vertical dotted lines indicate the LOD thresholds.
variation of a single phenotype (Supplemental Fig. 1), suggesting that Cst15.1 affects capsanthin content at more than one stage.

The color of pepper fruits starts to change at 45 DAF (turning-color stage), and this change is completed at 90 DAF (full-maturity stage); one QTL was detected at each of the two stages when the analyses were conducted with the two cultivations at each ripening stage as phenotypes (Table 3). Hence, the two QTLs may have distinct effects on fruit ripening. However, it is very difficult to identify the candidate genes for the QTLs because the existing regions of the QTLs on the pepper genome (http://peppergenome.snu.ac.kr) are too large to narrow down. On the other hand, CCS gene for capsanthin-capsorubin synthase, a key enzyme for capsanthin biosynthesis (Supplemental Fig. 3), begins to be expressed when the fruits starting to ripe (Lefebvre et al. 1998). Also, CCS gene was mapped to chromosome 6 (Thorup et al. 2000), and Cst 13.1 was also mapped to the same chromosome. Additionally, lycopene ε-cyclase gene (LCY-E, Supplemental Fig. 3), that probably act in the lutein synthesis pathway not in the capsanthin synthesis pathway, was mapped to chromosome 9 (Thorup et al. 2000) as with Cst 15.1. However, to detect the candidate gene of Cst 13.1 and Cst15.1 and to clarify the relationship of these QTLs with CCS and CLY-E, it is necessary to use the high-resolution QTL mapping and transcript quantification.

In spite of small $h^2$ values for capsanthin content at 45 and 90 DAF (Table 2), the QTLs detected in this study explained more than 15% of the total phenotypic variation, suggesting that the marker sets flanking these QTLs derived from ‘S3586’ would be efficient tools to breed peppers with high capsanthin content by marker-assisted selection. Because the SM-DH map covers only 75% of the entire genome, it may be necessary to check whether other QTLs cause the SM-DH map covers only 75% of the entire genome.
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