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Fine mapping and candidate gene analysis of *Brtri1*, a gene controlling trichome development in Chinese cabbage (*Brassica rapa* L. ssp *pekinensis*)

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ABSTRACT. Trichomes are derived from the epidermis and constitute an ideal system for studying cell division in plants. Here, a Chinese cabbage doubled haploid (DH) line (FT) without trichomes was crossed with another DH line (PurDH-1) with trichomes to develop an F_2 population for fine mapping of trichome control genes. Genetic analysis showed that the trichome phenotype was controlled by a single dominant gene, *Brtri1*. Using 1226 glabrous individuals in the F_2 segregation population, *Brtri1* was localized to a 16.84 kb region between markers Pur6-31 and Pur6-39 on chromosome A06. One of the four complete open reading frames within the mapping region, *Bra025311*, encodes a MYB transcription factor and is highly homologous to the trichome regulatory gene *GL1* in *Arabidopsis thaliana*. It was thus regarded as a candidate gene for *Brtri1*. Comparative sequencing showed a 5-bp

deletion in the third exon of *Bra025311* in FT, resulting in a frame-shift mutation. No expression of *Bra025311* was detected in FT. A co-dominant indel marker close to this mutation site was developed for marker-assisted selection in Chinese cabbage breeding.

Key words: *Brassica rapa*; Trichome; Fine mapping; Clone

INTRODUCTION

As the outermost extensions of plant epidermis, trichomes are widely present on the surface of most terrestrial plants (Hülskamp et al., 1994). Trichomes vary in morphology; they may be made from a single or multiple cells, branching or non-branching, and glandular or non-glandular. Trichomes have an important protective role as a physical barrier against abiotic and biotic stresses, including heat, drought, freezing, UV radiation, excess light, and fungal infections (Hauser, 2014).

Trichomes constitute an ideal system for exploring the molecular mechanisms of plant cell division, regulation of the cell cycle, and cell polarity (Szymanski et al., 2000). The trichomes of *Arabidopsis thaliana* are typically single-celled and non-glandular; their ontogeny has been clarified based on cloning and functional analyses of numerous trichome mutant genes. Some key regulatory genes controlling trichome development in *Arabidopsis* have been cloned, including the positive regulation factors *GLABRA1* (*GL1*) (Herman and Marks, 1989), *GLABRA3* (*GL3*) (Payne et al., 2000), *ENHANCER of GLABRA3* (Zhang et al., 2003), *Transparent Testa Glabra 1* (*TTG1*) (Chopra et al., 2014), *GLABROUS INFLORESCENCE STEMS* (*GIS*) (An et al., 2012), *GIS2* (Gan et al., 2007), *GIS3* (Sun et al., 2015), and Zinc Finger Protein 5, 6, and 8 (Gan et al., 2007; Zhou et al., 2011; 2013). In addition, the following MYB transcription factor-type negative regulation factors have been cloned: *Triptychon* (*TRY*) (Schnittger et al., 1999), *CAPRICE* (*CPC*) (Schellmann et al., 2002), *ENHANCER of TRY and CPC1* (*ETC1*) (Kirik et al., 2004a), *ETC2* (Kirik et al., 2004b), *TRICHOMELESS1* (*TCL1*) (Wang et al., 2007), and *SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE* (*SPL*) (Yu et al., 2010). *GL1*, *TTG1*, and *GL3* encode an R2R3-type MYB transcription factor, a WD40 protein, and a bHLH transcription factor, respectively. *GL1*, *TTG1*, and *GL3* combine to form a WD40-bHLH-MYB protein complex that regulates the expression of downstream genes to initiate the formation of trichomes (Pesch et al., 2015). A similar regulation pattern exists in other plants (Wan et al., 2014). Mutation of *GL1* and *TTG1* result in full loss of trichomes, while a *GL3* mutation decreases trichome number. MYB transcription factors *TCL1* and *TRY* compete with *GL1* at the protein level in binding with *GL3*, preventing the development of trichomes. *SPL9*, one of the target genes of miRNA156, activates the negative regulation factors *TCL1* and *TRY* by directly binding to their promoters, to inhibit the growth of trichomes (Yu et al., 2010).

Chinese cabbage (*Brassica rapa* L. ssp *pekinensis*) is one of the most important vegetables in east Asia. The genome sequence of Chinese cabbage has been released on the *Brassica* database website (http://brassicadb.org/brad/) (Cheng et al., 2011). Trichomes are ubiquitous in most germplasms of Chinese cabbage. As in *Arabidopsis*, trichomes usually exist throughout the whole Chinese cabbage plant, except for the cotyledons and epicotyls. Despite numerous studies having been conducted on the genetic features of the trichome trait in Chinese cabbage, the explanations of the underlying genetics vary. Some studies

have reported that the Chinese cabbage trichomes are controlled by a single dominant gene, whereas others have shown that trichomes are a quantitative phenotype, controlled by several major QTLs (Zhang et al., 2009; Song et al., 1995). Certain studies have implied that the mechanisms of formation of trichomes in *Brassica* crops and in *Arabidopsis* might be highly conserved (Nayidu et al., 2014). Zhang et al. (2009) cloned a gene (*Bra009770*) located on chromosome A06. This gene controls trichome formation and seed coat color, and is highly homologous with *TTG1* in *Arabidopsis*. A major QTL of leaf hairiness has been identified on chromosome A06 in Chinese cabbage, and a 5-bp deletion in exon 3 of the *GL1* ortholog (*BrGL1*) located in this QTL was detected in hairless germplasms with brown seeds (Li et al., 2011). In this study, based on simple sequence repeat (SSR) markers and a large-scale F_2 population, a gene located on chromosome A06 controlling trichome development of Chinese cabbage was fine mapped. Furthermore, this gene's sequence features and spatial expression pattern were validated as those of a candidate gene.

MATERIAL AND METHODS

Plant materials

PurDH-1 is a doubled haploid (DH) line obtained by microspore culture from a hybrid of Chinese cabbage and pak choi (*Brassica campestris* ssp *chinensis* Makino; also known as bok choy). Leaves of PurDH-1 are purple and covered by single-cell trichomes. The purple leaf phenotype of PurDH-1 was inherited from pak choi, whereas the trichome phenotype was inherited from Chinese cabbage. FT is a non-trichome DH line of Chinese cabbage (Figure 1).

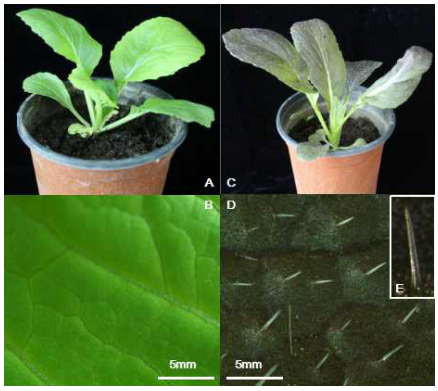

Figure 1. Morphological leaf characteristics of Chinese cabbage: glabrous DH line FT (**A**, **B**) and DH line PurDH-1 with trichomes (**C**, **D**, **E**).

Construction of mapping population

For genetic analysis of the trichome trait in Chinese cabbage, an F_2 population was constructed from a cross between PurDH-1 (with trichomes) and FT (glabrous) lines. All F_1 plants had trichomes. The F_2 segregation population was built with PurDH-1 as the maternal line and FT as the paternal line. Seeds of the F_2 generation were sowed into seedling trays (3.5 x 3.5 cm) in the greenhouse under natural lighting and temperature conditions, and the

trichome trait was determined at the two-leaf stage. Glabrous plants (homozygous recessive plants) in the F_2 population were used as the mapping population for *Brtri1*. The genetic status of the plants was determined by statistical analysis of the number of plants with trichomes and glabrous plants in the F_2 population. Individuals without trichomes in the F_2 population were used as the mapping population.

DNA extraction and primary mapping

DNA was extracted from individual plants from the parental and mapping populations, according to the procedure described by Murray and Thompson (1980) with minor modifications. Based on the Chinese cabbage genome sequence, 56 SSR primers covering the ten chromosomes were designed to examine polymorphism in PurDH-1 and FT. The polymerase chain reaction (PCR) conditions were: 94°C for 3 min; 30 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 30 s; followed by 72°C for 5 min. PCR products were then electrophoresed on a 6.0% SDS-PAGE gel, and visualized with silver staining (Sanguinetti et al., 1994). Thirty individuals from the mapping population were used to determine marker linkage, as well as which chromosome *Brtri1* is located on. Another 100 individuals from the mapping population were used for linkage analysis, in which recombination ratios were converted to genetic distance (cM) (Kosambi, 1943).

Fine mapping of *Brtri1* and sequence analysis of the candidate gene

Stable markers with clear bands flanking *Brtri1* were used for linkage analysis in the mapping population. Sequences within the primary localized region were downloaded from the BRAD website, in order to design SSR primers for fine mapping. The *Brtri1* candidate gene was selected based on functional information about coding genes within the fine mapping region. Primers were designed using Primer Premier version 5.0 (5'-GLA-L: ATGAGAACGAGGAGAAGAACAGAG-3' and 5'-GLA-R: CTAGAGGCAGTAGCCAGTATCACC-3'). Full-length DNA and cDNA sequences of the candidate gene in PurDH-1 and FT were amplified using high-fidelity Taq polymerase (TaKaRa, Dalian, China) following the manufacturer instructions. Sequences of the candidate gene in PurDH-1 and FT were aligned using ClustalW (Larkin et al., 2007), and compared. Two forward primers (P1: 5'-CACTTAACTTGTTAGTTGGGGCTA-3' and P3: 5'-TAGTTATCTGGCGTATTATTGTAACAA-3') and two reverse primers (P2: 5'-CTTCTTC AACTGTCCACAACCCTT-3' and P4: 5'-TTGTTACAATAATACGCCAGATAACTA-3') were designed to amplify the upstream regulatory sequences of Bra025311 in PurDH-1 and FT.

Analysis of *Brtri1* expression pattern

Total RNA of leaves, cotyledons, and roots of PurDH-1 and FT were extracted using Trizol (Invitrogen, Camarillo, CA, USA) and were treated with DNase RQI (Promega) to remove any DNA contamination. Aliquots of RNA (1 µg) were reverse-transcribed using SuperScript® III Reverse Transcriptase (Invitrogen) according to the manufacturer instructions. For real-time (RT) PCR, cDNAs were diluted to 100 µL, and 1 µL was added to 10 µL of SYBR Green PCR mix (TIANGEN, Beijing, China) together with 0.5 µL each primer (100 nM final concentration) in 20 µL reactions. The gene specific primers of

the candidate gene were CM-L (5'-GAAAACTGGGCTGAAGAGGTGT-3') and CM-R (5'-GCTTGTGGAGACGAATAATGAGG-3'). The results were normalized using actin as an internal control (with Actin-L: 5'-GGTCGTGACCTTACTGATTACCTCA-3' and Actin-R: 5'-GAAGTCTCCATCTCCTGCTCGT-3'). PCR and detection were performed on a Bio-Rad iQ5 cycler using the following conditions: 95°C for 60 s, followed by 40 cycles of 95°C for 10 s and 60°C for 20 s. Melt curve analysis (55-95°C) was performed to confirm the specificity of the PCR amplification. The qRT-PCR experiments were performed on three independent samples. The relative expression of the candidate gene was calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Semi-quantitative RT-PCR was performed with the same primers and undiluted cDNA products to detect *Brtri1* expression in leaves of PurDH-1 and FT. The PCR cycling conditions were 94°C for 5 min; followed by 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 60°C, and extension for 30 s at 72°C; followed by a final extension at 72°C for 5 min. The PCR products were electrophoresed on 2.0% agarose gel containing ethidium bromide and photographed under UV light.

Molecular marker for molecular marker-assisted selection of glabrous trait

Primers (Indel-L: 5'-TATTGGTGTTGACGTGTTGTACACA-3' and Indel-R: 5'-CACAGATTTTGTCTTGTTGATGATG-3') were designed based on the sequence differences of the *Brtri1* candidate gene in PurDH-1 and FT. Sequence polymorphisms were screened between 12 Chinese cabbage inbred lines with trichomes, and FT, to examined the usefulness of the marker in molecular marker-assisted selection of glabrous germplasm. The PCR conditions were predenaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, and finally 72°C for 5 min. The PCR products were then electrophoresed on a 6.0% SDS-PAGE gel, and silver stained prior to examination.

RESULTS

Genetic analysis

In the F_2 population, 3801 individuals had trichomes and 1226 individuals were glabrous. The segregation ratio fitted the expected 3:1 ($\chi^2_{0.05} = 0.955 < 3.841$), indicating that the trichome phenotype was controlled by a dominant gene, designated *Brtri1*.

Primary localization of *Brtri1*

Of 56 SSR primer pairs, 18 pairs produced polymorphic sequences in the two parent lines, based on electrophoresis, and all of these were co-dominant markers. The SSR marker Pur6-6, which was located on chromosome A06, was closely linked with *Brtri1*. Only a single recombinant individual was identified among the 30 mapping individuals. Thus, *Brtri1* was determined to be located on chromosome A06. Among the subsequent 100 mapping individuals, five more recombinant individuals were found, which further verified the linkage of the marker Pur6-6 with *Brtri1*. Ten more pairs of primers upstream and downstream of Pur6-6 were designed, and details of their location are presented in Table 1. Electrophoresis results showed that only three of them were polymorphic among the parents. Among the 130 mapping individuals, three different recombinant individuals were identified with Pur6-33, suggesting that *Brtri1* is located between these two markers.

Table 1. Sequences of primers used for mapping *Brtri1*.

Markers	Forward (5'-3')	Reverse (5'-3')	Location
Pur6-6	GTCGCACTTCGCTAAACCTAACT	AGACCCCTGCAATCAATTTCATA	20456341-20456538
Pur6-26	TTAAGGAAATTAGGGTAGGCAA	TAAATCAAAAACAATGGATGGAC	21244225-21244488
Pur6-31	CAACAGACTTTTGGAAACTACTCG	CACATTCATAACTGGGCTACGTAC	21190887-21191199
Pur6-29	TCTTCTCAACAACAATCATTACCCA	GACAAACAGCTTAGACTTGCTGCTA	21254491-21254718
Pur6-33	ATTGTCACATAATCTTGAAGCCATAC	CTTGCACACATCGAGGATAAATACTC	21365843-21366173
Pur6-35	GCAGTTTATCGCTTCGTTCTACC	TGCAGAGAAGAATCTGGGAGGT	21172007-21172383
Pur6-39	TGTACATCACTAATCTCGCCGTG	GAATCCAGAACCAGACCCTTG	21174045-21174762

Fine mapping of *Brtri1*

Given the stability of the bands amplified by primers Pur6-6 and Pur6-33, 1096 individuals from the mapping population were screened with these two marker primers, resulting in 52 and 18 recombinant individuals, respectively. These individuals were used as the population for further fine mapping. Based on the sequence between primers Pur6-6 and Pur6-33 taken from the BRAD database, 20 more pairs of SSR primers and indel markers were designed for fine mapping. After examination, five of these primer pairs produced polymorphic sequences, including Pur6-26, Pur6-29, Pur6-31, Pur6-35, and Pur6-39, from which nine, nine, three, three, and one recombinants were obtained, respectively. Thus, *Brtri1* was localized between marker Pur6-39 (at a distance of 0.12 cM) and Pur6-31 (at a distance of 0.04 cM) in a region 16.84 kb long (A06: 21,174,045-21,190,887 bp) (Figure 2).

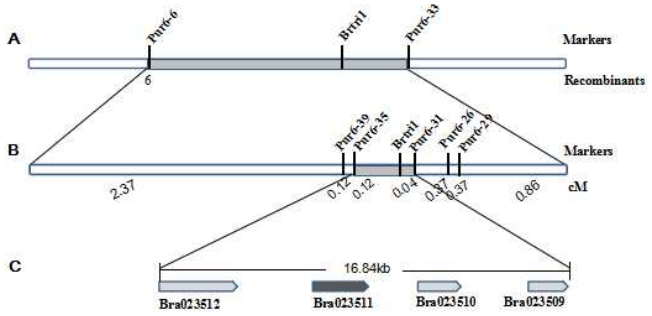

Figure 2. Genetic physical map and predicted open reading frames in the *Brtri1* region. **A.** Using 130 F_2 recessive glabrous individuals, *Brtri1* was mapped to the region between Pur6-6 and Pur6-33 on A06. **B.** Using 226 F_2 glabrous individuals, *Brtri1* was localized to the region between Pur6-39 and Pur6-31, with three and one recombination events, respectively. Numbers show the genetic distance between markers and *Brtri1* in cM. **C.** Location of *Brtri1* was narrowed down to a 16.84-kb region that contained four annotated genes.

Sequence analysis of *Brtri1*

Based on the genomic data held in the BRAD database, there are four genes within the fine mapping region, including *Bra025309*, *Bra025310*, *Bra025311*, and *Bra0235312* (Table 2). The predicted Bra025311 protein has a conserved MYB domain, belonging to the R2R3-type MYB transcription factor, and is highly homologous with the GL1 protein in *Arabidopsis*. Because the *GL1* phenotype is the same as the phenotype we studied, *Bra025311* was selected as the candidate gene of *Brtri1*. We cloned the whole length cDNA and DNA sequences of *Bra025311* from PurDH-1, with lengths of 678 and 1567 bp, respectively (Figure

3). Only the whole length DNA was obtained from FT. We then used the reverse transcription product of FT as template to amplify the internal control, *Actin*, from which the amplified band was the expected size, suggesting that the quality of the reverse transcription product of FT was reliable. This implied that *Bra025311* is possibly not expressed in FT. Sequence analysis showed that the *Bra025311* cDNA sequence from PurDH-1 was the same as that published in the BRAD database, except for 11 single nucleotide polymorphism (SNPs) in the third exon. In PurDH-1, *Bra025311* has three exons and two introns, and the boundary sequences of the introns and exons are in accordance with the GT-AG rules. The Bra025311 protein of PurDH-1 exhibits 73% identity with the *Arabidopsis* GL1 protein. *Bra025311* from PurDH-1 and Chiffu-401 (BRAD database) both encode a 225-amino acid protein, containing two SANT domains (SWI3, ADA2, N-CoR, and TFIIIB). At the DNA level, there were three SNPs and four indel sites between PurDH-1 and FT, but the only frame-shift mutation was a 5-bp deletion in the third exon of FT (Figure 4).

Table 2. Annotation of genes in *Brtri1* location region.

Gene ID	Orthologous gene in *Arabidopsis*	Function	E-value (BLASTx)
Bra025309	AT3G27890	NADPH: QUINONE OXIDOREDUCTASE	1.00E-81
Bra025310	AT4G39240	kelch repeat-containing F-box family protein	3.00E-105
Bra025311	AT3G27920	GL1 (ATMYB0)	9.00E-95
Bra025312	AT3G27925	Serine-type endopeptidase	0

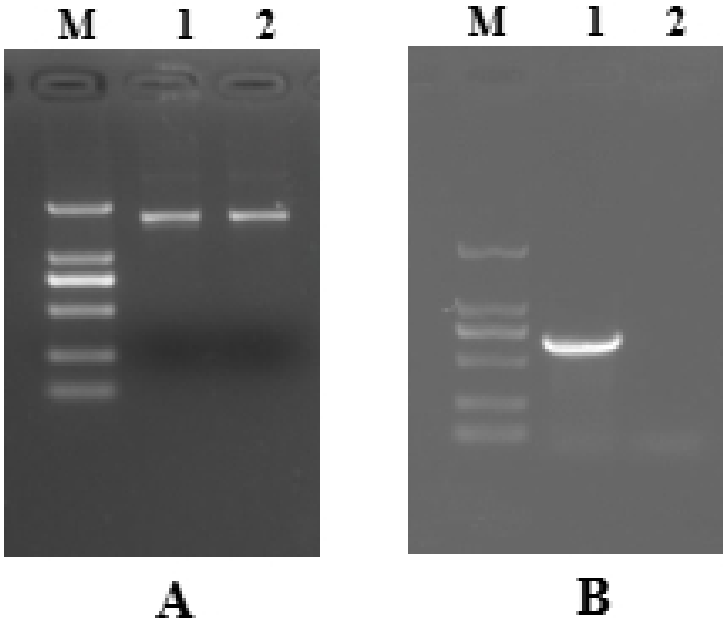

Figure 3. Cloning of the full-length DNA and cDNA sequence of the *Brtri1* candidate gene in FT and PurDH-1. **A.** Full-length DNA sequence. **B.** Full-length cDNA sequence. *Lanes 1* and *2* indicate PurDH-1 and FT, respectively.

Figure 4. Major *Bra025311* sequence differences between FT and PurDH-1. The three exons are underlined. The frame indicates the 5-bp deletion site.

Primers P1 and P2 were designed to amplify the upstream regulatory sequences of *Bra025311* in PurDH-1 and FT. P1 and P2 were located at -1063 to -1040 bp and +50 to +73 bp, respectively, where the A of the initiation codon ATG is designated as +1. An approximately 1100 bp product was obtained from PurDH-1, which was consistent with the expected length from the BRAD database. No products were obtained from FT using the same primers. Consequently, forward primer P3 (-527 to -500 bp) and reverse primer P4 (-500 to -527 bp) were designed, and the primer combinations P3 + P2 and P1 + P4 were used to amplify the relevant sequences of *Bra025311* in FT. A PCR product was obtained from FT using P3 + P2, and no obvious differences existed in the sequences of FT and PurDH-1. No PCR product was obtained from FT using P1 + P4 (data not shown).

Analysis of the spatial expression patterns of *Brtri1*

qRT-PCR and RT-PCR were used to examine the differences in expression of *Bra025311* between PurDH-1 and FT. The qRT-PCR results showed that the expression level of Bra025311 in PurDH-1 leaf was almost 420 times higher than that of FT leaf (Figure 4). In PurDH-1, the highest expression level of *Bra025311* was in the leaf, followed by the root, and the lowest was in cotyledons, which is consistent with trichome locations. The RT-PCR results indicated that *Bra025311* was not expressed in FT leaves (Figure 5).

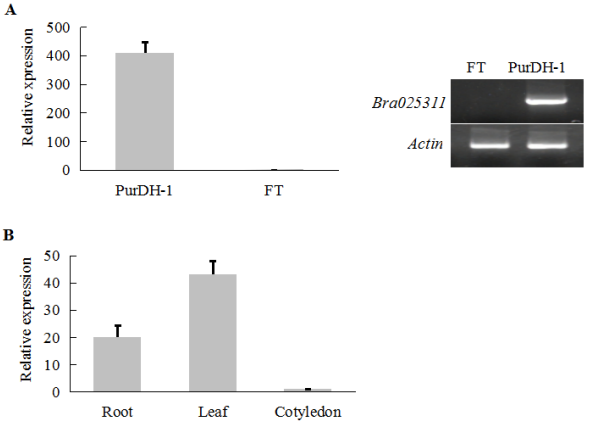

Figure 5. Expression analysis of Bra025311 in PurDH-1 and glabrous DH line FT. **A.** Expression patterns in leaves of PurDH-1 and FT using qRT-PCR and RT-PCR. **B.** Expression patterns of Bra025311 in leaf, root, and cotyledon of PurDH-1 using qRT-PCR. The expression level was normalized to the reference gene *Actin*.

Design of molecular marker based on the candidate gene

One pair of primers was designed based on the 5-bp indel site on the third exon of Bra025311 between PurDH-1 and FT. Electrophoresis results showed that in each of the 12 inbred lines with trichomes, the product was 302 bp long, whereas the product from FT was 297 bp, which could be clearly distinguished by SDS-PAGE (Figure 6). This suggested that this co-dominant indel marker can effectively distinguish the heterozygous, homozygous recessive, and homozygous dominant genotypes, and that it can be used effectively in marker-assisted selection of the glabrous phenotype. This marker was designated TRM1.

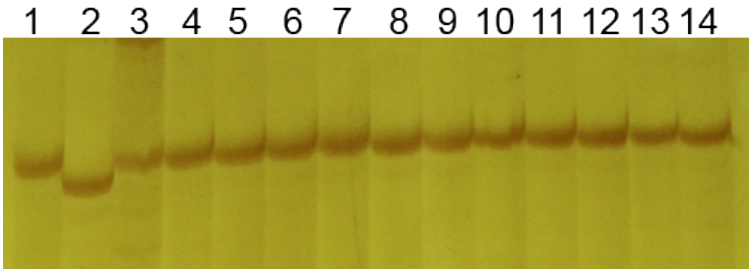

Figure 6. Polymorphic indel marker TRM1 in different Chinese cabbage germplasms. *Lane 1*: PurDH-1, *Lane 2*: FT. *Lanes 3-14*: different Chinese cabbage DH lines with trichomes.

DISCUSSION

Trichome ontogeny is strictly regulated by complex gene networks and mutations of key genes might produce trichome-deficient or glabrous phenotypes. Analysis of mutants has clarified the mechanism of trichome formation in *Arabidopsis* (Pattanaik et al., 2014). Trichome development is conserved across plants, but has elements of specificity in different groups. Both *Arabidopsis* and Chinese cabbage belong to Brassicaceae, and they are closely related, suggesting that their mechanisms of trichome formation might be conserved (Wang et al., 2011). One gene reported from Chinese cabbage, *Bra009770*, the homolog of *TTG1* on chromosome A06, regulates trichome development and the seed coat color (Zhang et al., 2009). Map-based cloning is one of the most effective ways to isolate genes controlling important agronomic traits (Wei et al., 2013; Ngu et al., 2014; Zou et al., 2016). In our paper, we attempted to clone the gene controlling trichome development in Chinese cabbage using this method. The candidate gene *Brtri1* was mapped to chromosome A06. Considering that the two DH lines we used have no significant differences in seed coat color, *Brtri1* would appear to be a novel gene controlling the development of trichomes in Chinese cabbage.

MYB transcription factors are coded by an ancient and highly conserved gene family, and play a number of roles in plant growth and development (Nguyen and Lee, 2016). Many MYB transcription factors have been shown to participate in the regulation of trichomes and root-hair, with positive or negative regulatory functions (Wang and Chen, 2014). *GL1* operates upstream of regulatory networks controlling the initiation of plant trichomes, and its mutation can result in the loss of trichomes (Dai et al., 2016). Using fine mapping, *Brtri1* was localized to a 16.84-kb long region. Within this region, *Bra025311*, encoding a R2R3-type MYB transcription factor and showing 95% homology with *GL1*, was regarded as the candidate gene of *Brtri1*. A 5-bp deletion within the coding sequence of this gene in FT resulted in a frame-shift mutation. This mutation (both the gene and its location) is consistent with previous research, but the expression pattern of *Bra025311* in FT is different from previous *research* (Li et al., 2011). In PurDH-1, the expression pattern of *Bra025311* was similar to that of *GL1*, whereas *Bra025311* was not expressed in FT. Given that trichomes are present in the vast majority of Chinese cabbage resources, the glabrous character of FT was thought to be due to a knock-out mutation. There are two possible reasons for the loss of function of *Bra025311* in FT. One is the deletion in the third exon, which led to a frame-shift mutation. Another possible reason is the mutation in the promoter. We also attempted to amplify the upstream regulatory sequence of *Bra025311* in PurDH-1 and FT. The 1100-bp sequence obtained from

PurDH-1 was consistent with that from the BRAD database; however, no products were obtained from FT. After trying different primers, a 500-bp PCR product was amplified in FT, which suggested that the mutation might occur in the regulatory sequences in FT. This may be the reason behind the loss of *Bra025311* function in FT. We will investigate our results further through overexpression the *Bra025311* mutation in FT in future research. Since only one single copy of the homologous gene (*Bra025311*) of *GL1* exists in the Chinese cabbage genome (as validated in the BRAD database), we expect the *Bra025311* mutation to affect the formation of the GL1-GL3-TTG1 complex, resulting in the glabrous phenotype. In other studies, the leaves and flower buds of FT were employed as the plant materials for RNA-seq analysis (Huang et al., 2015; 2016). However, no reads on *Bra025311* have been detected in FT, which further suggests that *Bra025311* is not expressed in FT (Huang et al., 2015; 2016).

Marker-assisted selection is one of the most effective techniques that could speed up the breeding process of crops. Most germplasms of Chinese cabbage are covered with trichomes. However, varieties without trichomes are more popular with consumers. According to traditional breeding strategies, an excellent germplasm can be converted to a glabrous phenotype by crossing it with a glabrous resource with saturated backcross. However, because the glabrous phenotype is recessive, individuals need to be self-pollinated in each generation. We have developed one co-dominant indel marker based on the sequence differences of the *Brtri1* candidate gene between germplasms with and without trichomes. This marker could be used to evaluate the heterozygous genotype in backcross populations, obviating the need for evaluation of genotypes by self-pollination, which would significantly increase breeding efficiency.

Conflicts of interest

The authors declare no conflict of interest.

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